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Fish Diversity and Phylogenetics in the Lakes of Bromo Tengger Semeru National Park Based on eDNA

Wilsen Chuanata[1], Ari Susilowati[1], Agung Budiharjo[1], Justin M.J. Travis[2]

[1]Graduate Program of Bioscience, Faculty of Mathematics and Natural Sciences, Universitas Sebelas Maret. Jl. Ir. Sutami 36A Surakarta 57126, Central Java, Indonesia. Tel./fax.: +62-271-663375,
[2]School of Biological Sciences, University of Aberdeen, Aberdeen, Scotland, UK.

*Corresponding Author: arisusilowati@staff.uns.ac.id

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Abstract. Indonesia is home to 1,248 species of freshwater fish, which play a vital role in sustaining the ecosystem. Lakes Ranu Pani, Ranu Regulo, and Ranu Darungan, located within the Bromo Tengger Semeru National Park, lack data on freshwater fish. This study aimed to assess fish diversity in these lakes using environmental DNA (eDNA) metabarcoding and to establish phylogenetic relationships among the species present. Water samples were filtered using 0.45 µm mixed cellulose ester filter paper, after which the collected DNA was extracted utilizing a DNA miniprep kit. The 12S rRNA gene was amplified using the MiFish primer, and the amplicon DNA was sequenced with the Illumina MiSeq 2x250 platform. The sequences were aligned using Blast+ for species identification and subsequently analyzed for alpha and beta diversity. A phylogenetic tree was constructed with MEGA12. The findings revealed eight species from five families, with *Gambusia affinis* being the most abundant. Ranu Pani exhibited low species diversity, while Ranu Regulo and Ranu Darungan displayed moderate diversity. Beta diversity analysis indicated similarities between Ranu Pani and Ranu Regulo, but distinct communities were found in Ranu Darungan. Phylogenetic analysis showed close relationships between species from the Cichlidae and Osphronemidae families, while a more distant relationship was observed with the Cyprinidae family. These baseline data can serve as a reference for future research on developing conservation strategies, monitoring environmental changes, and supporting biodiversity management.

Keywords: 12S rRNA; Diversity; eDNA; Freshwater Fish; Metabarcoding

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INTRODUCTION

The richness of freshwater fish species contributes significantly to Indonesia's biodiversity; the total number of species is approximately 1,248 (Dina et al., 2022). Kalimantan, Sumatra, and Java are part of the western region in Indonesia, which is an important hotspot for freshwater fish. Java island is composed of a diverse and complex freshwater system, characterized by its mountainous regions and numerous rivers and lakes (Hasan et al., 2022). Semeru (3,676 meters above sea level) is the highest mountain on Java, located at the border of Malang and Lumajang, East Java (Hendrawan et al., 2023). This mountain has rich biodiversity, making it a part of the Bromo Tengger Semeru National Park (BTSNP). Ranu Pani, Ranu Regulo, and Ranu Darungan are lakes located on the slopes of Mount Semeru.

Generally, fish sampling in lakes is performed using traditional direct sampling methods such as netting and electrocution (Millar et al., 2023). Advancements in technology now allow for the collection of biodiversity information through environmental DNA (eDNA). eDNA samples can be obtained from water sources (Sahu et al., 2023). Nowadays, the use of eDNA for biomonitoring purposes has increased (Hansen et al., 2018), and offers a promising method for species identification. Sampling using eDNA has many advantages, such as being a non-invasive technique, which reduces the potential of disturbance and harm to the environment. Additionally, it is time-efficient, more sensitive (Fukuzawa et al., 2023), and does not require direct contact with the target organism (Suryobroto et al., 2022).

The environmental DNA (eDNA) method, followed by metabarcoding analysis, allows researchers to detect fish diversity by identifying species through the 12S rRNA mitochondrial gene

marker (Xiong et al., 2022). Mitochondrial DNA is an advantageous gene marker due to the high variability of sequence that differs between species and distinctiveness at the species level (Cahyadi et al., 2018). MiFish is a primer designed to target the 170 bp-long hypervariable region of the 12S gene in both freshwater and marine fish (Yamamoto et al., 2024). This region stores essential information, enabling only short partial sequences to accurately identify fish to the species level (Miya et al., 2020; Xu et al., 2024), and effective even at lower DNA concentrations (Collins et al., 2019). Considering the potential degradation of DNA from environmental samples, a small barcode size (less than 200 bp) is recommended to improve PCR success rates (Li et al., 2024). Environmental DNA is often found in low concentrations; hence, the use of cytochrome c oxidase subunit I (COI) gene is less effective, as it requires higher DNA concentrations for reliable results (Collins et al., 2019). Research on freshwater fish metabarcoding in China shows that compared to the 16S rRNA or COI gene markers, the 12S rRNA marker is generally more successful in detecting species diversity (Zhang et al., 2020).

