Identifications Mitochondrial DNA Control Region Sequence of Captive and Wild Tilapia Species Existing in Ranu Klakah

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Abstract. Tilapia has a significant economic value, making it one of the important freshwater fish species in aquaculture. The goal of this study was to identify the many types of tilapia that can be found in the wild and captivity (Floating Net Cages), as well as to evaluate the water quality in Ranu Klakah, Lumajang. The CO1 gene found in mitochondrial DNA served as a molecular marker for the Polymerase Chain Reaction (PCR) methodology employed in this investigation. Twenty-five tilapia samples were taken from captive fish, and another twenty-five were taken from wild tilapia. Following morphological and meristic examination, DNA analysis of the fish samples was performed. The analytical results from the 1st BASE DNA Sequencing Service were used to determine the sequences of each sample. Following BLAST analysis, the sample sequences were compared to the sequences in GenBank. The analysis's findings indicated that the species of tilapia identified as being raised in captivity were *Oreochromis niloticus x Oreochromis aureus*, also known locally as tilapia Srikandi and *Oreochromis niloticus*. Tilapia is a species of Oreochromis niloticus that lives in the wild. Ranu Klakah's water quality is still fairly good. Waters' pH value is 7, DO level is between 11.8 mg/L and 13.3 mg/L, BOD level is between 1.8 mg/L and 3.1 mg/L, ammonia concentration is less than 0.15, nitrate concentration is 0 mg/L, and nitrate in waters ranges from 25 mg/L to 50 mg/L, according to observations of temperature parameters.

Keywords: Tilapia · *mtDNA* · identification · captive · wild

1 Introduction

Numerous fish species that may be found in both freshwater and saltwater habitats are members of the Cichlidae family, which includes the tilapia. Many kinds of fish, including *Oreochromis niloticus, Oreochromis aureus,* and *Oreochromis mossambicus,* are great for aquaculture because they can reproduce easily and adapt to a variety of habitats. The Nile tilapia (*Oreochromis niloticus*) is one of the most widely dispersed and farmed aquaculture species in the world, being raised throughout Africa, Asia, North and South America, and Europe [1, 2]. One of the lakes that has a place for tilapia cultivation and

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wild nature is in Ranu Klakah which is located in Tegal Randu Village, Klakah District, Lumajang Regency. The identification of species in both captive and wild tilapia in Ranu Klakah has not, however, been the subject of any investigation to yet. The species of tilapia must therefore be further identified using morphological and molecular methods.

Ranu Klakah is one of the lakes that is used as a tourist destination for tourists at an affordable price. In addition to being a tourist attraction, Ranu Klakah can be used as a fishing ground for fishermen and anglers. The lake known as Ranu Klakah is also a location for fish cultivation. From the daily activities of the residents in Ranu Klakah, it might produce changes in the quality of lake water. When economic growth and population increase, a lot of industrial and agricultural wastewater and domestic sewage inflow into the lake, causing the deterioration of lake water quality. In addition, lake water quality is significantly affected by climate change and hydrodynamic conditions, leading lake water pollution control meeting numerous obstacles. Choosing a rapid and effective water quality assessment method can assist the management department comprehend the water environmental quality and pinpoint the major points of pollution mitigation [3, 4].

The Nile tilapia (*Oreochromis niloticus*) is a worldwide important aquaculture species that is quickly becoming recognized as a farmed product. Due to the Nile tilapia's historical popularity as a fishery target and for aquaculture production, it has been purposefully introduced into numerous locations where it was not previously present. Even if the genetic roots of such introductions are frequently hazy or unknown, they may have a significant influence on the genetic diversity of wild populations. Because of significant genetic variability, choosing strains or specific genotypes presents the biggest difficulty to tilapia producers in terms of increasing productivity. Fish farmers can estimate their economic production using performance and morphological qualities [5, 6]. For taxonomic and evolutionary investigations, fish morphology has traditionally been the main source of data. According to the fundamental theory of evolution, every species is thought to be going through a micro and macro evolutionary process that manifests as significant genetic variations at the levels of species-specific chromosome morphology and structure, gene-controlled protein structure, and polygene-controlled morphometrics and metrics. There is some morphological differentiation that can be seen in populations that are closely related genetically. However, it has been found that physical description alone is insufficient to infer genetic links both within and between species [7–9].

