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Exploration of freshwater fish biodiversity in Diatas and Dibawah Lakes using molecular approaches

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ABSTRACT: Data and information on freshwater fish biodiversity in Diatas and Dibawah Lakes (Twin Lakes), West Sumatra, are still limited. This study aimed to provide fish biodiversity data using conventional (morphology and DNA barcoding) and non-invasive methods (environmental DNA Metabarcoding). Individual specimens and tissue samples were directly captured for DNA barcoding. Water samples were collected at six sites for eDNA metabarcoding. Universal primers (Fish F1 and Fish R1) were used for DNA barcoding and eDNA metabarcoding. A total of 23 fish species were identified using conventional methods. Cyprinidae was the family with the highest species richness. Of the total 23 samples obtained, nine species from the 11 species in the Cyprinidae and Danionidae groups (the most abundant and native species) were analyzed for DNA barcoding. A total of 35 DNA barcodes from nine species were successfully generated and deposited in the BOLD System and GenBank. Only seven species of the 23 species (30%) were detected using eDNA metabarcoding. Other species were detected (124 species) and suspected as false positives, and other species were not detected (16 species) and suspected as false negatives. The low detection of eDNA may be influenced by several main factors: the use of universal primers, incomplete reference libraries, low DNA quality, contamination, and the absence of target species in samples. Monitoring biodiversity can be conducted using conventional methods (Morphology and DNA barcoding), combined with non-invasive methods (eDNA metabarcoding) as a complementary approach. Thus, researchers can provide more reliable biodiversity assessments in the future.

1. INTRODUCTION

Freshwater fishes are an important component of inland aquatic ecosystems that play a role in maintaining trophic structures and ecological functions (Reid et al., 2019). However, various global studies show that freshwater fish populations have experienced a significant decline in recent decades (Reid et al., 2019; Su et al., 2021). Indonesia is one of the global centers of freshwater fish biodiversity, including West Sumatra. Aquatic ecosystems in West Sumatra, including lakes and rivers, are not exempt from these threats. West Sumatra has five major lakes: Singkarak Lake, Maninjau Lake, Diatas Lake, Dibawah Lake, and Talang Lake (Roesma, 2011; Ajiwibowo et al., 2019). Despite their importance, anthropogenic pressures and overexploitation can reduce freshwater fish diversity, potentially disrupting the stability and threatening the sustainability of lake ecosystems in West Sumatra (Roesma, 2011; Ajiwibowo et al., 2019).

Two of the five lakes in West Sumatra have been reported to have experienced a decline in fish species richness. Previous studies have reported a decline in fish species in Maninjau (33 to 14 species) and Singkarak Lakes (30 to 16 species) (Roesma, 2011; Roesma, 2013). Diatas and Dibawah Lakes, or known as the Twin Lakes, are also suspected to be experiencing similar conditions. The surrounding areas in Twin Lakes are intensive horticultural centers closely associated with pesticide use. Pesticide residues entering water bodies can degrade habitat quality, worsening aquatic conditions through eutrophication and reducing dissolved oxygen. Over time, it can decline populations and change fish community structure, with a tendency for more dominant pollution-tolerant species (Liess et al., 2021). This threatens native fish populations in Twin Lakes. However, scientific information on fish biodiversity in Twin Lakes is still unavailable, and existing information is largely based on local community knowledge. This lack of scientific information raises concerns that valuable biodiversity data may be lost before the fish fauna of these lakes can be fully documented.

Biodiversity exploration has been carried out for a long time using conventional methods by direct capture (Santanumurti et al., 2024). However, these approaches have limitations, particularly in distinguishing morphologically similar/cryptic species, dealing with damaged/ incomplete specimens, and the decline of taxonomic experts (Wang et al., 2024). Over the past two decades, biodiversity exploration has increasingly employed molecular approaches, namely DNA barcoding and eDNA metabarcoding (Hebert et al., 2003; Taberlet et al., 2018). DNA barcoding identifies species based on short DNA fragments obtained from directly collected organisms (Tizard et al., 2019; Ali et al., 2020; Jiang et al., 2024). Meanwhile, eDNA metabarcoding is a non-invasive approach that detects DNA released by organisms into the environment without capturing, injuring, or killing the organism (Hanfling et al., 2016; Taberlet et al., 2018). DNA barcoding and eDNA metabarcoding has been used to monitor fish biodiversity in various inland aquatic ecosystems (Valentini et al., 2016; Yamamoto et al., 2017; Valdez-Moreno et al., 2019; Ali et al., 2020; Andriyono et al., 2021; Othman et al., 2023; Roesma et al., 2018; Roesma et al., 2019; Roesma et al., 2021a; 2021b; 2023a; 2023b; 2025). The previous biodiversity monitoring in Maninjau and Singkarak Lakes showed that several fish species previously reported using conventional methods were also detected by eDNA with universal COI primers (Roesma 2011; 2013; Roesma et al., 2021a; 2021b; 2023a; 2023b; 2025). The use of universal primers and the limitation of local reference barcodes are likely responsible for the undetection of some native species and the detection of previously unreported species (false positives and false negatives) by eDNA. However, universal COI primers remain chosen because most available genetic data for freshwater fishes from West Sumatra deposited in GenBank are based on the COI gene. Based on this, it is necessary to conduct a study using conventional and non-invasive methods in the Twin Lakes.

This study aims to (i) document freshwater fish diversity in Twin Lakes using conventional morphology and DNA barcoding, (ii) assess fish diversity using eDNA metabarcoding, and (iii) evaluate the congruence and limitations of these molecular approaches. This study represents an initial step toward building a local genetic reference database and evaluating the potential and limitations of DNA barcoding and eDNA approaches for supporting future freshwater biodiversity monitoring in Twin Lakes.

2. MATERIALS AND METHODS

2.1 Study Area

Diatas and Dibawah are tectonic lakes known as the Twin Lakes and are located in Solok Regency, West Sumatra. Diatas Lake covers an area of approximately 12.3 km², at an altitude of approximately 1,530–1,600 meters above sea level, with a maximum depth of approximately 44 meters. The inlet water comes from several small rivers in the surrounding area, with

one main outlet being the Gumanti River, which flows into the Batanghari. Meanwhile, Dibawah Lake covers an area of approximately 11.2 km², at an altitude of approximately 1,462 meters above sea level, with a maximum depth of approximately 326.8 meters. The inlet water comes from several small rivers in the surrounding area, with one main outlet being the Batang Lembang River, which flows into the Singkarak Lake. Both lakes are surrounded by hills, agricultural land, and plantations. This creates a characteristic highland aquatic environment that is both ecologically important and vulnerable to the pressures of human activity in its catchment area. Sampling sites for DNA barcoding consist of four stations in Diatas Lake and four stations in Dibawah Lake (Figure 1). Sampling sites for eDNA metabarcoding consist of three stations in Diatas Lake and three stations in Dibawah Lake (Figure 1).