The diversity of freshwater fish in Indonesia is important for completing the existing data and carrying out conservation efforts. However, such data collection has not yet been conducted to assess fish diversity on Mount Semeru. This study aims to determine the biodiversity of freshwater fish in Ranu Pani, Ranu Regulo, and Ranu Darungan by analyzing fish eDNA. A phylogenetic relationship of the fish species was also assessed by constructing a phylogenetic tree. This research provides valuable information that can be used as a base for sustainable conservation efforts.

METHODS

Sample Collection and Measurement of Environmental Parameters

Samples of water were collected using a sterilized 1 L bottle sampler at 0.5 m depth (Leblanc et al., 2020). Sampling was done at the three lakes within BTSNP: Ranu Pani and Ranu Regulo on the northern slopes, and Ranu Darungan on the southern slopes of Mount Semeru (Figure 1). Each lake was made into 2 stations. The eDNA was isolated from water samples using a 0.45 µm mixed cellulose ester filter paper (Milipore) and a Nalgene rapid flow device connected to a peristaltic pump (Bessey et al., 2020). Filter paper containing eDNA was preserved in a 1.8 mL cryotube containing 1.5 mL Longmire buffer solution (Williams et al., 2016). The cryotubes were stored in a cool box to prevent DNA degradation during transport to the laboratory.

Environmental parameters, such as water temperature and pH, were measured using a water thermometer and pH meter at each sampling site. Laboratory analyses were performed at the Laboratory of the Faculty of Mathematics and Natural Sciences at Universitas Sebelas Maret. The study was conducted from July to November 2024.

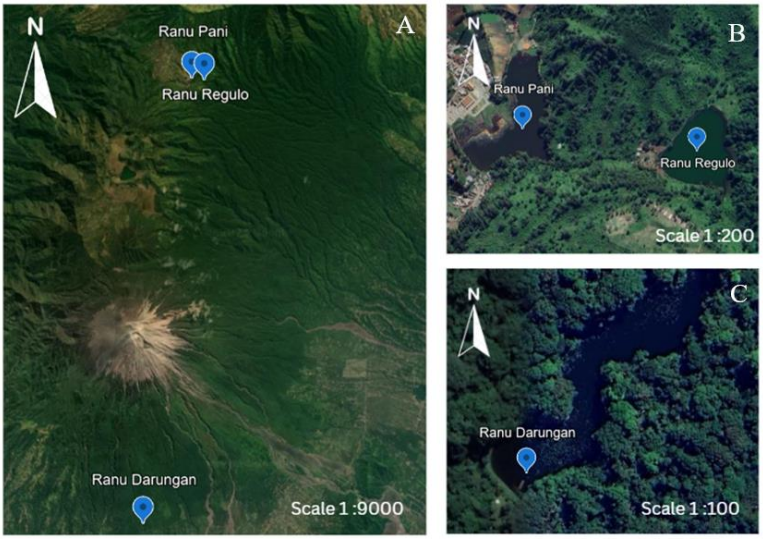

Figure 1. Research location in the BTSNP. (A) The location of Ranu Pani and Ranu Regulo Lake on the northern slope of Mt. Semeru, Ranu Darungan on the southern slope of Mt. Semeru; close-up view of: (B) Ranu Pani and Ranu Regulo lakes, (C) Ranu Darungan lake.

eDNA Extraction and Quality

The filter paper containing eDNA was cut into smaller pieces to improve the release of eDNA. The eDNA extraction was performed using the ZymoBIOMICS™ DNA Miniprep Kit (Zymo Research, USA), following the protocol guidelines provided by the manufacturer. To verify the eDNA extraction, gel electrophoresis was performed on a 1% agarose gel (Rahmi et al., 2023) at 85 volts for 60 minutes. Genomic DNA analysis was performed using a biophotometer to determine the concentration and purity of the DNA obtained. An amount of 5 µl eDNA sample was diluted with distilled water to a final volume of 100 µl in a new cuvette, and the eDNA concentration and purity were measured at a wavelength of A260/A280 (Peletiri et al., 2021).