DNA-based method is more precise and useful than morphological observation and description. DNA barcoding of all living species is based on the sequence of a single mitochondrial protein-coding gene, cytochrome c oxidase subunit I (COI). Mitochondrial DNA (mtDNA) is a circular double-stranded DNA that is 15–20 kb long and typically codes for 37 genes, including 13 PCGs (protein-coding genes), 22 tRNAs (transport RNAs), and 2 ribosomal RNAs (rRNAs). Due to its quick variation, maternal inheritance, rapid evolution, and absence of recombination, mtDNA has been employed extensively for genetic study, taxon categorization, phylogenetic evolution research, and population studies to date [1, 10]. In this study, we describe the identification of tilapia species found in captive and wild populations in Ranu Klakah using mtDNA sequence information that was then compared with information in the GenBank database.

2 Methods

2.1 Water Sampling and Fish Collecting

In May 2022, water samples from a lake in Ranu Klakah, Lumajang, East Java, were taken. Three observation stations were used for water sampling: the inlet, the floating net cage (KJA), and the outlet. The water quality is assessed once the sample is gathered in a bottle. Temperature, pH, ammonia, nitrate, and nitrite are among the water quality parameters that can be assessed in-situ using a test kit, whereas BOD values are measured ex-situ.

In this study, 50 samples of tilapia were gathered from captive (KJA) and wild (up to 25 samples each). At the Central Laboratory Life Sciences (LSIH), Brawijaya University, fish are captured using fishing equipment such as nets and then preserved in a cool box to be used in morphometric and meristic molecular identification activities.

2.2 Morphometric and Meristic Measurement of Tilapia

Morphological identification, which tries to identify the general characteristics of the caught fish, is done by looking at body shape and body color. Then, to complete the needed data, morphometric measurements and meristic computations were also performed. The following morphometric parameters were measured in this study: height, weight, standard length, total length, head length, base of tail length, face length, nose length, jaw length, and forehead length. Using a ruler, the length of the fish was calculated. While calculating the radius of the fish's fins is the meristic method of measuring fish.

2.3 DNA Extraction and Amplification

Muscle tissue total DNA was extracted utilizing a DNA extraction kit technique (GeneAll Exgene Clinic SV mini, 100p). Tissue samples were briefly digested with 20 µl proteinase K at 56 ºC for an overnight period. The final mixture was centrifuged, and the supernatant was extracted using BL buffer, precipitated in absolute ethanol, and then dissolved in AE buffer. A spectrophotometer and agarose gel electrophoresis were used to evaluate the DNA's purity and concentration. The primers forward CO1 (5' - GGT CAA CAA ATC ATA AAG ATA TTG G – 3') and primer reverse (5' - TAA ACT TCA GGG TGA CCA AAA AAT CA -3') were used to perform PCR for mtDNA. The PCR mixture included 0.5 l of ddH2O, 5 l of pcr mix, 5 µl of BSA (10 mg/ml), 0.5 l each of primers F and R (10 pmol/l), and 1 µl of DNA template. Utilizing the TaKara Thermal cycler (Bio Rad) device, DNA molecules were amplified. The PCR procedure involves a Hot Start stage lasting 5 minutes at 95 °C, a denaturation step lasting 1 minute at 95 °C, 35 cycles of annealing lasting 1 minute each at 54 °C, and a final extension step lasting 1 minute each at 72 °C.

2.4 DNA Sequencing

The results of samples that have been amplified using the PCR method and obtained positive samples containing DNA bands are then packaged for the sequencing process.

DNA samples were sent to PT. Genetic Science Indonesia then processed by the 1st BASE DNA Sequencing Service located in Singapore. The 1st BASE DNA Sequencing Service in Singapore then processes the genetic science from Indonesia. The electroferogram that is produced as a result of the sequencing process. Then, using the BioEdit and MEGA programs, the results can be corrected and a phylogenetic tree can be analyzed.

