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Research Article

The Biodiversity Assessment of Sediment Community in Balekambang Lake, Dieng Plateau, Indonesia, using Environmental DNA (eDNA) Metabarcoding Approach

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ABSTRACT

A sediment community is vital for the health of the surrounding community. Microbes play an essential role in chemical cycling. Meanwhile, diatoms, unicellular photosynthetic algae found in the water bodies and sediments, are used as water bioindicators for their fast responses to changes in water quality. Assessing the sediment community is crucial to understanding ecosystem dynamic. With the newest technology in DNA identification, this research aims to identify the community in the Balekambang Lake, Dieng Plateau, Central Java, Indonesia. This preliminary study tested the Environmental DNA (eDNA) Metabarcoding method to determine the eukaryotes in the sediment community. This study utilized the High-Throughput Sequencing method to massively identify the organism communities in the sediment, targeting the 18S rRNA gene. This study captured millions of sequences, including Eukaryota, Excavata, Amoebozoa, Opisthokonta, Rhizaria, and Alveolata supergroups. This method identified 14 genera and 13 species of multicellular and unicellular organisms from the Balekambang Lake sediment samples. Although this study could not identify more organism taxa due to a high number of "unidentified" groups in the sampling area, the results show the importance of the eDNA Metabarcoding technique for biodiversity assessment in the sediments.

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INTRODUCTION

Freshwater sediments store high levels of biodiversity and provide organic substances for the sustainability of freshwater ecosystems and their surroundings. Microorganisms, including bacteria and invertebrates such as insects, nematodes, and crustaceans, generally dominate sediments (Sun et al. 2018). The composition of organisms in sediment can be used as a bioindicator for the sediment. Some taxa typically used as bioindicators are bacteria, nematodes, arthropods, and diatoms. Bacteria serve a bioindicators of biological, physical, and chemical of the soil. Soil condition and health are generally characterised by diverse soil microbiomes (Ray et al. 2020; Santos & Olivares 2021). Nematodes and arthropods are usually used as indicators of soil health based on their abundance and diversity in the soil (Menta & Remelli 2020). Diatoms are also often used in waters as bioindicators of water quality because they are sensitive to changes in water conditions (Masouras et al. 2021).

The dynamic nature of sediment ecosystems in freshwater environments highlights the need for rapid data assessment to see the diversity and changes in the ecosystem (Stefanidis & Papastergiadou 2024). The silting of lakes is an inevitable consequence of sedimentation (Hauer et al. 2018). Typically, sediments from rivers and streams are transported into the lake, moving through dense bottom currents, intermediate layers, and surface flows. However, when there is an excessive influx of terrestrial sediments, it can lead to the formation of a fluviolacustrine delta (Vonk et al. 2016). Lake sediments play a crucial role in the lake ecosystem, serving as natural archives that record environmental changes in the catchment area. Land-use modifications are well documented within these sediments, which can be analysed using multiple indicators, much like entries in a diary (Soeprobowati et al. 2021).

Research on the sediment biodiversity from the freshwater ecosystem in Indonesia is mainly done with traditional methods: morphological-based identification. Sediment's diatom biodiversity has been studied from Rawapening Lake (Soeprobowati et al. 2012), Warna Lake (Soeprobowati et al. 2018), Pengilon Lakes (Sari et al. 2021), Galela Lake (Soeprobowati et al. 2023), Cebong Lake (Soeprobowati et al. 2022; Putri et al. 2023), Bengawan Solo, Brantas (Andriyono et al. 2023) and Balekambang Lake (Soeprobowati et al. 2023). The conventional method of identifying diatom species using a light microscope is time-consuming, as it requires examining a minimum of 300 frustules for accurate analysis (Soeprobowati et al. 2016). Therefore, applying eDNA and metabarcoding is a promising method for environmental assessment (Bailet 2020; Gregersen et al. 2023).

Molecular research in Indonesia is still considered expensive—for example, freshwater diatom data. Although diatoms are key species determining water health, the diatom molecular database from Indonesia is still limited. Research using a molecular approach, such as DNA Barcoding, is currently being developed because it provides a fast and massive method for detecting organisms from the environment or identifying cryptic species (Andriyono et al. 2020; Joesidawati et al. 2023). Newer methods with environmental DNA can also detect the presence of organisms without having to see the organisms directly. This is important because some essential environmental organisms are complicated to detect (Kelly et al. 2016; Kelly et al. 2017).