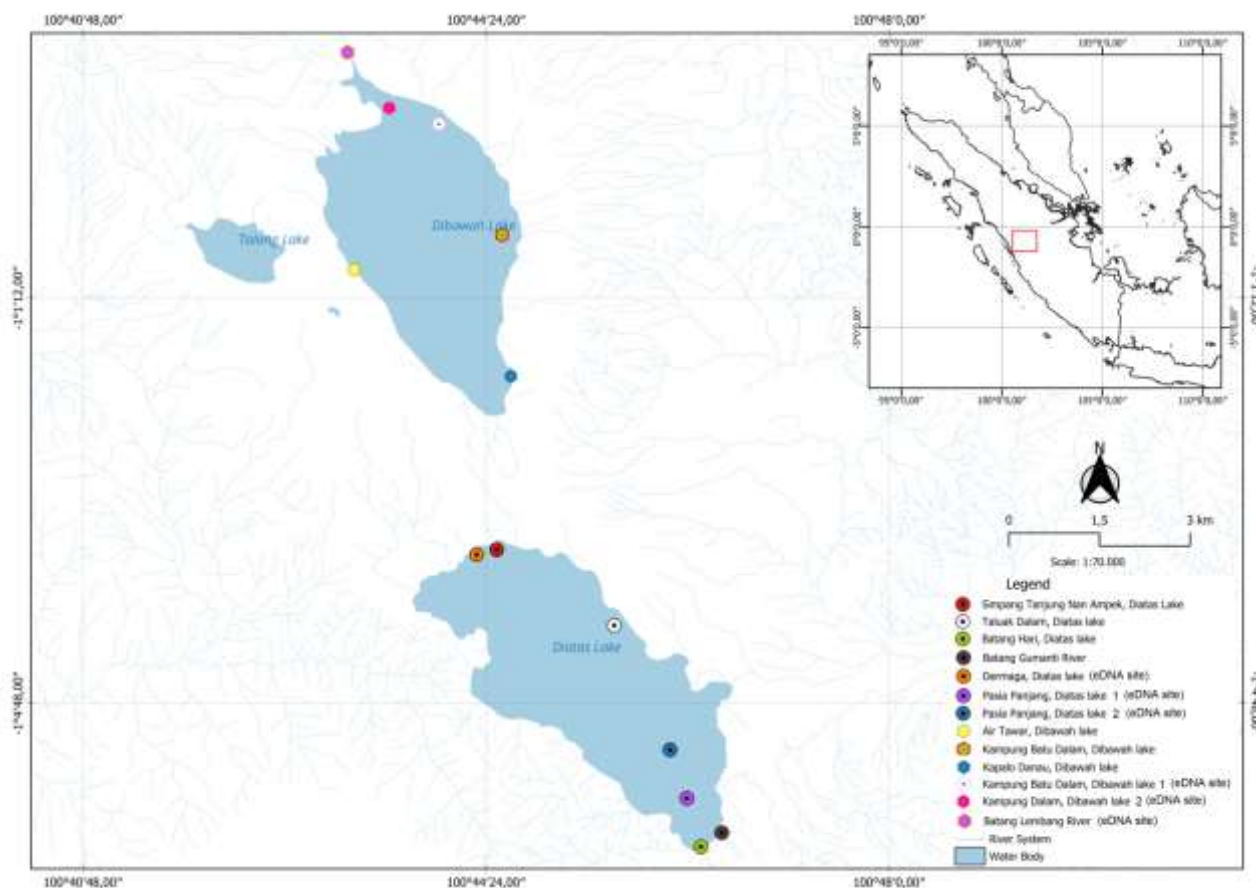


Figure 1. Map of sampling location

2.2 Sample Collection

Sample Collection for DNA Barcoding

Samples were collected from four sites in Diatas and Dibawah Lakes, respectively. The sample collection obtained a legal permit from the Biology Department of Andalas University and the surrounding local people. All procedures followed ethical guidelines for the use of animals in research. Samples were collected using fishing gear such as gill nets with a mesh size of 0.5–2 inches and electrofishing, with the help of local fishermen. Sampling was carried out as single time-point sampling during the research period. Sample collection was carried out using fishing gear representative of each location. The duration of sample collection depended on the fish species found. If the fish species found were uniform, sample collection was stopped. The total time spent was approximately 1.5 hours. The number of individuals obtained varied for each species, ranging from 1 to 25 individuals per species in each lake. Collected individuals were placed in labeled plastic bags containing water. Representative individual samples of each species were photographed, counted, and labeled. Several tissue samples from each species were taken and stored in ethanol PA.

All collected individuals were fixed in 10% formalin for several weeks. After that, the samples were washed using running water and stored in a box containing 70% ethanol for long-term storage at the Genetics and Biomolecular Laboratory, Department of Biology, Andalas University, Padang, West Sumatra, Indonesia. DNA barcoding of fish samples from Diatas and Dibawah Lakes was conducted in 2018. Limited funding was a consideration in selecting certain species for DNA barcoding. The target DNA barcoding for all samples collected was Cyprinidae and Danionidae fish, the families with the most native species in West Sumatra. Species identification followed standard freshwater fish identification guides (Nelson et al., 2016; van der Laan, 2018). Emphasise that barcoding was restricted to native, abundant Cyprinidae and Danionidae due to funding limits, and that this constrains direct comparisons between barcoded and non-barcoded taxa.

Sample Collection for eDNA Metabarcoding

eDNA sampling locations focused on areas utilized by local fishermen as fishing grounds and locations that were suitable for sample collection. In each lake, water samples were collected at three different locations. Sampling was conducted once (single-time-point sampling) during the study period. At certain locations, samples were collected from the center site using a boat, and water samples were taken from the surface (Goldberg and Strickler, 2017). Water samples were collected using gloves to avoid contamination (Laramie et al., 2015; Carim et al., 2016). At each location, 2 L of water was collected as an independent sample and was not combined with other samples (Andruszkiewicz et al., 2017). This study did not use field blanks or replication. Water samples were collected using sterile bottles and stored in a cooler. After sampling at all locations, samples were immediately transported to the genetics and biomolecular laboratory, Department of Biology, Universitas Andalas. Water samples were immediately filtered within 24- 48 hours (Carim et al., 2016; Goldberg and Strickler, 2017). Each water sample per point was filtered using a 0.22 μ m membrane filter with a diameter of 47 mm (Millipore, MA, USA) inserted into a 250 mL disposable analytical test filter funnel (Nalgene, USA) and filtered using a vacuum machine (Goldberg and Strickler, 2017; Carim et al., 2016). This study did not use filtration blanks. After filtration, the membrane filter was stored in a microtube containing absolute ethanol and stored at -20°C until DNA extraction (Goldberg and Strickler, 2017; Carim et al., 2016). eDNA sampling in this study was limited by spatial and temporal replication, and the absence of control samples. The lack of spatial and temporal replication limits our ability to estimate detection probabilities, thereby constraining our interpretation of potential false positives and false negatives. This was due to limited research funding, which can be considered a methodological limitation. Accordingly, the interpretation of species detections in this study is presented as a preliminary and initial exploration to evaluate the potential of the eDNA method to detect fish diversity in Twin Lakes. This limitation should be considered when interpreting the results.

2.3 Laboratory Procedure

Laboratory Procedures for DNA Barcoding

DNA was isolated using the Invitrogen DNA Isolation Kit (Thermo Fisher Scientific, USA) according to the manufacturer's protocol. DNA quality was assessed using 1.2% agarose gel electrophoresis. Successful DNA extraction was checked by the presence of DNA bands. The isolated DNA results were amplified using universal primers COI Fish FI (5'-TCAACCAACCACAAAGACATTGGCAC-3') and Fish RI (5'-TAGACTTCTGGGTGGCCAAAGAATCA-3') (Ward et al., 2005). DNA amplification was conducted in a total volume of 25 μ L containing three μ L DNA isolate, nine μ L DDH₂O, 11 μ L PCR Supermix, and one μ L forward and reverse primers (10 μ M), respectively (Roesma et al., 2018; Roesma et al., 2019; Roesma et al., 2023c; Roesma et al., 2025). PCR cycles were conducted with an initial denaturation at 95°C for 2 minutes. Next, the denaturation stage at 94°C for 0.5 min, annealing at 54°C for 0.5 min, extension at 72°C for 1 min for 35 cycles, and final extension at 72°C for 10 min (Ward et al., 2005). PCR products were visualized on a 2% agarose gel electrophoresis. The good PCR products were sent for sequencing to First Base Laboratories in Malaysia. Sequencing results consisted of forward and reverse chromatograms, which were further analyzed using appropriate DNA sequence analysis software.

Laboratory Procedures for eDNA Metabarcoding

DNA isolation was performed on six Twin Lakes water samples without extraction blanks. eDNA from the water samples was extracted using the Quick-DNA Magbead Plus Kit (Zymo Research, D4082), and Genomic DNA Quantification by Nanodrop and Qubit. DNA quality was evaluated prior to amplification. DNA amplification was performed using negative controls without positive controls to detect possible contamination during the amplification process. Positive controls were not included in the PCR setup due to resource constraints, and therefore the possibility of PCR inhibition or amplification

failure cannot be fully excluded. The target amplicon size for Fish F1 and Fish R1 was approximately 600–800 bp, consistent with the standard COI barcode region. The primers used were universal primers to amplify all samples simultaneously. Furthermore, available reference data for local fish in West Sumatra mostly cover the COI gene using these primers. Amplification was performed on all samples simultaneously in one batch. PCR amplification of target regions was performed using KOD-Multi & Epi-TM (Toyobo, KME-101) in a single-step PCR. PCR products were purified and QC (size and concentration) checked. PCR products were assessed by electrophoresis on 1% TBE agarose. Library preparation was performed following the Oxford Nanopore Technologies protocol using the Native Barcoding Kit 96 V14 (SQK-NBD114.96). The cleaned PCR products were then subjected to end-repair and dA-tailing, followed by ligation of unique native barcodes using the Native Barcoding Kit 96 V14 (SQK-NBD114.96), without PCR-based indexing, enabling multiplexed Oxford Nanopore sequencing. The cleanup process was conducted according to the manufacturer's instructions, and the resulting libraries were loaded onto the Oxford Nanopore flow cell for sequencing. The identity of each sample was then bioinformatically distinguished (demultiplexed) based on the barcode sequence during sequencing data analysis. All procedures, from DNA isolation to bioinformatics analysis, were conducted at the Genetika Science laboratory, Jakarta.

2.4 Data Analysis

Data analysis for DNA Barcoding

The forward and reverse sequencing results were combined using DNA STAR software and trimmed to remove low-quality regions at the ends of the sequences (Li et al., 2019). The minimum sequence length after trimming was set at 600–630 bp. Sequences with a proportion of ambiguous bases (N) $\leq 1\%$ were still analyzed. Remaining ambiguous bases were manually inspected and, if possible, corrected based on the electrophoregram results. All sequences were then translated into amino acids using AliView to ensure the absence of stop codons or frameshifts, thus minimizing the possibility of pseudogene sequences (NUMTs). Sequence alignment was performed using AliView and then manually edited using BioEdit (Hall, 2011; Reguant, 2020). For samples containing more than one sequence, a consensus sequence was formed based on the majority nucleotide match at each position, and only positions with clear and consistent electrophoregrams were retained. Amino acid translations were checked to verify the absence of sequencing errors. All sequences were compared with the reference sequence in GenBank using BLAST. Species identification was accepted using the COI gene similarity value criteria at the species level based on Kartavtsev et al. (2016). The genetic distance of each individual and species was observed using the p-distance method in MEGA 11 (Tamura et al., 2021). The haplotype and nucleotide diversity (π) were calculated using DNASP software (Rozas et al., 2017). DNA barcodes were generated by submitting sequences to the DNA Barcode Web Tool. Sequences were registered in the Barcode of Life Data System (BOLD) following the given guidelines. Each sequence was assigned a Barcode Index Number (BIN) and linked to GenBank to obtain an accession number (Kartavtsev, 2018). All DNA barcodes are archived in the BOLD System and GenBank databases.