12S rRNA Gene Amplification and Sequencing

The MiFish universal primer sets are used for amplification (Miya et al., 2015) with the addition of Illumina overhang for sequencing purposes. The primers consisted of the forward primer U-F 5'-TCGTCGGCAGCGTCAGATGTGTATAAGAG ACAGGTCGG TAAA ACT CGTGCCAGC'-3 and reverse primer MiFish U-R 5'-GTCTCGTGGGCT CGGAGA TGTGTATAAGAGACAGCATAGTGGGGTAT CTAATCCCAGTTTG-'3. PCR mixture, with a total volume of 50 µL, consisted of 25 µL of 2x MyTaq HS Redmix PCR master mix (Bioline, UK), 2 µL of 10 µM upstream primer, 2 µL of 10 µM downstream primer, 19 µL of nuclease-free water, and 2 µL of eDNA as the template. The polymerase chain reaction (PCR) was carried out according to Xin et al., (2024) with optimization. The process begins with initial denaturation at 95°C (3 minutes) followed by 35 cycles, each consisting of denaturation at 98°C (20 seconds), annealing at 62°C (30 seconds), and elongation at 72°C (20 seconds). After completing 35 cycles, a final elongation step was performed at 72°C for 10 minutes. The expected size of the PCR product was between 300 and 400 bp and was verified by gel electrophoresis on a 1% agarose gel (Rahmi et al., 2023) at 85 volts for 60 minutes. The PCR product was purified using a gel extraction kit (Fermentas). The purified PCR product was then sent to Macrogen Singapore for sequencing with the Illumina MiSeq 2x250 platform.

Data Analysis

The sequence in the form of fastq, which is still raw data, is processed with Cutadapt to cut the adapter, primer, and poly tail-A attached to the sequence (He et al., 2020). The cleaned sequence is preprocessed through the quality filtering stage to remove low-quality sequence data based on the quality score of each read, denoising to identify and correct sequencing errors while maintaining biological variation, merging to combine forward reads and reverse reads into a single sequence and finally chimera removal to remove chimeras or artifacts formed due to the combination of DNA fragments from two or more sources during PCR using DADA2 software (Hakimzadeh et al., 2023). Then, Amplicon Sequence Variants (ASVs) with a sequence length exceeding 200 bp will be removed using R. Each ASV is aligned with the NCBI database using the BLAST+ algorithm.

The ASV data obtained were then analyzed using Ubuntu software with the QIIME2 pipeline application, including the Shannon-Wiener and Simpson indices for alpha diversity and the Bray-Curtis Index for beta diversity analysis. In metabarcoding, alpha diversity refers to the variety of species found in a single sample, while beta diversity pertains to the differences in species composition between samples (Calle, 2019). Community diversity can be evaluated by considering species richness (number of species) and evenness (relative abundance of species) using the Shannon-Wiener diversity index (Yuan et al., 2024). Species distribution was confirmed using FishBase (http://www.fishbase.se/). The phylogenetic tree was constructed in MEGA 12 software using the Neighbor-Joining model with 1,000 bootstrap applications (Hariyanto et al., 2019) in MEGA 12 software.

RESULTS AND DISCUSSION

Environmental Conditions on Sampling Site

The water temperature and pH measured at each research location varied. Water temperatures in Ranu Pani, Ranu Regulo, and Ranu Darungan were 26°C, 23°C, and 28°C, while the pH was 10, 8.3, and 7.7, respectively.

eDNA and PCR Product Quality

According to Bairoliya et al. (2022), the concentration of eDNA in freshwater typically ranges from 0.5 to 25.6 µg/L. The quality of eDNA is crucial for successful eDNA metabarcoding. It is important to note that eDNA can be contaminated or degraded. A good purity ratio for eDNA is between 1.7 and 1.9 based on the

A260/A280 measurements (Peletiri et al., 2021). Only two out of six samples had a good eDNA concentration and purity after extraction, which indicates that the DNA at these locations have a low DNA concentration (Table 1), but this is not a significant issue, as the 12S rRNA gene can still be effectively analyzed even with low eDNA concentrations (Collins et al., 2019).

The electrophoregram of the extracted eDNA showed the genome size exceeding 10,000 bp, indicating that the extraction was successful (Figure 2A). Meanwhile, the electrophoregram of the PCR product, following gel purification, displayed a single visible DNA band with a size greater than 250 bp (Figure 2B). This size is obtained since the 170 bp product has an additional overhang from the MiFish primer.