Environmental DNA (eDNA) is one of the most adopted methods used by ecologists to assess the targeted taxa in the environment (Lim et al. 2016; Bailet et al. 2020). The amplicon-based method (DNA Metabarcoding) in eDNA utilizes the High Throughput Sequencing (HTS) approach. NGS allows a massive and cheaper way to sequence millions of small DNA fragments to identify the community structure and abundance directly from environmental samples (Madduppa et al. 2021). Additionally, several studies have successfully implemented eDNA to detect diatom biodiversity through sedi-

ment samples (Kutty et al. 2022; Gregersen et al. 2023).

This is the preliminary study to test the eDNA Metabarcoding method in the freshwater lake community in Central Java. Indonesia. This study aims to identify the eukaryotic community in the Balekambang Lake, Dieng Plateau, Central Java, Indonesia. In future research, this method will be used as an additional approach to assessing the sediment community in freshwater, brackish, and marine ecosystems in Indonesia.

METHODS

Field Sampling

Samples were collected from Balekambang Lake, in the Dieng Plateau, Central Java, Indonesia on July 26, 2022 (sampling coordinatesa -7.2077891172104955, 109.90839964907225). Ten grams of surface sediment were collected using a sterile spatula and preserved in 10 mL 96 % ethanol (Persaud et al. 2021). Four samples (sample ID: S2006, S2007, S2008, and S2009) were collected from four sampling points (Figure 1) in Telaga Balekambang, Dieng. The sampling coordinates are -7.207655, 109.908093 (S2006), -7.207648, 109.908129 (S2007), -7.207614, 109.908325 (S2008) and -7.207634, 109.908399 (S2009). The samples were stored in a cool box and transported to Diponegoro Biodiversity Project Laboratory at Laboratorium Terpadu, Universitas Diponegoro, Semarang, Central Java.

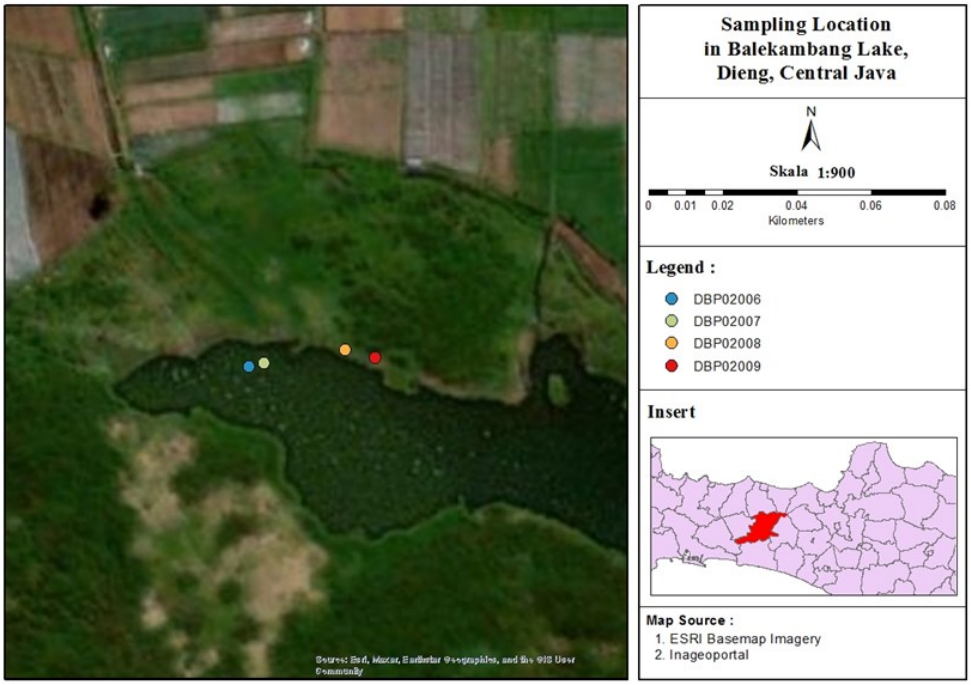

Figure 1. Map of the sampling location in Balekambang Lake, in Dieng Plateau, Central Java, Indonesia on July 26, 2022