Bioinformatic Analysis for eDNA Metabacoding

Nanopore sequencing was performed using MinKNOW software version 23.04.05 Oxford Nanopore Technologies (<https://nanoporetech.com/about-us/news/introducing-new-minknow-app>). Basecalling was performed using Guppy version 6.5.7 with high accuracy mode (Wick et al., 2019). Primers were removed when basecalling was processed. After basecalling, which contains sequence quality score information. The Q-score threshold follows the default setting of the High-Accuracy Mode. The quality score in Phred Q is related to the probability of basecalling error. FASTQ files were filtered using Filtlong software (<https://github.com/rrwick/Filtlong>) to obtain good-quality sequences and visualized using NanoPlot (de Coster et al., 2018). Quality and read-length filtering were performed using NanoFilt and filtered within a range of 600–800 bp according to the estimated amplicon length. Other settings followed the software defaults. Sequencing reads were filtered using Filtlong with a minimum read length of 700 bp and a minimum quality score of Q12. All reads meeting these criteria were retained for further analysis. Clustering and denoising were not performed because, as of 2023, there is no BI analysis method for Nanopore data that can perform clustering and denoising consistently and effectively. The filtered data reads were classified using the Centrifuge classifier based on sequence similarities (Kim et al., 2016). Filtering was performed by applying an alignment score threshold (identity $\geq 90\%$). The Centrifuge assignment parameter uses a single primary assignment setting, which limits the number of multiple hits to 1 primary assignment. Therefore, multiple hits are automatically reported using the lowest common ancestor of the multiple hits. Conflicting assignments are resolved by centrifugation with a single primary assignment, so species names are given when hits occur only once. The OTU/ASV

clustering method for Nanopore data in 2023 was still not possible, so its derivative methods (chimera removal, low complexity, de-replication) could not be performed. The reference fish database index was built using the MitoFish database. Because it uses a fish database, non-fish sequences will not be annotated. Downstream analysis and visualizations were performed using Pavian (<https://github.com/fbreitwieser/pavian>), Krona Tools (<https://github.com/marbl/Krona>), and RStudio using R version 4.2.0 (<https://www.R-project.org/>).

3. RESULTS AND DISCUSSION

3.1 Fish database, Morphological, and Taxonomic Notes

Based on the results of morphological identification, a total of 23 fish species were recorded in the Twin Lakes, including 20 fish species in the Diatas Lake and 18 fish species in the Dibawah Lake (Table 1). All types of fish identified belong to six orders, 13 families, 21 genera, and 23 species. Based on the data obtained, the order Cypriniformes was the most species-rich group, comprising 13 species (56.6%), predominantly from the family Cyprinidae. Meanwhile, the genus *Rasbora* was represented by the highest number of species, with two species recorded. Overall, based on IUCN Red List data, 19 species were classified as Least Concern (LC), one species as Data Deficient (DD), two species as Vulnerable (VU), and one species as Not Evaluated (NE).

Among the 23 fish species recorded, seven species are native fish with locality types originating from West Sumatra, namely *Barbonymus schwanefeldii*, *Mystacoleucus padangensis*, *Neolissochilus sumatranus*, *Rasbora spilotaenia*, *Nemacheilus pfeifferae*, *Glyptothorax schmidtii*, and *Homalopterula gymnogaster*. Meanwhile, introduced fish species in Diatas and Dibawah Lakes include *Anabas testudineus*, *Cyprinus carpio*, *Oreochromis niloticus*, and *Clarias gariepinus*. Other species are native Indonesian fishes with type localities from Java that are widely distributed across other regions of Indonesia, including West Sumatra, such as *Clarias batrachus*, *Dermogenys pusilla*, *Hemibagrus nemurus*, *Cyclocheilichthys apogon*, *Hampala macrolepidota*, *Osteochilus vittatus*, *Rasbora lateristriata*, *Tor douronensis*, and *Monopterus albus*.

Table 1. List of freshwater fishes in Diatas and Dibawah Lakes, West Sumatra

No.	Family	Genera	Scientific Name	Local Name	Location	IUCN Status
1	Anabantidae	Anabas	<i>Anabas testudineus</i>	Puyu	Diatas and Dibawah Lakes	LC
2	Channidae	Channa	<i>Channa gachua</i>	Bakok	Diatas Lakes	LC
3	Zenarchopteridae	Dermogenys	<i>Dermogenys pusilla</i>	Julung-julung	Dibawah Lakes	LC
4	Cichlidae	Oreochromis	<i>Oreochromis niloticus</i>	Nila	Diatas and Dibawah Lakes	LC
5	Balitoridae	Homalopterula	<i>Homalopterula gymnogaster</i>	Silurian	Diatas and Dibawah Lakes	LC
6	Cyprinidae	Barbodes	<i>Barbodes banksi</i>	Kapareh	Diatas and Dibawah Lakes	LC
7		Barbonymus	<i>Barbonymus schwanefeldii</i>	Balingka	Diatas and Dibawah Lakes	LC
8		Cyclocheilichthys	<i>Cyclocheilichthys apogon</i>	Catua	Diatas and Dibawah Lakes	LC
9		Cyprinus	<i>Cyprinus carpio</i>	Rayo	Diatas and Dibawah Lakes	LC
10		Hampala	<i>Hampala macrolepidota</i>	Sasau/barau	Diatas and Dibawah Lakes	LC

11		Mystacoleucus	<i>Mystacoleucus padangensis</i>	Bilih	Dibawah Lakes	VU
12		Neolissochilus	<i>Neolissochilus sumatranus</i>	Gariang	Diatas Lakes	LC
13		Osteochilus	<i>Osteochilus vittatus</i>	Asang	Diatas and Dibawah Lakes	LC
14		Tor	<i>Tor douronensis</i>	Gariang	Diatas Lakes	NE
15	Danionidae	Rasbora	<i>Rasbora cf. lateristriata</i>	Bada	Diatas and Dibawah Lakes	VU
16			<i>Rasbora spilotaenia</i>	Bada	Diatas Lakes	LC
17	Nemacheilidae	Nemacheilus	<i>Nemacheilus pfeifferae</i>	Tali-tali	Diatas and Dibawah Lakes	DD
18	Aplocheilidae	Aplocheilus	<i>Aplocheilus panchax</i>	Kapalo timah	Diatas and Dibawah Lakes	LC
19	Bagridae	Hemibagrus	<i>Hemibagrus nemurus</i>	Baung Tageh	Diatas and Dibawah Lakes	LC
20	Clariidae	Clarias	<i>Clarias batrachus</i>	Lele	Dibawah Lakes	LC
21			<i>Clarias gariepinus</i>	Limbek	Diatas and Dibawah Lakes	LC
22	Sisoridae	Glyptothorax	<i>Glyptothorax schmidtii</i>	Situkah	Diatas Lakes	LC
23	Synbranchidae	Monopterus	<i>Monopterus albus</i>	Belut	Diatas and Dibawah Lakes	LC

3.2 DNA Barcoding

Amplification of the COI gene was successfully performed on freshwater fish collected in Diatas and Dibawah Lakes using universal primers for freshwater fish. The success of COI gene amplification was confirmed by the presence of DNA fragments on agarose gel electrophoresis with DNA fragment sizes ranging from 650 to 700 bp. The target DNA barcoding for all samples collected is Cyprinidae and Danionidae fish, the families with the most native species in West Sumatra. Of the total 23 samples obtained, nine species from the 11 species in the Cyprinidae and Danionidae groups (the most abundant and native species) were analyzed for DNA barcoding. Two species from the groups were excluded from analysis due to PCR and sequencing failures. A total of 35 DNA barcodes from nine species were successfully generated (Table 2). The number of barcoded individuals varied across species. Among all the barcoded species, *T. douronensis* had the fewest barcodes, as only one individual was found in the Batang Gumanti River. Likewise, *B. schwanefeldii*, *N. sumatranus*, and *R. cf. lateristriata* each had only two individuals. Sequencing results showed sequence lengths ranging from 650 to 690 bp. The FASTA sequence data were subsequently translated into amino acids using an online DNA-to-protein translation tool. Amino acid translation was examined to verify the absence of stop codons or translation errors in all samples. No insertions, deletions, or stop codons were detected in any of the sequences, indicating the absence of pseudogenes.

The FASTA sequences were then subjected to BLAST analysis to determine sequence similarity with reference sequences in GenBank, NCBI. The BLAST analysis results for the nine species from Dibawah and Diatas Lakes, West Sumatra, are presented in Table 2. Sequence similarity values between the obtained DNA sequences and GenBank reference data ranged from 92% to 100%. Based on the similarity values, the molecular identification results support the morphological identification results, with values ranging from 100% to 98.9%. However, in *Rasbora cf. lateristriata*, the closest similarity value to GenBank data is only 92% with *Rasbora lateristriata*. Therefore, we determined the species name using cf. (*Rasbora cf. lateristriata*). Interestingly, the BLAST results for *M. padangensis* show a similarity value of 100% with *M. padangensis* and *M. marginatus*. Meanwhile, the *N. sumatranus* and *T. douronensis* species have a similarity value of 99.9% with *T. tambroides*. DNA barcodes for *N. sumatranus* are unavailable in GenBank and the BOLD System. *N. sumatranus* and *T. douronensis* are included in

the Mahseer fish group, which has morphological similarities and is distinguished by the median lobe character. Morphological identification has been conducted based on the identification books, which consider differences in the median lobes and other characters of each species. However, molecular identification results revealed high similarities between the two species. Based on these results, we confirm that *M. padangensis* and *M. marginatus* are the same species, as previously reported in the literature. Meanwhile, for *N. sumatranus* and *T. douronensis*, the species names are still based on morphological identification results until further studies are conducted to determine appropriate names for all members.