2.5 Data Analysis

With the aid of BioEdit software, the electropherogram data from 1st BASE that was used for the analysis of the sequencing data was edited and aligned to provide consensus/combined sequences. Using the BLAST method (Basic Local Alignment Search Tool) on the NCBI website, the consensus sequences are then compared with the GenBank sequences. The phylogenetic tree of the sequences that have revealed the species is next examined. In this study, a phylogenetic tree was constructed using the Maximum Parsimony method with 1000x bootstrap and the species *Oreochromis mozambicus* was added as an outgroup to see the relationships between the identified sample species. The phylogenetic analysis was carried out using MEGA (Molecular Evolutionery Genetics Analysis) software.

3 Results and Discussion

3.1 Water Quality

In June 2022, measurements of water quality were made with chemical and physical parameters. Temperature was detected in physical parameter, whereas pH, Dissolved Oxygen (DO), Biological Oxygen Demand (BOD), Nitrite, Nitrate, and Ammonia were measured in chemical parameters. The outcomes of the water quality data shown in Table 1 are as follows. According to government regulation number 82 of 2001, the findings of the water quality test will be compared to quality criteria [11].

Table 1. Water Quality Measurement Results

No	Station	Temperature (ºC)	pH	DO (mg/L)	BOD (mg/L)	Nitrite (mg/L)	Nitrate (mg/L)	Ammonia (mg/L)
	Quality standards	26–30 ºC	7	> 4 mg/L	3 mg/L	0,5 mg/L	20 mg/l	0,5 mg/L
1	Floating Net Cages	27	7	13,3	3,1	0	50	< 0.15
2	Water springs source (inlet)	27,6	7	13	2,8	0	50	< 0.15
3	Outlet	28	7	11,8	1,8	0	25	< 0.15

The table demonstrates that the temperature findings fall within a range of values between 27 and 28 °C. Despite the chemical factors, all stations should have a pH value of 7. The DO results that were then obtained ranged from 11.8 to 13.3 mg/L, and the BOD results had values between 1.8-3.1 mg/L. Finally, the value for nitrite was determined to be 0 mg/L, the range for nitrate was 25 to 50 mg/L, and the value for ammonia was 0.15 mg/L. These findings provide an explanation for why the majority of metrics are still at their ideal values. There are certain parameters that are above the ideal ranges, with the exception of BOD and nitrate. The high concentration of BOD, which does not support aquatic life, is probably to blame for the loss in the lake's aquatic population. While a high BOD number implies contamination, a low BOD value suggests pure water in the lake [12].

The main factors contributing to nitrate nutrient enrichment include runoff, erosion, household waste, and leaching of fertile agricultural land. Nitrate levels in natural waters are often seldom higher than 0.1 mg/L. Eutrophication happens when the amount of nitrate in the water surpasses 0.2 mg/L, which encourages the rapid growth of algae and phytoplankton. Anthropogenic contamination happens if the water's nitrate level is more than 5 mg/L [13].

These bodies of water are able to support a large number of aquatic plants because they have an abundance of nutrients, particularly nitrogen and phosphorus. Aquatic plants or algae will typically predominate in the body of water. The water usually seems pure when aquatic plants are in charge. The water usually seems darker when algae are in charge. The fish and other aquatic life in these waters receive oxygen thanks to the photosynthesis that the algae perform. On rare occasions, an extreme algae bloom will happen, and the bottom-dwelling bacteria and respiration will cause fish to die. Both natural eutrophication and human environmental effect are possible [14].

3.2 Tilapia Morphology from Captive and Wild

Only three of the 25 species of tilapia that may be caught in captivity with nets are believed to exhibit distinct physical traits. Fish number 5 measures 20 cm in length, 6.5 cm in height, and weighs 144 grams. Its body is covered in irregular, darker-colored black blotches. Fish number 13 has the following characteristics: a total length of 13.5 cm, a height of 5.5 cm, and a weight of 86 grams. Its color from the body to the head is also darker than that of the other fish. While fish number 20, which measures 18.3 cm in length overall, has the feature of having a red rash on its neck, 6 cm tall and 96 grams in weight (Fig. 1).