Table 1. eDNA concentration and purity

Parameter	Ranu Pani		Ranu Regulo		Ranu Darungan	
	P1	P2	R1	R2	D1	D2
Concentration DNA (µg/L)	0.518	0.245	0.465	0.515	0.075	0.140
Purity DNA	1.64	1.53	2.24	1.87	1.70	1.65

Note: P1: Ranu Pani 1, P2: Ranu Pani 2, R1: Ranu Regulo 1, R2: Ranu Regulo 2, D1: Ranu Darungan 1, R2: Ranu Darungan 2.

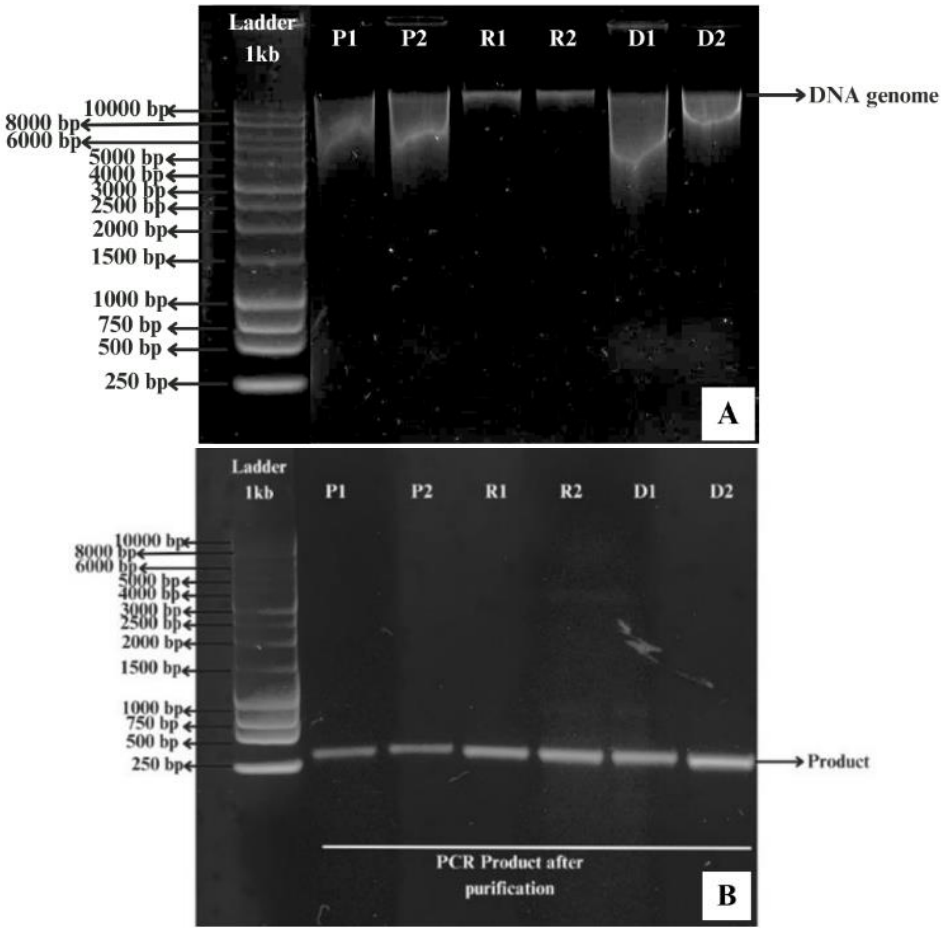

Figure 2. Electrophoregram (A) eDNA genome, (B) PCR product after purification. P1: Ranu Pani 1, P2: Ranu Pani 2, R1: Ranu Regulo 1, R2: Ranu Regulo 2, D1: Ranu Darungan 1, D2: Ranu Darungan 2.

Table 2. List of fish species in BTSNP identified by the 12S rRNA gene region.

Family	Species	Common name	Occurrence	Total reads						Genbank Accession number
				P1	P2	R1	R2	D1	D2	
Channidae	*Channa gachua*	Red seam snakehead	Introduction	2	0	0	1002	1537	4870	MK371068.1
Cichlidae	*Oreochromis niloticus*	Nile tilapia	Introduction	24	257	0	1674	68550	7171	LC776886.1
	Amphilophus amarillo	Red devil	Introduction	0	0	0	4	289	478	KY315559.1
Cyprinidae	*Cyprinus carpio*	Common carp	Introduction	1880	2203	1663	0	0	604	LC552361.1
	Barbodes binotatus	Spotted barb	Native	17	0	0	385	6955	59412	LC069419.1
Osphronemidae	*Trichopodus microlepis*	Moonlight gourami	Introduction	0	0	0	5	3134	619	NC_027238.1
Poeciliidae	*Gambusia affinis*	Mosquitofish	Introduction	95787	95268	95986	94122	17265	24349	PP620812.1
	Poecilia reticulata	Guppy	Native	0	0	62	314	12	246	LC146200.1

Note: P1: Ranu Pani 1, P2: Ranu Pani 2, R1: Ranu Regulo 1, R2: Ranu Regulo 2, D1: Ranu Darungan 1, R2: Ranu Darungan 2.