Each sequence registered in the BOLD System is assigned a Barcode Index Number (BIN). Each sequence with high nucleotide sequence similarity is assigned the same BIN code. From 35 sequences representing nine species, eight BIN codes were obtained (Table 2). Each species resulted in a different BIN code, except for the mahseer species *N. sumatranus* and *T. douronensis*, which shared two identical BIN codes. Within a species, all individuals have a single BIN code, indicating that conspecific individuals share highly similar DNA sequences. All COI gene sequences from fish collected in Diatas and Dibawah Lakes, West Sumatra, were registered in the BOLD System. These sequence data represent the genetic richness of freshwater fishes in West Sumatra and can be used as reference sequences for future species identification.

Table 2. Scientific Name, No. of sample, Length of sequences, BIN Code, Sequence identities, and Blast result

No.	Scientific name	No. of samples	Length of COI (bp)	BIN Code	Sequence Identities	Blast Result from Genbank Database
1	<i>Barbodes banksi</i>	4	640	AAU3665	100%	<i>Barbodes banksi</i>
2	<i>Barbonymus schwanenfeldii</i>	2	670	AAU0688	99.3%	<i>Barbonymus schwanenfeldii</i>
3	<i>Cyclocheilichthys apogon</i>	12	664	AAU3852	99.8%	<i>Cyclocheilichthys apogon</i>
4	<i>Mystacoleucus padangensis</i>	6	660	AFG9182	100%	<i>M. marginatus</i> and <i>M. padangensis</i>
5	<i>Neolissochilus sumatranus</i>	2	664	ACC0378	99.9%	<i>Tor tambroides</i>
6	<i>Osteochilus vittatus</i>	3	664	ACC0089	99.3%	<i>Osteochilus hasseltii</i> and <i>Osteochilus vittatus</i>
7	<i>Rasbora</i> cf. <i>lateristriata</i>	2	667	ADP0830	92%	<i>Rasbora lateristriata</i>
8	<i>Rasbora spilotaenia</i>	3	667	ADY6054	98.9%	<i>Rasbora spilotaenia</i>
9	<i>Tor douronensis</i>	1	668	ACC0378	99.9%	<i>Tor tambroides</i>

Genetic distance values were calculated using the p-distance model. Average genetic distance increased progressively from species to higher taxonomic levels. The average genetic distance at the species, genus, family, and order levels was 0.0%, 9.9%, 14.7%, and 22.8%, respectively. The intraspecific genetic distance for all species that have more than one sample was 0.0%. This value indicates high genetic similarity among individuals within the species. The mean interspecific genetic distance within the genera was 9.9%. The interspecific genetic distance was approximately ten times greater than the intraspecific genetic distance. Small genetic distances between different genera within the same family were observed between *N. sumatranus* and *T. douronensis*. These genetic distance values indicate that the COI gene analysis does not strongly support the separation of the two taxa as distinct species.

3.3 eDNA Metabarcoding

Isolation of eDNA samples from Diatas and Dibawah Lakes has been successfully performed, yielding an average DNA concentration of 30.37 ng/ul measured by NanoDrop and 25.28 ng/ul measured by Qubit. NanoDrop and Qubit results indicate that the samples passed quality control (QC) and were suitable for subsequent DNA amplification. The amplification results showed the appearance of all bands corresponding to the target DNA size and the absence of DNA bands in the negative PCR control. These results indicate that the laboratory work carried out was free of contamination. All samples successfully proceeded to the library preparation and sequencing stage using the Third Generation Sequencing technique

(GridION: Oxford Nanopore). The NGS sequencing output was generated in FASTQ format, a text-based format for storing nucleotide sequences and assessing their quality. In FASTQ files, raw sequences were filtered and trimmed based on Q-scores to obtain good quality. Sequences with low Q scores will be trimmed and not included in the analysis. The FASTQ quality metrics visualized using Nanoplot on the sequencing results of eDNA samples from Diatas and Dibawah Lakes are presented in Table 3.

Table 3. Quality of FASTQ data

Data FASTQ	S1	S2	S3	S4	S5	S6
Mean read length	761.9	765.2	766.4	764.6	765.6	765.1
Mean read quality	14.9	15	14.9	15	14.9	15
Number of reads	8,501	11,506	9,719	10,136	8,924	10,884
Median read length	762	761	762	761	762	762
Median read quality	15.2	15.2	15.2	15.2	15.1	15.3
Total bases	6,477,149	8,804,431	7,448,408	7,749,935	6,831,813	8,327,085

Note: S1: Dermaga, Diatas Lake, S2: Pasia Panjang, Diatas Lake 1, S3: Pasia Panjang, Diatas Lake 2, S4: Kampung Batu Dalam, Dibawah Lake 1, S5: Kampung Batu Dalam, Dibawah Lake 2, S6: Kampung Dalam, Dibawah Lake

Total bases refers to the total number of nucleotide bases generated from all sequencing reads. The number of reads refers to the total count of sequencing reads obtained. Mean read length represents the average length of all sequencing reads. Mean read quality represents the average value of the quality of the sequence read (Q score). Median read length refers to the middle value of read lengths when all reads are ordered by length. Median read quality refers to the average value of the middle value of the quality of the sequence read (Q score). Based on the sequencing results, a total of 8,501-11,506 sequencing reads were obtained across the six eDNA sampling sites.

The eDNA sequencing results were used to detect fish taxa. Therefore, the data obtained were only fish groups. Among all the sequences read, 287 species were detected, consisting of 131 freshwater fish and 156 marine fish (Supplementary Table 1). Meanwhile, freshwater fish were detected and grouped into fish that had been previously reported (7 species) and fish that had not been previously reported (124 species). All marine fishes are not discussed further because their detection is implausible and suggests contamination, misassignment, or other causes. The freshwater fish that were detected using eDNA and previously reported in Diatas and Dibawah Lakes are shown in Table 4. A total of seven species (30%) were detected using eDNA out of a total of 23 species reported using conventional methods. The seven species include *A. testudineus*, *Aplocheilichthys panchax*, *C. batrachus*, *C. gariepinus*, *M. albus*, *O. niloticus* and *O. vittatus*. Among the seven species, there are two native species with type localities in Indonesia (*O. vittatus* and *C. batrachus*), and three are introduced species (*O. niloticus*, *A. testudineus*, and *C. gariepinus*).

Of the seven species detected with eDNA, not all were found at all sample collection locations. One species was found at most four locations (*A. testudineus*, *O. niloticus*, and *C. gariepinus*) and at least two locations (*C. batrachus*). The Pasia Panjang, Diatas Lake (S2) was the location with the highest number of species (five) and number of reads (18) compared to the other locations. *O. vittatus* is the species with the highest number of reads (13) detected in Pasia Panjang, Diatas Lake (S2) using the eDNA method. Generally, the number of reads for the species detected at each site was almost similar, except for *O. vittatus*, which had the highest number of reads (13). Of the seven species detected, three species were introduced (*A. testudineus*, *O. niloticus*, and *C. gariepinus*), which were found at four of the six sites (Table 4). No introduced species dominated any of the sites. Among all species, only *N. sumatranus* did not have a DNA barcode in GenBank and the BOLD System.

Table 4. Species list in Twin Lakes obtained using conventional sampling (2018) compared with eDNA metabarcoding

No.	Scientific Name	Sequence recovered from eDNA per sites						Barcode Species from this study	Barcode species in GenBank
		S1	S2	S3	S4	S5	S6		
1	<i>Anabas testudineus</i>	1	1	2	2	0	0	—	√
2	<i>Channa gachua</i>	0	0	0	0	0	0	—	√
3	<i>Dermogenys pusilla</i>	0	0	0	0	0	0	—	√
4	<i>Oreochromis niloticus</i>	1	2	1	0	1	0	—	√
5	<i>Homalopterula gymnogaster</i>	0	0	0	0	0	0	—	√
6	<i>Barbodes banksi</i>	0	0	0	0	0	0	√	√
7	<i>Barbonymus schwanefeldii</i>	0	0	0	0	0	0	√	√
8	<i>Cyclocheilichthys apogon</i>	0	0	0	0	0	0	√	√
9	<i>Cyprinus carpio</i>	0	0	0	0	0	0	—	√
10	<i>Hampala macrolepidota</i>	0	0	0	0	0	0	—	√
11	<i>Mystacoleucus padangensis</i>	0	0	0	0	0	0	√	√
12	<i>Neolissochilus sumatranus</i>	0	0	0	0	0	0	√	—
13	<i>Osteochilus vittatus</i>	0	13	0	0	1	1	√	√
14	<i>Tor douronensis</i>	0	0	0	0	0	0	√	√
15	<i>Rasbora</i> cf. <i>lateristriata</i>	0	0	0	0	0	0	√	√
16	<i>Rasbora spilotaenia</i>	0	0	0	0	0	0	√	√
17	<i>Nemacheilus pfeifferae</i>	0	0	0	0	0	0	—	√
18	<i>Apocheilus panchax</i>	0	0	2	1	0	2	—	√
19	<i>Hemibagrus nemurus</i>	0	0	0	0	0	0	—	√
20	<i>Clarias batrachus</i>	0	1	0	0	1	0	—	√
21	<i>Clarias gariepinus</i>	1	0	0	1	1	1	—	√
22	<i>Glyptothorax schmidtii</i>	0	0	0	0	0	0	—	—
23	<i>Monopterus albus</i>	2	1	0	0	0	1	—	√

Among the 23 species known to inhabit the Twin Lakes, West Sumatra, 16 species were not detected using eDNA metabarcoding. Among the 16 undetected species are several native species of West Sumatra, which include economically important species such as *B. schwanefeldii* (kapick),

H. gymnogaster (Silurian), *M. padangensis* (bilih), *C. apogon* (Catua), *R. lateristriata* (Bada), and *R. spilotaenia* (Bada). Furthermore, there are 124 species that have never been reported from Twin Lakes. Among the 124 other detected species, three categories can be distinguished: (1) species known to inhabit rivers and other lakes in Sumatra; (2) species detected that have never been reported from Sumatra Island but are known from other islands in Indonesia; and (3) species detected that have never been reported to occur in Indonesia (Supplementary Table 1).