In Lake Ranu Klakah, 25 wild tilapia fish have been discovered. Following that, three tilapia were discovered that differed morphologically from other fish. The three fish with the numbers 8, 16, and 17 were supposed to have distinct morphologies. Morphological features of fish number 8 include a pelvic fin that appears yellow and lower fish body scales that have a brighter color. The dorsal and tail fins of fish number 16 appear to have a reddish color change, while the fish's head and anal fins appear green. The fish's body color is also fairly dark. The last fish is fish number 17, which has a crimson tint all over its fins and a fish head that is sharper than the heads of the other two fish (Fig, 2).

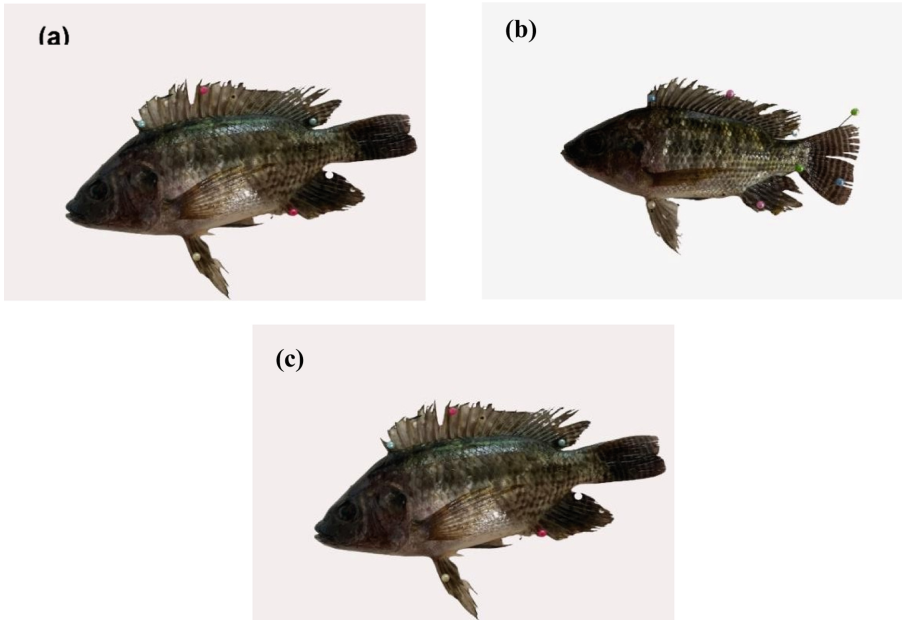

Fig. 1. Differences in Fish Morphology (a) Fish No. 5; (b) Fish No. 13; (c) Fish No. 20