Species Identification

The study identified 80 amplicon sequence variants (ASVs) by clustering sequences that exhibited over 95% similarity. These ASVs correspond to the sequences of eight species from five families of freshwater fish. According to Table 2, not all fish species were found at the three research locations. Five out of the eight species were present in Ranu Pani. Meanwhile, in both Ranu Regulo and Ranu Darungan, all species were observed.

MiFish Primer is a universal primer widely used in fish metabarcoding that specifically targets the 12S rRNA gene within the 170 bp region. The primer can be used to detect marine and freshwater species (Miya et al., 2015). This study showed that MiFish primers successfully identify eight species of freshwater fish in BTSNP. Studies involving MiFish primers in Indonesia are primarily focused on marine fish (Andriyono et al., 2019; Ambeng et al., 2024), this research gives a valuable reference for identifying freshwater fish species in Indonesia.

Several species encountered in this study are introduced or non-native. The introduction of non-native species can cause biological invasions, which have a negative impact on the environment and the economy (Gallardo et al., 2016). The invasive species can be a significant threat to the ecosystem function and contribute to the decline of native species (Shuai & Li, 2022). This will lead to the disruption of food webs and ultimately harm the stability of ecological function.

Only 2 out of the 8 detected fish species are native to Indonesia, namely *Channa gachua* and *Barbodes binotatus*. Other species, namely *Gambusia affinis*, *Oreochromis niloticus*, *Poecilia reticulata*, *Cyprinus carpio*, and *Amphilophus amarillo,* are not native to Indonesia. Still, they are introduced for various purposes, such as biocontrol, source food, and as ornamental fish (Andriyono & Fitrani, 2021; Xiang et al., 2021). The existence of *O. niloticus* in the BTSNP, an introduced fish species, provides great value to local residents who utilize the fish as a food source. *G. affinis* is introduced from North and Central America to various regions in the world for biocontrol purposes, especially mosquitoes, because it is an omnivorous animal that eats various types of insects and has good adaptability (Chalabia et al., 2022). Meanwhile, *P. reticulata* and *C. carpio* are ornamental fish with attractive colors (López Fuentes et al., 2021; Andrian et al., 2024).

Introduced species pose a serious threat to ecosystem sustainability. For instance, *A. amarillo* disrupts Lake Toba's habitat (Lumbanraja and Nasution, 2024) and has also been found in Ranu Regulo and Ranu Darungan, albeit in small numbers. Despite their low population, these invasive species could harm the ecosystem over time. Controlling their numbers is crucial to prevent potential ecological damage. *Trichopodus microlepis* is the only fish species reported to be absent from Indonesia, according to FishBase (http://www.fishbase.se/). Sequence alignment indicates that this species may belong to the gourami group from Indonesia. However, due to

the limited availability of Indonesian fish sequence data in the database, the alignment relies on species found outside of Indonesia. Nonetheless, this does not eliminate the possibility that *T. microlepis* may already exist in various regions of Indonesia, as several studies have identified it through eDNA metabarcoding techniques (Wibowo et al., 2023).

Relative abundance of the species

The most abundant species observed in Ranu Pani in both P1 and P2 locations were *Gambusia affinis*, with relative abundance of 98.032% and 97.501%, respectively. This species was also the most abundant fish community in Ranu Regulo, with a relative abundance of 98.236% in site R1 and 96.328% in site R2. The abundance of this fish is related to its high reproductive rate and good adaptability (Chalabia et al., 2022). With limited food resources in Ranu Pani and Ranu Regulo, *G. affinis* has a larger population than other species because it is a small fish that does not require many resources. In contrast, Ranu Darungan has two species that are the most abundant. *Oreochromis niloticus* dominated at site D1, with a relative abundance of 70.157%, while *Barbodes binotatus* was the most widespread at site D2, with a relative abundance of 60.804%. Ranu Darungan, with more abundant food resources and better environmental conditions than the other two lakes, supports large fish to dominate. Other species were present but found with lower relative abundances, as illustrated in Figure 3.