DISCUSSION

This study successfully identified 23 fish species using conventional methods, including direct capture in Diatas and Dibawah Lakes. Among all the fish species identified, Cyprinidae were the most abundant group in these lakes. Cyprinidae, as the family with the highest species richness in Sumatra, has also been reported in previous studies (Marnis et al., 2024). Studies on freshwater fish diversity in western Indonesia have shown that fish assemblages are dominated by the family Cyprinidae (Hadiaty, 2011; Hadiaty, 2014), whereas those in eastern Indonesia are dominated by the family Gobiidae (Hadiaty, 2009; Hadiaty, 2012). Cyprinidae is the largest freshwater fish family and is distributed worldwide except Australia, Madagascar, New Zealand, and South America (Nelson, 2016). In terms of utilization potential from species in Twin Lakes, 20 species (87%) have potential as food fish, seven species (30%) as ornamental fish, and five species (22%) as both food and ornamental fish. Other species are native Indonesian fishes with type localities from Java that are widely distributed across other regions of Indonesia, including West Sumatra, such as *C. batrachus*, *Dermogenys pusilla*, *H. nemurus*, *C. apogon*, *H. macrolepidota*, *O. basseltii*, *R. lateristriata*, *T. dourenensis*, and *M. albus*. These fish are economically valuable and provide an important source of livelihood for the communities surrounding these two lakes.

This study is the first preliminary exploration using conventional methods, incorporating morphological and molecular identification (DNA barcoding), conducted in Diatas and Dibawah Lakes (2018). The number of species found in Twin Lakes is lower than in other lakes in West Sumatra, such as Maninjau and Singkarak Lakes. The first exploration in Maninjau and Singkarak Lakes reported 33 and 30 species, respectively, but these numbers have continued to decline. The most recent survey (Roesma, 2011) reported only 14 species in Maninjau Lake and 16 species in Singkarak Lake. This may also have occurred in Twin Lakes, but the lack of monitoring studies makes it impossible to determine whether there has been a decline in fish species currently. Climate change, environmental degradation, and increasing anthropogenic pressures have made it increasingly difficult for native fish to be obtained in large quantities. Consequently, the market prices of native fish have risen. Therefore, biodiversity monitoring is necessary to obtain data and information on the richness of freshwater fish in Twin Lakes and to store their genetic data (DNA barcodes) in GenBank and the BOLD System. In this study, only the Cyprinidae and Danionidae groups were used for DNA barcoding, with our focus on the native fish in the lakes, which are generally the Cyprinidae and Danionidae groups. Limited funding for this research caused the selection of native lake species for DNA barcoding. Although DNA barcoding of species from other families has not been performed, DNA barcoding for most other species in Twin Lakes is available for other locations in West Sumatra (Roesma et al., 2018; Roesma et al., 2022). These data can also serve as a comparison for identification. The availability of DNA barcodes is important for the success of future biodiversity monitoring. These data will serve as a reference for monitoring using the non-invasive eDNA method.

A total of 35 sequences from nine species were generated in this study. The results of the sequences showed almost 100% similarity with GenBank, NCBI data except for the species *R. cf. lateristriata*. Morphological identification showed *R. cf. lateristriata* similarities to *R. lateristriata*. However, BLAST results showed a similarity value of only 92% with *R. lateristriata* in GenBank data. According to researchers, this value indicates a high sequence divergence for the same species. However, other researchers, Kartavtsev et al. (2016), stated that sequence divergence of 0- 11% in the COI gene for vertebrate groups indicates differences at the population level for the same species. Therefore, we assigned the name *R. cf. lateristriata* for this species because it has morphological similarities with *R. lateristriata* but has a significant genetic distance. Further study on this sample is needed using other genes to confirm the taxonomy of this species. BLAST results for *M. padangensis* show a similarity value of 100% with *M. padangensis* and *M. marginatus*. This value indicates a high genetic similarity between *M. padangensis* and *M. marginatus*. Morphologically, the two species show clear differences (Roesma, 2011). However, the results of phylogenetic studies using the COI and Cyt b genes showed overlapping positions between *M. padangensis* and *M. marginatus* with genetic distances of 0.0%-0.3% (Roesma, 2011; Roesma et al., 2025). Therefore, *M. padangensis* is the same species as *M. marginatus*, and *M. padangensis* is a synonym of *M. marginatus* (Roesma, 2011; Roesma et al., 2025).

Each species was generally assigned a single BIN code, indicating that conspecific individuals share highly similar DNA sequences, except for the mahseer species *N. sumatranus* and *T. dourenensis*, which shared identical BIN codes and were identified as *T. tambroides*. These results indicate a high level of genetic similarity in the COI gene sequences of both species. These results have also been reported in previous studies, which showed the high sequence similarities of the COI gene in the mahseer group (*Neolissochilus* and *Tor*) (Roesma et al., 2025). Morphologically, the mahseer group (*T. tambroides*, *T. dourenensis*, *T. tambra*, and *N. sumatranus*) is difficult to distinguish. In this study, morphological identification was conducted

based on identification books, which consider differences in the median lobes and other characteristics of each species. Thus, the possibility of morphological identification errors was avoided. Furthermore, the samples used were labeled correctly between individual samples and their tissue samples before molecular analysis. Molecular identification results revealed high similarities between *N. sumatranus* and *T. douronensis*, with genetic distances of 0.5%. However, the previous study using other mtDNA genes (Cyt b) also showed a low genetic distance (0.0-1.0%) between the Mahseer group (*T. tambroides*, *T. douronensis*, *T. tambra*, and *N. sumatranus*) in West Sumatra (Roesma, 2011). The low genetic distance in Cyt b definitely does not separate them into different species (Roesma, 2011). Thus, studies using mtDNA genes (COI and Cyt b) do not support the separation of *Tor* spp. and *N. sumatranus* into different species. The taxonomy of the Mahseer group remains unresolved due to persistent morphological similarities between species groups and a lack of comprehensive genetic data. To clarify taxonomic relationships and ensure accurate identification within the Mahseer group, especially for *N. sumatranus*, comprehensive morphological, genomic, and geographical studies are imperative.

Average genetic distance increases progressively from species to higher taxonomic levels. Similar patterns of the genetic distance have been reported in other fish DNA barcoding studies, with values of 0.4%, 9.9%, and 15.5% at the species, genus, and family levels in Australia (Ward et al., 2005) 0.4%, 7.2%, and 12.8% in Bangladesh (Ahmed et al., 2020), and 0.3%, 12.1%, and 17.4% in Vietnam (Thu et al., 2019). The intraspecific genetic distance for all species was 0.0%. This value indicates high genetic similarity among individuals of the same species and low genetic variation in the COI gene. However, this value may vary if more locations and samples are obtained and other genes are analyzed. These results represent preliminary data from the COI gene, indicating that fish in Diatas and Dibawah Lakes share high genetic similarity within populations, respectively. The mean interspecific genetic distance within the genera was 9.9%. The interspecific genetic distance was approximately ten times greater than the intraspecific genetic distance. According to Kartavtsev et al. (2016), the genetic differences between species ranged around $11.06\% \pm 0.53$. Small genetic distances between different genera within the same family were observed between *N. sumatranus* and *T. douronensis*. These genetic distance values indicate that the COI gene analysis does not strongly support the separation of the two taxa as distinct species. Similarly, low genetic distances among closely related species have been reported in other studies, such as three *Trigonostigma* species (Panprommin and Panprommin, 2017) and four cichlid species (Panprommin et al., 2019).