Genetic Data Preparation

A total of 0.25 grams of sediment was extracted using Quick-DNA™ Fecal/Soil Microbe MiniPrep Kit from ZymoBIOMICS™ following the extraction protocol. Thirty microlitres (uL) of DNA extraction were sent to sequencing facility for library preparation and a sequencing using the NGS platform. The 18S rRNA genes were targeted using V4_18SNext.For and V4_18Snext.Ref primers (Manzari et al. 2015) and sequenced with MiSeq Illumina platform. Only four (S2006, S2007, S2008, and S2009) out of five samples were successfully sequenced and continued into bioinformatic analysis steps.

Data Analysis

Forward and reverse FASTQ data were merged, primers were removed, qual-

ity was filtered and analysed using *QIIME2* version 2019.1.0. (Quantitative Insights into Microbial Ecology 2 program, https://qiime2.org/). The Divisive Amplicon Denoising Algorithm 2 (*DADA2*) software, integrated into *QIIME2*, was used to filter, trim, de-noise, merge the data, and remove the chimeric sequences using the consensus method (Callahan et al. 2016). The ASVs (Amplicon Sequence Variants) were produced by training a feature classifier in *QIIME2* against the PR2 database (Guillou et al. 2013), adopting a default confidence threshold of 0.7. The taxonomic composition of each sample was summarized using *phyloseq* (McMurdie & Holmes 2013) in RStudio (R Development Core Team). The *ggplot2* (Wickham et al. 2016) in RStudio (R Development Core Team) was used to generate stacked bar plots summarizing taxonomic composition and sequence abundance based on the total abundance in the samples. For each sample from each location, we used http://bioinformatics.psb.ugent.be/webtools/Venn/ to create a set of Venn diagrams to determine how many ASVs were shared between sampling locations. The rarefaction curves were created with the Ranacapa package using the *grade* command (Kandlikar et al. 2018).

RESULTS

A total of 113,889 reads were obtained from this study. The mean of the read per sample is 28,472.25 reads, with a total of 145 ASVs. The reads range from 1,939 to 51,172 sequences per sample. Samples were then rarefied to an equal number of reads (1,939 reads) to minimize bias in data analysis. At the end, 7,756 reads and 120 ASVs were used for downstream analysis.

The taxa composition based on read abundance shows a slightly different composition between the four samples (Figure 2). Overall, six supergroups were identified, namely Eukaryota, Excavata, Amoebozoa, Opisthokonta, Rhizaria, Alveolata, and an unidentified group. Excavata were the dominant taxa in three samples (S2007, S2008, and S2009). Meanwhile, a sample with ID S2006 shows the dominance of Opisthokonta.