Alpha and Beta Diversity

The Shannon-Wiener index value has a categorical scale to interpret the diversity value, namely low ($H'<1.0$), medium ($1.0<H'\leq3.0$), and high categories ($H'>3$) (Rosalina et al., 2023). Based on alpha diversity analysis, the value of the Shannon-Wiener diversity index in Ranu Pani is categorized as low, while Ranu Regulo and Ranu Darungan are moderate, with both lakes having higher total ASV than Ranu Pani (Table 3). Simpson's index, also known as the dominance index, is a measurement tool to evaluate the degree of dominance of individuals in a community. The value of this index ranges from 0 to 1. Smaller index values indicate that no species dominates, while larger values indicate the dominance of certain species. This implies a low diversity or species richness level in the community (Kitikidou et al., 2024). Simpson's index value has a categorical scale to interpret dominance, the categories are low ($0 \leq C \leq 0.4$), medium ($0.4 < C \leq 0.6$), and high ($0.6 < C \leq 1$) (Rosalina et al., 2023). Based on this index, Ranu Pani and Ranu Regulo are classified as high-category, primarily due to the dominance of *G. affinis*. In contrast, Ranu Darungan is categorized with a moderate dominance index for D1 and a low dominance index for D2.

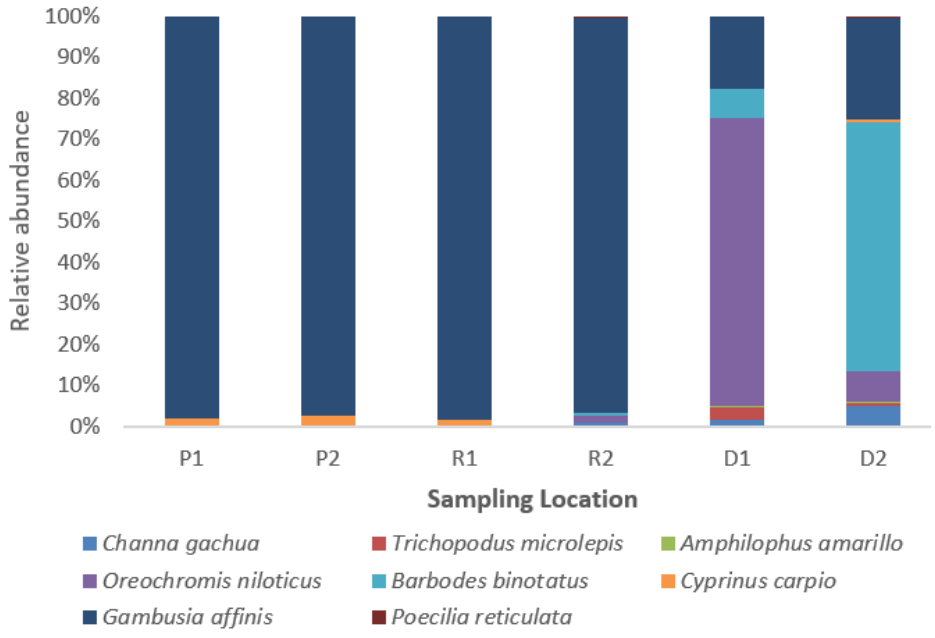

Figure 3. Relative abundance at the species level. P1: Ranu Pani 1, P2: Ranu Pani 2, R1: Ranu Regulo 1, R2: Ranu Regulo 2, D1: Ranu Darungan 1, R2: Ranu Darungan 2

Table 3. Alpha diversity indices and amplicon sequence variants of fish found in Ranu Pani, Ranu Regulo, and Ranu Darungan

Location	Shannon-Wiener	Simpson	ASV
P1	0.26	0.94	8
P2	0.43	0.90	12
R1	1.13	0.74	20
R2	1.00	0.71	20
D1	1.70	0.52	26
D2	2.35	0.28	25

Note: P1: Ranu Pani 1, P2: Ranu Pani 2, R1: Ranu Regulo 1, R2: Ranu Regulo 2, D1: Ranu Darungan 1, D2: Ranu Darungan 2

In Ranu Pani, the low Shannon-Wiener index and high Simpson index indicate a lack of fish species diversity, primarily due to the dominance of certain species. This situation is closely linked to the severely polluted environmental conditions in Ranu Pani, which allow only a limited number of fish species to survive. In contrast, Ranu Regulo is categorized as having a moderate Shannon-Wiener index, suggesting greater fish diversity. Ranu Darungan exhibits a moderate diversity index while the dominance index is low, indicating that the environment supports a wider variety of evenly distributed species. This finding aligns with which stated that a high diversity index implies a well-structured and stable ecosystem capable of supporting a wide range of aquatic organisms. Conversely, a low diversity index is a sign of ecological imbalance and decreased habitat suitability for sustaining aquatic life.