This study represents the first exploration of fish biodiversity in Diatas and Dibawah Lakes using molecular methods with DNA barcoding and eDNA metabarcoding (non-invasive). The results of this study provide information on the types of species and DNA Barcodes of freshwater fish in Diatas and Dibawah Lakes, West Sumatra. DNA Barcodes have been registered in the BOLD System and GenBank, NCBI, and contribute to baseline data on fish genetic diversity, particularly in West Sumatra. The availability of DNA Barcodes in the BOLD System and GenBank, NCBI, represents essential reference data for future biodiversity monitoring using the non-invasive eDNA metabarcoding method. The eDNA method can be used for an initial, rapid assessment to monitor freshwater fish. However, its accuracy is highly dependent on the precision of the field sampling (single time point, limited spatial replication), lab/primer constraints (long COI fragment, no positive controls, no blanks), and bioinformatics (no denoising, incomplete reference library). Building a local reference library through the provision of DNA barcodes is a crucial step to improve identification performance and reduce detection errors. The eDNA method is more appropriately considered a complementary method, not a replacement for conventional surveys. The eDNA approach, as an initial screening stage for mapping diversity and identifying target species, requires conventional surveys and DNA barcoding to validate the eDNA results. Thus, these two methods complement each other, with eDNA increasing detection sensitivity, while specimen-based surveys ensure taxonomic validity.

eDNA metabarcoding combined with NGS techniques has been successfully used to identify species in Diatas and Dibawah Lakes. However, only seven species (30%) were identified using the non-invasive eDNA metabarcoding approach. The seven species include *A. testudineus*, *A. panchax*, *C. batrachus*, *C. gariépinus*, *M. albus*, *O. niloticus*, and *O. vittatus*. The Pasia Panjang, Diatas Lake (S2) was the location with the highest number of individual detections compared to the other locations. *O. vittatus* is the species with the highest number of individuals (13) detected using the eDNA method, and *C. batrachus* is the species with the lowest number of individuals (2) detected. *O. vittatus* is a species that inhabits various habitats and has a wide distribution in Indonesia. The Marine and Fisheries Service routinely releases *O. vittatus* in West Sumatra to enhance fishermen's catches (Limbu et al., 2024). Therefore, this fish can be detected using both conventional (DNA barcoding) and eDNA metabarcoding methods. *O. niloticus* is an introduced species with significant economic value in lakes in West Sumatra. Biodiversity monitoring using eDNA was conducted in 2023, after five years of DNA barcoding. This monitoring

was conducted to determine the ability of the eDNA method to detect species without direct capture. Although only seven species were detected using eDNA, discussion with local fishermen revealed 23 species previously reported as still found in Twin Lakes, although in low numbers and not always captured. Some eDNA sample collection was conducted at fishing locations, hoping to capture eDNA from the surrounding fish remains. However, sequencing results reported only a small number of detected species. Among the 16 undetected species are several native species of West Sumatra, including economically important species. Species that were not detected in the Twin Lakes using eDNA metabarcoding but were actually present in the waters were classified as false negatives. Other eDNA studies have also reported false-negative detections (Goldberg et al., 2016; Shelton et al., 2016; Andruszkiewicz et al., 2017; Roesma et al., 2021a; Roesma et al., 2021b; Roesma et al., 2023a; Roesma et al., 2023b). Based on the references, the main factors may contribute to false-negative detections, including field sampling (single time point, limited spatial replication), lab/primer constraints (DNA quality, molecular process, no positive controls), and an incomplete reference library (Dejean, 2011; Shelton et al., 2016; Andruszkiewicz et al., 2017; Roesma et al., 2021a; Roesma et al., 2021b).

In this study, sample collection was limited in terms of a single time point and limited spatial replication (number of sampling points and locations); therefore, the water obtained was not sufficient to represent all the target DNA present. Furthermore, the collection was only conducted in one season, which could have affected the appearance of fish at the location. The uneven distribution of fish throughout the lake also affected the success of eDNA detection. According to Andruszkiewicz et al. (2017), water sample collection without repetition was not representative of all DNA in the lake waters because of the possibility that the eDNA had not been mixed homogeneously in all water samples. The amount of target DNA must be increased by collecting more water samples with multiple replications at a different location to increase target DNA detection accuracy. The presence of inhibitors during the PCR process and low DNA quality due to degradation also influenced the low fish detection in this study. The amount of target DNA falling below a detection threshold, or DNA molecules with low concentration and quality, caused the presence of false detections (Shelton et al., 2016). The use of universal primers in this study is suspected to be the cause of the undetected target species due to the long primer size, while the DNA has been fragmented into shorter sizes. Consequently, the relatively long amplicon length targeted by the primers may reduce detection sensitivity, particularly for taxa represented by highly degraded DNA. This limitation may bias detection toward species that are abundant, recently present, or that shed larger quantities of DNA. This contributes to the relatively high false-negative rate observed in this study, as some taxa present in the environment may not be detected due to the failure to amplify longer DNA fragments. The use of universal primers for the COI gene aims to detect all fish species from various taxa. In addition, the available database for freshwater fish in West Sumatra is based on the COI gene, which has been registered in GenBank and the BOLD System. Other universal primers used for eDNA, such as MiFish, are designed to amplify regions in the 12S rRNA gene. However, 12S rRNA gene data for fish in West Sumatra are incomplete, which also reduces the detection rate. Therefore, if possible, using the shorter amplicon markers in future eDNA studies will improve detection sensitivity.

Besides, the undetected native fish species in Twin Lakes may also be due to the limited availability of DNA barcode databases in the BOLD System and GenBank. However, although only nine species were barcoded in this study, DNA barcodes for other species are also available from other regions in West Sumatra. The limited local database of freshwater fish from Twin Lake in GenBank and the BOLD System may have led to misassignments or assignments only assigned at the genus/family level. The previous study conducted in Maninjau Lake found only a few species and undetected native species using the eDNA method, possibly due to the inability to capture native species DNA from the collected water samples or the low DNA concentration, which prevented further PCR (Roesma et al., 2021a). The future studies only detected ten species (30%) using eDNA metabarcoding, compared with 30 species previously reported in Singkarak Lake using conventional methods (Roesma et al., 2023a; 2023b). In addition to detecting seven previously reported species in Twin Lakes, eDNA also detected a species not previously reported in Twin Lakes. The detection of species that have never been reported to exist in an ecosystem can be classified as false positives. Other studies have also reported false positive detections using the eDNA method (Andruszkiewicz et al., 2017; Valdez-Moreno, 2019; Euclide et al., 2021; Roesma et al., 2021a; Roesma et al., 2023a; 2023b; Schwentner et al., 2021). Based on the references, the main factors may contribute to false-positive detections, including lab/primer constraints (contamination during field sampling or laboratory procedures, low primer specificity for detecting target DNA; competition between target DNA and non-target DNA) (Lahoz-Monfort et al., 2016; Ruppert et al., 2019; Sard et al., 2019; Euclide et al., 2021), and bioinformatics (no OTU clustering, Chimera removal, and denoising, mis assignment reads because incomplete reference databases) (Hanfling et al., 2016).

This study did not collect field blanks, filtration blanks, and extraction blanks due to limited research funding. This condition is one of the limitations in this study, which causes false-positive detections. The absence of controls makes it impossible to detect whether contamination has occurred during sample collection in the field until DNA isolation. However, in the PCR process, a negative control is used to detect contamination, although a positive control is not used. The amplification results showed no amplified DNA bands in the negative control sample, indicating no contamination occurred during PCR. Future studies using eDNA are recommended to use field blanks, filtration blanks, and extraction blanks. The use of universal primers Fish FI and RI can detect other taxonomic groups that are not the target, thus detecting false positives. Universal primers are used because they can bind to various DNA templates in a single sequencing process. However, the use of universal primers also impacts the detection of other fish groups that are not targeted, such as marine and freshwater fish groups that have never been found in Indonesia, especially in West Sumatra (Supplementary Table 1). As explained, these primers were used because the available database for fish in West Sumatra was derived from the COI gene. Designing more specific primers is necessary to detect fish in West Sumatra. These primers have also been used in other studies monitoring fish and metazoans (Schwentner et al., 2021), which also detected non-fish and non-metazoan groups. Meanwhile, monitoring marine vertebrates (Andruszkiewicz et al., 2017) using specific primer sets resulted in the detection of more fish groups compared than non-target taxa. In addition to the use of non-specific primers, errors in assigning reads also resulted in false positives. The absence of OTU clustering, Chimera removal, and denoising in bioinformatic analysis may increase the risk of sequencing artefacts. These artefacts may lead to mis-assignments and potentially contribute to the increase in false-positive detections, particularly among taxa represented by low read counts. Therefore, the biodiversity estimates reported in this study are interpreted with great caution, particularly with respect to rare taxa detected at low read counts.

Based on the study results and various references, recommendations are made for biomonitoring in areas with limited available species data, especially in Twin Lakes: (i) suggesting field sampling frequency (eg, annual dry versus wet season surveys), (ii) developing a local reference database before large-scale surveys, (iii) strict laboratory quality control and bioinformatics, integrating molecular results with ecological field data, and (iv) determining priority species for future monitoring (such as native type-locality species and economically important taxa). The implications of this study for monitoring and conservation in Twin Lakes, West Sumatra, include providing baseline data for conservation policies, accelerating the identification of community changes, and supplying long-term data for sustainable inland water management in the area.

4. CONCLUSION

This study identified 23 freshwater fish species, including several native species, in West Sumatra. COI barcoding provides a valuable local reference library (35 DNA Barcodes). Only seven species of the 23 species (30%) were detected using eDNA metabarcoding. The low detection of eDNA may be influenced by several main factors: the absence of target species in samples, contamination, low DNA quality, the use of universal primers, and incomplete reference libraries. eDNA results must be interpreted cautiously, considering some factors that influence the detection. Biodiversity monitoring can be conducted using conventional methods (Morphology and DNA barcoding), combined with non-invasive (eDNA metabarcoding) as a complementary method. Thus, researchers can provide more reliable biodiversity assessments in the future.

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AUTHOR CONTRIBUTIONS

Dewi Imelda Roesma: Conceptualization, Methodology, Investigation, Validation, Writing Original Draft, Reviewing, and Editing.

Djong Hon Tjong: Data curation, Reviewing, and Editing.

Dyta Rabbani Aidil: Methodology, Writing Original Draft, Reviewing, and Editing.

Delia Selvi Yanti: Writing and Editing.