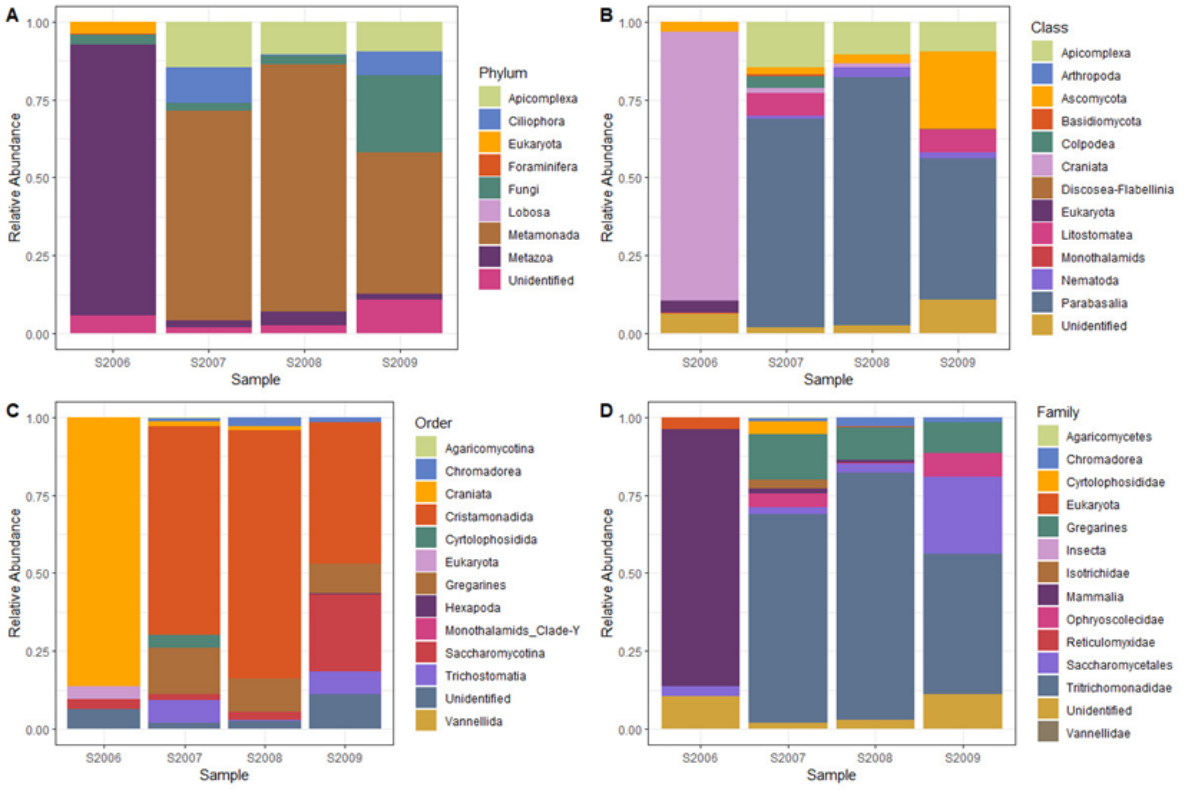

Figure 2. A bar plot illustrating the taxonomic composition based on the read abundance of eukaryotic taxa from each sample. The plot represents the taxonomic hierarchy at different levels: (A) Phylum, (B) Class, (C) Order, and (D) Family.

At the species level, sample S2006 was dominated by human DNA (*Homo sapiens*) comprising approximately 20 % of the total reads across all samples and 80 % of reads in the S2006 sample alone. The other three samples, S2007, S2008, and S2009 were dominated by *Tritichomonas muris* (Family Tritichomonadidae). The other dominated taxa are *Gregarines* sp. and *Candida apicola*.

The study identified 14 different genera (Figure 3, Table 1.) and identified 13 species (*Tritrichomonas muris*, *Candida apicola*, *Gregarines* sp., *Gregarina cloptoni*, *Ophryoscolex* sp., *Entodinium* sp., *Cyrtolophosididae* sp., *Homo sapiens*, *Candida tropicalis*, *Isotricha prostoma*, *Distolabrellus veechi*, *Ripella* sp., *Haplomyxa saranae)* and unidentified group. Sample S2006 was dominated by the Homo genus, with an unidentified group comprising 212 reads (11 % of the total sample reads). Meanwhile, sample S2007 was dominated by the *Tritrichomonas* and *Gregarines* genera (67 and 15 % of reads from each total sample reads, respectively). The *Tritrichomonas* genus also dominated samples S2008 and S2009. However, sample S2009 also has a dominant *Candida* genus and an Unidentified genus (25 and 11 % of reads from each sample reads, respectively).