The alpha diversity index in this study was influenced by environmental factors measured at each location. Water pH can be easily affected by nature due to biological processes and anthropogenic activities such as industrial, agricultural, and atmospheric deposition (Dewangan et al., 2023). The ideal pH for fish habitats ranges between 6.5 and 9 (Marium et al., 2023). This study discovered that the very high pH of water in Ranu Pani is the least favorable for fish development and may increase the chance of inhibiting growth and reproductive capabilities. Ranu Regulo and Ranu Darungan maintain pH values within this range. Human activity is likely to be the leading cause of Ranu Pani's high water pH value. Most of the water in Ranu Pani has been polluted by waste from human activities such as tourism and agriculture, traveling, and camping

Beta diversity analysis is commonly used to evaluate the similarity of communities from different locations (Campos et al., 2024). Beta diversity is assessed using the Bray-Curtis approach. Principal coordinate Analysis (PCoA) is one of the principles for analyzing the distance matrix between samples in a multidimensional space. The closer the samples are to each other, the more similar their communities (Shi et al., 2023). In Figure 4, Principal Component 1 accounts for 74.02% of the total variation among the samples, highlighting the differences between sample communities, while Principal Component 2 contributes only 24.08%. Consequently, the confidence value is 98.1%.

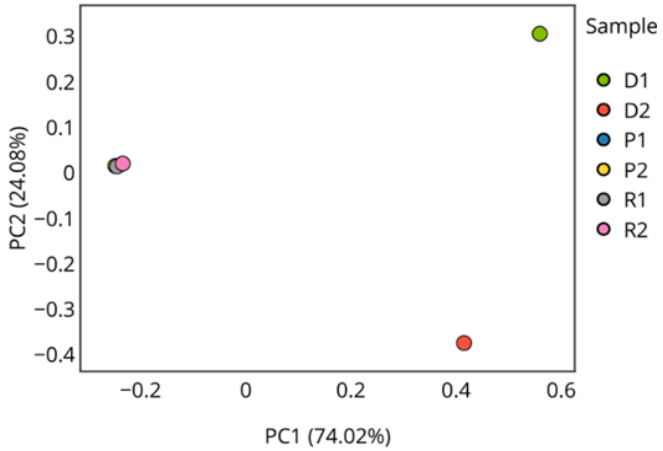

Figure 4. Beta diversity PCoA Bray-Curtis. P1, P2, R1, and R2 are overlapping. P1: Ranu Pani 1, P2: Ranu Pani 2, R1: Ranu Regulo 1, R2: Ranu Regulo 2, D1: Ranu Darungan 1, R2: Ranu Darungan 2, PC : Principal component

D1 and D2 originate from the same location, Ranu Darungan; however, the communities between them are not closely connected. This suggests a difference in community diversity between D1 and D2. In the PCoA diagram, P1, P2, R1, and R2 appear to overlap. This overlap indicates that their fish community structures are similar (Shi et al., 2023), as both locations are dominated by the same species, *G. affinis*. Additionally, variations in fish community structure may arise from differences in habitat characteristics, such as pH and temperature (Li et al., 2018).

Phylogenetic Relationship

The phylogenetic tree was constructed using the Neighbor-Joining model with 1,000 bootstraps. Based on Figure 5, the phylogenetic tree formed is divided into five branches, namely *B. binotatus* and *C. carpio* on branch A with bootstrap support 100%, *P. reticulata* and *G. affinis* on branch B with bootstrap support 87%, *C. gachua* on branch C with bootstrap support 34%, *O. niloticus*, and *A. amarillo* on Branch D with bootstrap support 76%, *T. microlepis* on branch E with bootstrap support 34%, and *Clarias* sp. as outgroup. *Clarias* sp. was chosen as the outgroup, as it is a freshwater fish that is widely distributed in Indonesia. The phylogenetic analysis indicates that branches A, B, and D have high confidence, while branches C and E are considered weak or not well-supported (Sulistyaningsih et al., 2018).