CONFLICT OF INTEREST

The authors have declared that no competing interest exists.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Table 1. Supplementary Data

No.	Species/Genus	TaxRank	NumUniqueReads					
			T1	T2	T3	T4	T5	T6
1	<i>Abudefduf concolor</i>	species			3		1	1
2	<i>Abudefduf declivifrons</i>	species		1	1		1	1
3	<i>Abudefduf taurus</i>	species		1				3
4	<i>Acanthocephala indica</i>	species			2		1	
5	<i>Acanthopagrus bifasciatus</i>	species						1
6	<i>Acentrogobius viridipunctatus</i>	species						1
7	<i>Acheilognathus imberbis</i>	species			1			
8	<i>Actinopterygii</i> sp. OC218	species	1			1		
9	<i>Aetobatus ocellatus</i>	species	2	1	1			
10	<i>Abliesaurus berryi</i>	species		1				
11	<i>Alburnus alburnus</i>	species	1					
12	<i>Alopias pelagicus</i>	species					1	
13	<i>Altigena yunnanensis</i>	species	2					
14	<i>Amblygobius phalaena</i>	species						2
15	<i>Amphiprion clarkii</i>	species	2					
16	<i>Amphiprion perideraion</i>	species					1	
17	<i>Anabas testudineus</i>	species	1	1	2	2		
18	<i>Anguilla bengalensis bengalensis</i>	subspecies	1		1			1
19	<i>Anguilla rostrata</i>	species		1				
20	<i>Anoxypristis cuspidata</i>	species					1	
21	<i>Amphiprion clarkii</i>	species				2		

22	<i>Apareiodon affinis</i>	species			1			
23	<i>Aplocheilichthys panchax</i>	species			2	1		2
24	<i>Aplocheilichthys</i> sp. Ap2G	species	1					
25	<i>Ariosoma shiroanago</i>	species	1	1	5	4	1	5
26	<i>Arius leptotacanthus</i>	species			1	1		
27	<i>Arius maculatus</i>	species	2	2		4		1
28	<i>Arius manillensis</i>	species					1	
29	<i>Arius microcephalus</i>	species				1		
30	<i>Astyanax</i>	genus						1
31	<i>Astyanax bimaculatus</i>	species				1		
32	<i>Astyanax lacustris</i>	species	2	2				2
33	<i>Auchenoglanis occidentalis</i>	species	3	1	11	1	1	6
34	<i>Bagarius bagarius</i>	species						2
35	<i>Balistoides viridescens</i>	species	2	1	2	2	2	
36	<i>Barbodes carnaticus</i>	species	2		3			
37	<i>Barbus</i> sp. FSA-2019	species						1
38	<i>Batasio merianensis</i>	species				1		
39	<i>Benthoosema pterotum</i>	species		1				
40	<i>Beryx splendens</i>	species						1
41	<i>Betta splendens</i>	species	5		5			
42	<i>Botia almorhae</i>	species		1				
43	<i>Boulengeromyrus knoepffleri</i>	species					1	
44	<i>Bregmaceros nectabanus</i>	species	1	1	1			
45	<i>Brevitrygon walga</i>	species		1		1		
46	<i>Brosmophyciops pautzkei</i>	species	1	2	1	1	4	1
47	<i>Butis gymnopomus</i>	species	65					
48	<i>Caranx sexfasciatus</i>	species			2			2
49	<i>Centropyge</i> sp. BOLD:AAI3793	species	1					
50	<i>Chaenogobius annularis</i>	species	25	19	18	13	19	68
51	<i>Chaetodon zanzibarensis</i>	species					1	
52	<i>Chaeturichthys stigmatias</i>	species				1		
53	<i>Chagunius chagunio</i>	species	17	1	5	2	4	18
54	<i>Channa punctata</i>	species			1		1	
55	<i>Cheilinus trilobatus</i>	species						1

56	<i>Chelonodontops bengalensis</i>	species				1		
57	<i>Chirocentrus nudus</i>	species						1
58	<i>Chitala ornata</i>	species	2		1			
59	<i>Clarias</i>	genus		2			2	
60	<i>Clarias batrachus</i>	species					1	
61	<i>Clarias gabonensis</i>	species	11	38	18	24	25	17
62	<i>Clarias gariepinus</i>	species	1			1	1	1
63	<i>Clarias gariepinus</i> × <i>Clarias batrachus</i>	species	1					
64	<i>Clupea pallasii pallasii</i>	subspecies	19	47	19	57	41	28
65	<i>Clupisoma prateri</i>	species	1	1				1
66	<i>Coccorella atlantica</i>	species	2	1	1		1	2
67	<i>Coptodon zillii</i>	species	2			3		2
68	<i>Coregonus</i>	genus				1		
69	<i>Coregonus albula</i>	species				1		
70	<i>Coregonus sardinella</i>	species				1	1	1
71	<i>Coryphaena hippurus</i>	species			1			
72	<i>Coryphaenoides acrolepis</i>	species	172	988	339	1886	1171	868
73	<i>Coryphaenoides suberrulatus</i>	species				1		
74	<i>Crenimugil sebeli</i>	species	2			2	1	
75	<i>Cruriraja triangularis</i>	species						1
76	<i>Cubiceps pauciradiatus</i>	species	14		41	2	10	5
77	<i>Curimatopsis</i>	genus	997	386	701	500	359	537
78	<i>Curimatopsis</i> aff. <i>crypticus</i> BFM-2016	species			1			
79	<i>Curimatopsis crypticus</i>	species	69	16	51	42	25	37
80	<i>Curimatopsis macrolepis</i>	species	3		1	2	1	3
81	<i>Curimatopsis</i> sp. Tapajos	species	1	1		1	1	1
82	<i>Cyclocheilichthys</i>	genus	3					
83	<i>Cyclocheilichthys repasson</i>	species	12	5			5	
84	<i>Cyclopsetta</i>	genus					1	
85	<i>Cynodonichthys tenuis</i>	species					1	
86	<i>Cynoglossus arel</i>	species	4		2	2	1	6
87	<i>Cynoglossus itinus</i>	species	1		2	1		
88	<i>Datnioides microlepis</i>	species			1	1		
89	<i>Dawkinsia tambraparniei</i>	species			1	3		

90	<i>Decapterus maruadsi</i>	species	3	4	1		5
91	<i>Decapterus</i> sp. SepS-6-110	species	1				
92	<i>Dendrophysa russelii</i>	species				1	1
93	<i>Dermogenys</i>	genus				1	
94	<i>Desmopuntius rhomboocellatus</i>	species			1		
95	<i>Deveximentum insidiator</i>	species					1
96	<i>Diodon</i>	genus			1		
97	<i>Dipturus laevis</i>	species	1		2	2	1
98	<i>Dissostichus eleginoides</i>	species				1	1
99	<i>Distichodus rostratus</i>	species	254	687	354	538	435
100	<i>Ebosia falcata</i>	species	2		1	2	
101	<i>Entomacrodus vermiculatus</i>	species					1
102	<i>Epinephelus coioides</i>	species	1				1
103	<i>Epinephelus moara</i> × <i>Epinephelus lanceolatus</i>	species	2	3		2	2
104	<i>Exyrias puntang</i>	species	20	17	124	18	55
105	<i>Flagellotomias boureei</i>	species	3	1	2	3	6
106	<i>Gadus morhua</i>	species			1		5
107	<i>Galaxias zebratus</i>	species	1	1	5	2	
108	<i>Garra annandalei</i>	species	1				1
109	<i>Gasteropelecus sternicla</i>	species					1
110	<i>Gerres erythrorus</i>	species				1	
111	<i>Glyptocephalus kitabarae</i>	species					1
112	<i>Glyptothorax poonaensis</i>	species				1	
113	<i>Gnathophis</i> sp. ZSI/SRC E-28	species	2				1
114	<i>Gobiidae</i> sp. 1 LZ-2021	species		1			2
115	<i>Gobiocypris rarus</i>	species	1				
116	<i>Guyanancistrus niger</i>	species					1
117	<i>Halichoeres hortulanus</i>	species		2		2	
118	<i>Harengula jaguana</i>	species	1		1	1	1
119	<i>Herklotsichthys lossei</i>	species				1	
120	<i>Heteropneustes fossilis</i>	species		2			
121	<i>Holocentrinae</i> sp. SepS-13-13	species	1				
122	<i>Hoplostethus mediterraneus</i>	species			1		
123	<i>Horaglanis krishnai</i>	species				3	

124	<i>Hyphessobrycon roseus</i>	species				1	
125	<i>Hypomesus olidus</i>	species	1	1	1		
126	<i>Hypophthalmichthys nobilis</i>	species			1		
127	<i>Iberochondrostoma oretanum</i>	species	1			1	
128	<i>Istiophoridae</i> sp. 388-42-150	species	3		1	1	6
129	<i>Istiophoridae</i> sp. 394-42-150	species		1		1	1
130	<i>Johnius belangerii</i>	species			1		
131	<i>Johnius trewavasae</i>	species	5		4	1	2
132	<i>Knodus</i> cf. <i>orteguasae</i> LBPV-23626	species					1
133	<i>Knodus heteresthes</i>	species				1	
134	<i>Knodus moenkhausii</i>	species	1		4	1	1
135	<i>Knodus orteguasae</i>	species			2		
136	<i>Knodus victoriae</i>	species			1	1	
137	<i>Labeo</i>	genus			1		
138	<i>Labeo gonius</i>	species				1	
139	<i>Labeo rohita</i>	species					1
140	<i>Labeo vulgaris</i>	species	17		2	2	
141	<i>Lagocephalus</i>	genus	3	1	3		
142	<i>Lagocephalus gloveri</i>	species					1
143	<i>Lagocephalus guentheri</i>	species					1
144	<i>Lagocephalus spadiceus</i>	species				1	
145	<i>Lamna nasus</i>	species	2	1	1		1
146	<i>Lampanyctus</i> sp. ASIZP0078800	species					1
147	<i>Leiognathus ruconius</i>	species	1				
148	<i>Leiopotherapon plumbeus</i>	species			1		
149	<i>Lepidocephalichthys guntea</i>	species		1			
150	<i>Lepidocephalichthys thermalis</i>	species	2			2	1
151	<i>Lesueurigobius friesii</i>	species		1			1
152	<i>Lethrinus microdon</i>	species					1
153	<i>Liobagrus reinii</i>	species					1
154	<i>Liopsetta glacialis</i>	species	108	180	133	135	107
155	<i>Loxodon</i>	genus	1				1
156	<i>Lutjanus fulviflamma</i>	species	1				
157	<i>Lutjanus griseus</i>	species					1