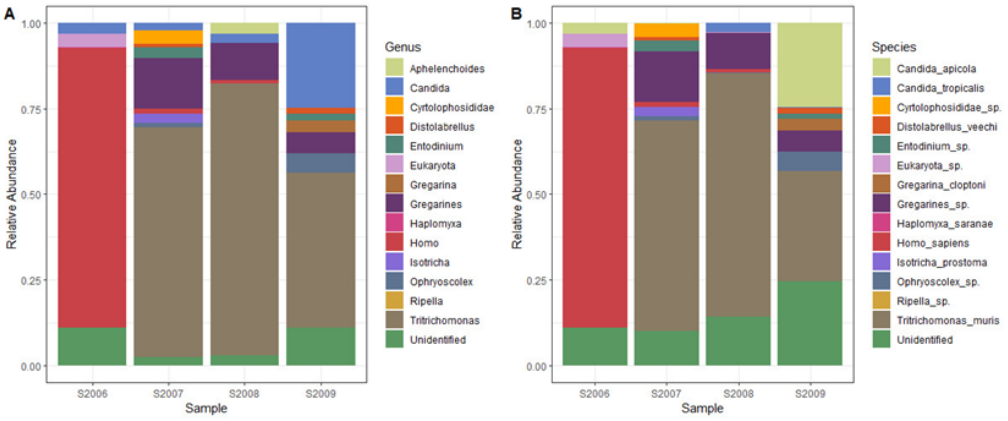

Figure 3. A bar plot showing the taxonomic composition based on the read abundance of eukaryotic taxa from each sample. The plot represents the taxonomic hierarchy at two levels: (A) Genus and (B) Species.

Table 1. The list of species present in each sampling location is from four eDNA Metabarcoding data from Dieng Plateau, Central Java, Indonesia.

Species name	Presence at ID Sample			
	S2006	S2007	S2008	S2009
Candida apicola	Yes	Yes	Yes	Yes
Candida tropicalis			Yes	Yes
*Cyrtolophosidida*e sp.		Yes		
Distolabrellus veechi		Yes		Yes
Entodinium sp.		Yes	Yes	Yes
Gregarina cloptoni				Yes
Gregarines sp.		Yes	Yes	Yes
Haplomyxa saranae	Yes			
Homo sapiens	Yes	Yes	Yes	
Isotricha prostoma		Yes		Yes
Ophryoscolex sp.		Yes	Yes	Yes
Ripella sp.				Yes
Tritrichomonas muris		Yes	Yes	Yes
Eukaryota sp.	Yes		Yes	
Unidentified	Yes	Yes	Yes	Yes
Total Species*	3	9	7	10

* *Exclude Unidentified and Eukaryota* sp.

Rarefaction, Alpha diversity, and Venn Diagram

The rarefaction curve of the 18S rRNA amplicons indicates that none of the samples reached a plateau (Figure 4), suggesting that additional sequencing depth is required to capture the full diversity of the amplified taxa.

Sample S2006 has the highest number of ASVs (41) but the lowest taxonomic diversity, with only three identified species: *Candida apicola*, *Haplomyxa saranae*, and *Homo sapiens*. *Haplomyxa saranae* species were only found in the S2006 sample. The majority of reads in sample S2006 were identified as *Homo sapiens* (1,583 reads or 82 % of the total sample reads). The study also identifies nine and seven species from samples S2007 and S2008, respectively. Sample S2009 has the highest number of identified species (10 species). *Species Gregarina cloptoni* and *Ripella* sp. were only found in sample S2009.

The Venn diagram shows that only one ASV (*Candida apicola*) was shared among all samples (Figure 5). Sample S2006 had the highest number of unique ASVs (37 ASVs), followed by sample S2009 with 24, S2007 with 23, and S2008 with 21.