The phylogenetic tree presented in this study established distinct family groups among the fish. *O. niloticus* and *A. amarillo* fish from the Cichlidae family have a close phylogenetic relationship with the *T. microlepis* from the Osphronemidae family. The high bootstrap value in clade A (100) indicates a strong kinship between *C. carpio* and *B. binotatus*. These two species are known to belong to the same family, Cyprinidae, while *G. affinis* and *P. reticulata* in clade B belong to the Poeciliidae family. The relationship between clades A and B suggests a closer common ancestor than the other clades. *Clarias* sp. used as an outgroup separates itself from all other taxa, suggesting that it has the most distant evolutionary divergence.

Some nodes in the phylogenetic tree in this study are weakly supported, but it demonstrates several characteristics of a good phylogenetic tree (dichotomous branching and some strong bootstrap support). The phylogenetic tree that was formed has some of the characteristics described by Lazuardhi et al. (2025), in the form of A phylogenetic tree with high bootstrap values, dichotomous branching, and consistently formed clades is are feature of a phylogenetic tree considered good and acceptable in systematics, but enough to describe the kinship of each species found.

This research provides novelty value in providing the first fish database in the BTSNP, which has never been done before, especially in these three lakes. The use of environmental DNA (eDNA) as a data collection method demonstrates that the eDNA metabarcoding technique can be effectively and efficiently implemented in freshwater habitats to obtain data on fish diversity. Furthermore, this method adheres to the same identification standards, as it references a database with 12S rRNA marker genes. In addition, this research can be the basis for further research on determining conservation strategies, monitoring environmental changes, and supporting biodiversity management. That way, conservation activities can be carried out more optimally.

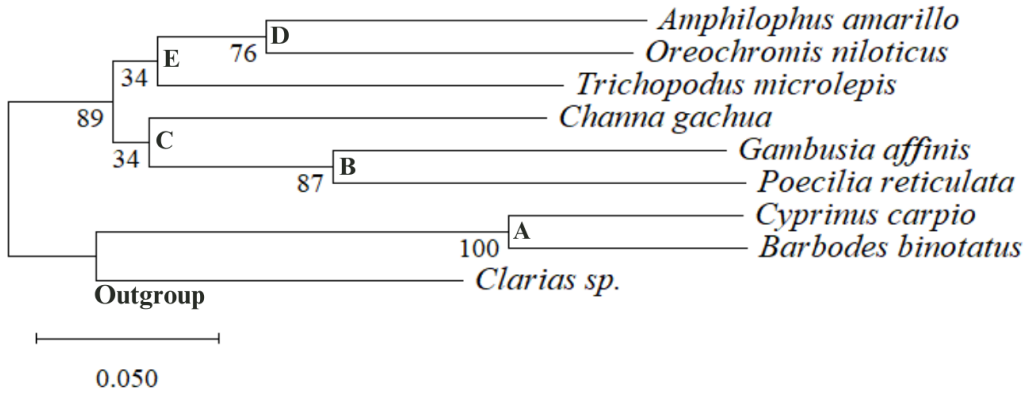

Figure 5. Phylogenetic relationship of freshwater fish from BTSNP based on 12S rRNA gene sequence by the Neighbor-Joining method using MEGA 12.

CONCLUSION

Based on this study, it can be concluded that the eDNA metabarcoding technique has successfully identified freshwater fish in the BTSNP. A total of 8 fish species from 5 families were identified using 12S rRNA gene markers. Fish diversity in Ranu Pani is categorized as low with high species dominance, while Ranu Regulo and Ranu Darungan have moderate fish diversity. The phylogenetic tree formed by the Neighbor-Joining method shows the closeness of fish from the Cichlidae family with Osphronemidae and has a distant kinship with the Cyprinidae family. This baseline data can serve as a reference for future research on conservation strategies, monitoring environmental changes, supporting biodiversity management, and guiding sustainable resource utilization.

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AUTHOR CONTRIBUTION STATEMENT

The contributions of each author are as follows: WC designed and conducted the research, generated the data, and drafted the manuscript. AS guided the development of the research design, secured funding, and contributed to writing the manuscript. AB also played a role in developing the research design and in writing the manuscript. JM.J.T reviewed and proofread the manuscript. All authors participated in discussions and approved the final version of the manuscript.

CONFLICT OF INTEREST STATEMENT

All authors have reviewed the manuscript and agree that they have no conflict of interest to declare.

USE OF ARTIFICIAL INTELLIGENCE (AI)-ASSISTED TECHNOLOGY

The authors declare that no artificial intelligence (AI) tools were used in the generation, analysis, or writing of this manuscript. All aspects of the research, including data collection, interpretation, and manuscript preparation, were carried out entirely by the authors without the assistance of AI-based technologies

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