158	<i>Lutjanus guttatus</i>	species			2			
159	<i>Lutjanus notatus</i>	species	1					1
160	<i>Lutjanus rivulatus</i>	species	231	163	122	159	247	548
161	<i>Lutjanus russellii</i>	species	1					
162	<i>Lutjanus</i> sp. MS-2015	species						1
163	<i>Malacocottus gibber</i>	species	1			1		
164	<i>Mallotus villosus villosus</i>	subspecies	2					
165	<i>Maurollicus</i> sp. SepS-26-1	species	2		2			4
166	<i>Microdevario kubotai</i>	species			2			1
167	<i>Microglanis parahybae</i>	species	1					
168	<i>Minous monodactylus</i>	species		1		1		
169	<i>Misgurnus anguillicaudatus</i>	species	2			1		
170	<i>Monopterus albus</i>	species	2	1				1
171	<i>Moolgarda cunnesius</i>	species	25		22	16	8	33
172	<i>Moolgarda perusii</i>	species	1		1	1		1
173	<i>Mugil cephalus</i>	species	2		1		3	
174	<i>Mugil thoburni</i>	species					1	
175	<i>Mugilogobius bitam</i>	species	3		1			
176	<i>Mugilogobius mertonii</i>	species	1		2	3	1	
177	<i>Mustelus mustelus</i>	species					1	
178	<i>Mystus vittatus</i>	species			1			
179	<i>Nemipterus randalli</i>	species	5	13	9	71	25	54
180	<i>Neopinnula americana</i>	species	1					
181	<i>Neolissochilus soroides</i>	species	1				2	1
182	<i>Nothobranchius torgasbevi</i>	species					1	
183	<i>Oligosarcus longirostris</i>	species						1
184	<i>Ohyra longicaudata</i>	species						2
185	<i>Omobranchus</i> sp. s MPC-2021	species	1					
186	<i>Omosudis lowii</i>	species	8	7	5		6	19
187	<i>Oncorhynchus keta</i>	species	2					1
188	<i>Opsariichthys</i> sp. 'chengtui'	species						1
189	<i>Oreochromis niloticus</i>	species	1	2	1			
190	<i>Oryzias melastigma</i>	species	1	1	1			
191	<i>Osteochilus</i>	genus		17			2	

192	<i>Osteochilus basseltii</i>	species	13			1	1
193	<i>Osteochilus kabajanensis</i>	species	125	1		17	
194	<i>Osteochilus</i> sp. CBM-ZF-12420	species	15			5	1
195	<i>Osteochilus</i> sp. CTOL3981	species	1				
196	<i>Osteochilus vittatus</i>	species	12			2	
197	<i>Pangasianodon gigas</i>	species	2			1	
198	<i>Pangasianodon hypophthalmus</i>	species	6	3	1	1	7
199	<i>Parablennius pilicornis</i>	species	1				
200	<i>Parodon nasus</i>	species				1	
201	<i>Parambassis ranga</i>	species	1				
202	<i>Paraqianlabeo lineatus</i>	species	1				
203	<i>Parioglossus raoi</i>	species	1				
204	<i>Perciformes</i> sp. BOLD:AAB0990	species				1	
205	<i>Pethia cumingii</i>	species		1			
206	<i>Pethia padamya</i>	species	1				
207	<i>Pethia ticto</i>	species	2	1	1	1	2
208	<i>Petrosirtes variabilis</i>	species					1
209	<i>Phenacobius teretulus</i>	species			2		
210	<i>Phoxinus</i> sp. 2 LC-2017	species	1	2	1	2	2
211	<i>Piaractus brachypomus</i>	species	1				
212	<i>Pimephales vigilax</i>	species	4	1	6	5	2
213	<i>Planiliza macrolepis</i>	species	1			1	
214	<i>Planiliza tade</i>	species				1	
215	<i>Platycephalus indicus</i>	species				1	
216	<i>Plectropomus maculatus</i>	species	3	5	6	2	8
217	<i>Pleurogrammus monoptygius</i>	species				1	13
218	<i>Plotosus canius</i>	species	1	2	1		
219	<i>Poecilia salvatoris</i>	species	1				
220	<i>Pomacentrus chrysurus</i>	species			1		
221	<i>Pomadasys argyreus</i>	species	2				
222	<i>Potamotrygon falkneri</i>	species	1		1		2
223	<i>Prietella phreatophila</i>	species					1
224	<i>Prochilodus lineatus</i>	species	2				
225	<i>Promethichthys prometheus</i>	species	4		3		

226	<i>Pseudocaranx dentex</i>	species	1					
227	<i>Pseudochromis elongatus</i>	species	1					
228	<i>Psilorhynchus balitora</i>	species		1				
229	<i>Ptychobarbus chungtienensis</i>	species	14	2	6	5	8	16
230	<i>Pungitius</i>	genus				1		
231	<i>Puntius chelynoides</i>	species			2			
232	<i>Puntius</i> sp. RR-2013c	species	1					
233	<i>Puntius titteya</i>	species	1					
234	<i>Rachycentron canadum</i>	species						1
235	<i>Rasbora rubrodorsalis</i>	species	1		1			1
236	<i>Rastrelliger kanagurta</i>	species	7	2	9	3	2	
237	<i>Rexea</i> sp. FNSIC073-11	species				4		5
238	<i>Rhinecanthus verrucosus</i>	species	2	1	1		2	1
239	<i>Rhinogobius formosanus</i>	species				2		
240	<i>Salmo platycephalus</i>	species			1	1		1
241	<i>Salmo salar</i>	species						1
242	<i>Salmo trutta</i>	species		1	1		1	1
243	<i>Saurida umeyosbii</i>	species			1			
244	<i>Schistura geisleri</i>	species						1
245	<i>Scombrops oculatus</i>	species	1					
246	<i>Scopelosaurus lepidus</i>	species	4		1		1	6
247	<i>Sebastiscus marmoratus</i>	species	1222	4288	1824	3110	2795	2548
248	<i>Selaroides leptolepis</i>	species				1	1	
249	<i>Seriola quinqueradiata</i>	species	2					
250	<i>Serrapinnus notomelas</i>	species	7		3	4	2	1
251	<i>Setipinna melanochir</i>	species					1	
252	<i>Siganus guttatus</i>	species	1				1	1
253	<i>Sigmops</i> sp. SepS-16-3	species					1	
254	<i>Sillago robusta</i>	species	139	45	160	42	39	135
255	<i>Siniperca loona</i>	species	2	1				
256	<i>Siniperca undulata</i>	species			1			
257	<i>Sperata aorides</i>	species	5	2	1		1	9
258	<i>Stolephorus andbraensis</i>	species	1		5			
259	<i>Stomias affinis</i>	species			1			2

260	<i>Suggrundus meerdervoortii</i>	species	1	1		2	1	1
261	<i>Synodontis</i> sp. TT-2020	species	1					
262	<i>Tanichthys micagemmae</i>	species			1			
263	<i>Teleostei</i> sp. U18_6	species			1		1	2
264	<i>Teleostei</i> sp. U5_11	species			2			1
265	<i>Teleostei</i> sp. U7_11	species	1					
266	<i>Terapon theraps</i>	species	1	1	2			3
267	<i>Tetraroge barbata</i>	species	1					
268	<i>Thunnus obesus</i>	species	3	7	10	5	3	7
269	<i>Tilapia</i> sp. ONDOTG 001	species	1		1			
270	<i>Tomicodon humeralis</i>	species		1				
271	<i>Tor putitora</i>	species		1				
272	<i>Tor tor</i>	species	2	1	4	4	1	2
273	<i>Torpedo panthera</i>	species					1	1
274	<i>Trachinotus falcatus</i>	species		1				
275	<i>Trachurus picturatus</i>	species						1
276	<i>Trachyrhamphus serratus</i>	species	2	3	4	3	3	3
277	<i>Trichiurus japonicus</i>	species		1				
278	<i>Trichomycterus diabolus</i>	species			1	1		
279	<i>Tridentiger barbatus</i>	species	1					1
280	<i>Trigonostigma heteromorpha</i>	species	9		1	1	2	2
281	<i>Triplophysa</i>	genus					1	
282	<i>Triplophysa bleekeri</i>	species				1		
283	<i>Triplophysa dalaica</i>	species			1			
284	<i>Uroconger</i> sp. FNSIC077-11	species			1			
285	<i>Vimba vimba</i>	species	1					2
286	<i>Vinciguerria</i>	genus	1		1	1	1	1
287	<i>Zoarces gillii</i>	species	2	3	6	5	1	2