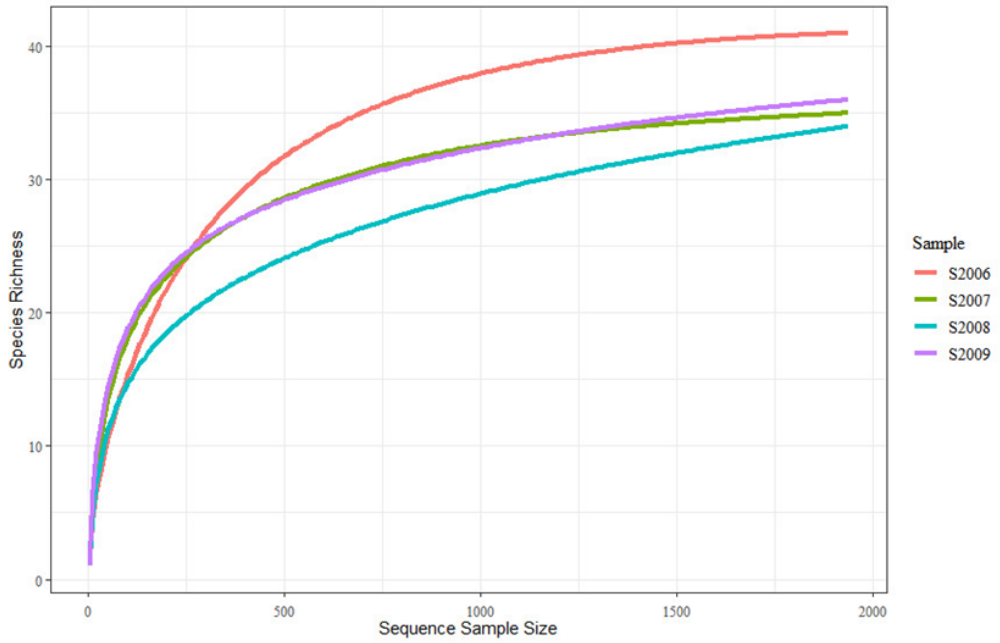

Figure 4. Rarefaction plot for each of the samples examined in this study. Species richness (left axis) plotted against sequencing depth (bottom axis).

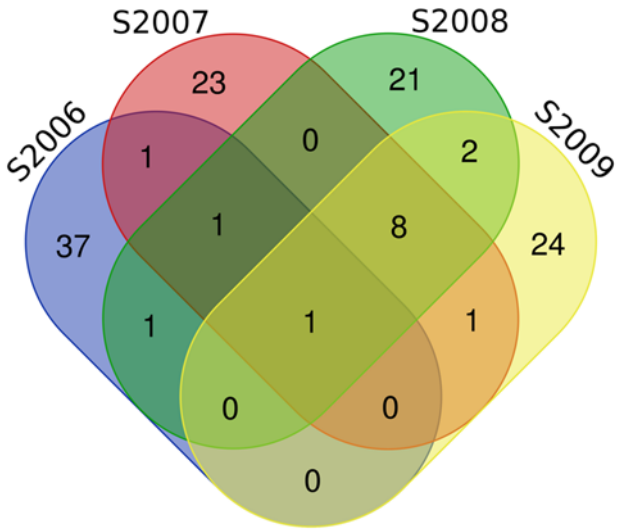

Figure 5. The Venn diagram illustrates the number of unique and shared Amplicon Sequence Variants (ASVs) among four samples.

DISCUSSION

The eukaryotic community in sediment plays a vital role in maintaining ecosystem health. Eukaryotes such as arthropods, apicomplexans, and diatoms are usually used as bioindicators and have fast responses to changes in the water quality. They play crucial roles in nutrient cycling, habitat provisioning, and serving as a food source for numerous organisms (B-Béres et al. 2023). Diatoms are found in both freshwater and marine environments, but they belong to different species. Freshwater diatoms differ from marine diatoms in terms of size, silica content, and species composition, reflecting the distinct environmental conditions in which they thrive (Litchman et al. 2009). Freshwater diatoms are also beneficial to see the past condition of the freshwater ecosystem (paleolimnology). The eukaryotic communities in sediment were identified and counted for morphology and abundance under a microscope. Unfortunately, despite many estimated extant species, our knowledge of this diverse biodiversity remains limited (Mann & Vanormelingen 2013). Currently, molecular approaches, such as DNA metabarcoding, provide a powerful tool for investigating unknown eukaryotic diversity and expanding our understanding of their distribution patterns (Juhel et al. 2022). The eDNA Metabarcoding method provides both species identification and read abundance data, which can be used to predict the dominance of specific taxa in the ecosystem (Rees et al. 2014; Beng & Corlett 2020; Andriyono et al. 2021; Garcia-Vazquez et al. 2021; Cahyani et al. 2024).

The utilization of the 18S rRNA gene as a marker for single-cell eukaryotes has been reported by several studies including diatoms (Evans et al. 2007; Pawlowski et al. 2016; Bailet et al. 2020), marine protists in Indonesian waters (Cahyani 2021), fish (Kumar et al. 2022), Coral-Zoothanthella (Shinzato et al. 2018) and fungi (Quandt et al. 2023). The V4 region of the 18S rRNA gene has a great potential to identify diatoms and other protist sequences (Zimmermann et al. 2015). However, the choice of genetic markers and database availability is always a challenge.

One interesting taxon captured by the eDNA Metabarcoding method is the *Haplomyxa saranae*. This species is a new naked freshwater foraminifer, identified in 2014 (Dellinger et al. 2014). This is an exciting finding and needs to be explored since Balekambang Lake is situated on the Dieng Plateau, with temperature ranges from 12-20 °C during the day (Fahma et al. 2024).

Agricultural fields, such as potatoes and other vegetables, surround the Balekambang Lake (Hakim et al. 2014). This could be why this study found *Distolabrellus veechi*, a nematode species usually found in agricultural soil (Bhat et al. 2020). The ecosystem surrounding the sampling area can also be the reason that this study found a dominant Candida taxon (*Candida apicola* and *candida tropicalis*). Candida is a yeast family that might relate to some potato diseases (Zheng et al. 2021).

Another dominant taxonomic group found in this study is Protozoa. This protozoan group consists of several species, *Cyrtolophosididae* sp., *Entodinium* sp., *Isotricha prostoma*, *Ophryoscolex* sp., *Ripella* sp., and *Tritrichomonas muris*. Most of these protozoans are found in the animal's intestines as parasites (Jouany & Ushida 1999; Escalante et al. 2016). This finding could be related to the conditions around Balekambang Lake, an area of agriculture and animal husbandry (Fahma et al. 2024; Hakim et al. 2014).

The eDNA Metabarcoding used in this study can capture hundreds of sequences, including Excavata, Amoebozoa, Opisthokonta, Rhizaria, and Alveolata supergroups. This method is able to identify 14 genera and 13 species from the Balekambang Lake sediment samples. In future studies, some things need to be improved, including the sampling and preservation technique and the bioinformatic analysis. Nevertheless, this study hopes to provide prelimi-

nary data on how important the eDNA Metabarcoding technique complements the morphological identification of eukaryotes including protists in the sediment.

The dynamics of sedimentary eDNA may be influenced by sediment substrates, as it is believed that DNA adsorption is affected by variations in surface area corresponding to particle size (Barnes et al. 2021). Sediments help slow down biologically driven DNA decay by adsorbing both DNase enzymes and DNA molecules, especially in low-oxygen environments such as deeper sediment layers. Consequently, sedimentary eDNA tends to have a longer lifespan compared to aqueous eDNA (Sakata et al. 2020). Sedimentary eDNA analysis has the potential to enhance future biomonitoring and ecological studies by offering insights across different timescales, providing a broader perspective on environmental changes (Sakata et al. 2020).

The application of eDNA research for ecosystem assessment in sediments is a promising approach. Time efficiency, low price, and the large amount of data are some of the advantages of the eDNA assessment method (Foster et al. 2020). However, the challenges ahead are marker selection, sample preparation, and database availability (database gaps for unidentified taxa) (Elbrecht et al. 2017; Casey et al. 2021). Therefore, the use of eDNA should become a complement and not replace conventional methods (such as morphological approaches) (Pereira et al. 2021).

This study provides an initial exploration of eDNA application in freshwater ecosystems, with a particular focus on freshwater sediments. Despite challenges, such as limited database availability and the need for improved sample processing techniques, it offers a promising method for rapid data collection. The findings from this study contribute to scientific knowledge and hold significant potential for conservation and ecosystem management efforts.

CONCLUSION

This research serves as an example to show how eDNA with the 18S rRNA marker can be used to assess eukaryotes in sediment and analyse the diversity of the sediment ecosystem in Balekambang Lake, Dieng, Central Java. This study identifies various taxa including nematodes, foraminifera, yeast and protozoan. Some of the dominant taxa, such as *Distolabrellus veechi*, *Candida apicola*, and *Candida tropicali*, are generally associated with conditions around lakes which are dominated by agriculture. This research provides a basic database for lake management in Balekambang, Dieng, such as the diversity and richness of the sediment taxa that can reveal the conditions of the surrounding environment and the changes it undergoes over time, offering valuable insights into ecosystem health and dynamics.

AUTHORS CONTRIBUTION

NKDC contributes in conceptualisation, methodology, conduct the analysis, visualization, and original draft preparation. Followed by EMK contributes in project administration, writing, reviewing, and editing the manuscript. MDAM with the data analysis, visualization, and writing, reviewing and editing the manuscript. MHAF, FAR, STN, TLNF, JJ and RH contribute in curating the data, writing, reviewing, and editing the manuscript. TRS plays role in funding acquisition, writing, reviewing, editing the manuscript.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest

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