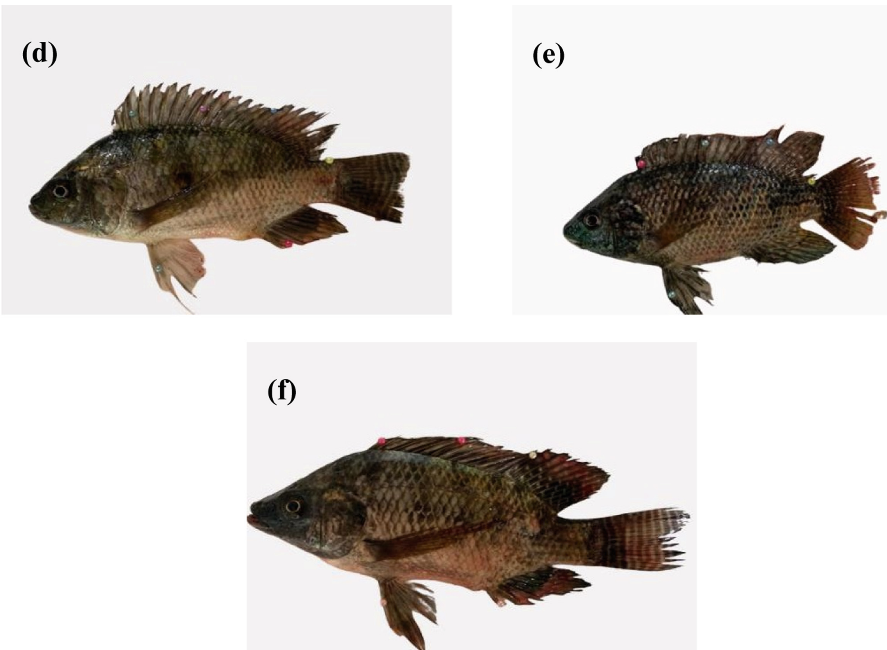

Fig. 2. Differences in Fish Morphology (d) Fish No. 8; (e) Fish No. 16; (f) Fish No. 17

Table 2. Morphometric Measurement Results of Captive Tilapia

Morphometric Characteristic	Fish No. 5	Fish no. 13	Fish no.20
Total Length (cm)	20	17.3	18,3
Fork Length (cm)	16.1	13.9	15.1
Head Length (cm)	5.4	4.4	5
Caudal fin length (cm)	2.3	2.1	2.3
Face Length (cm)	2.4	1.7	1.9
Nose Length (cm)	1,2	1	1
Jaw Length (cm)	1,8	1,4	1,5
Forehead Length (cm)	1,7	1,6	1,6
Body Height (cm)	8,3	7,4	6,1
Body Weight (gram)	231	193	118

Table 3. Meristic Calculation Results of Captive Tilapia

Meristic Characteristic	Fish No. 5	Fish no. 13	Fish no.20
Dorsal fin rays	XVI.11	XVI.11	XVI. 10
Pectoral fin rays	I. 11	I. 8	I. 12
Ventral fin rays	I. 5	I. 5	I. 6
Anal fin rays	III. 9	III. 6	II. 8
Caudal fin rays	16	16	15

This study included morphological observations together with morphometric measurements, which are shown in Table 2 and tilapia meristic calculations in Table 3, in addition to morphological observations.

Table 2 displays various results based on the morphometric properties of tilapia measured using morphometric techniques and collected by fishermen in floating net cages, Ranu Klakah. The number of meristic counts has a considerable change and is the same amount in Table 2, which displays the findings of the tilapia meristic calculation. Roman symbols are used to represent hard fin rays, while normal numerals are used to represent weak fin rays. The dorsal fin radius, which is equal to D XVI, 11; and the ventral fin rays, which are I, are commonalities between fish numbers 5 and 13. Several factors that influence the difference in numbers in the calculation of meristic fish are age, climate, sex, habitat and environmental conditions [15].

The three samples of Ranu Klakah's wild tilapia were collected, and the following are the morphometric and meristic data: (Table 4).

The table demonstrates that there are morphometric variations in wild tilapia. The length of the nose and the forehead are identical in wild tilapia numbers 16 and 17. The

Table 4. Results of morphometric measurements of wild tilapia

Morphometric Characteristic	Fish No. 8	Fish no. 16	Fish no.17
Total Length (cm)	24,1	23,1	19,2
Fork Length (cm)	18,2	18	14,9
Head Length (cm)	6	5,9	5
Caudal fin length (cm)	3,2	2,3	2,2
Face Length (cm)	2,7	2,5	2,3
Nose Length (cm)	1,2	1	1
Jaw Length (cm)	1,8	1,4	1,5
Forehead Length (cm)	1,7	1,6	1,6
Body Height (cm)	8,3	7,4	6,1
Body Weight (gram)	231	193	118

Table 5. Meristic Calculation results of wild tilapia

Meristic Characteristic	Fish No. 8	Fish no. 16	Fish no.17
Dorsal fin rays	XVII.12	XVII.12	XVI. ii. 9
Pectoral fin rays	I. 10	I. 10	I. 10
Ventral fin rays	I. 5	I. 5	I. 5
Anal fin rays	III. 7	II. 7	II. 9
Caudal fin rays	15	16	16

eighth wild tilapia is bigger than the other two fish. While compared to others, wild tilapia number 17 is the smallest.

Based on Table 5, it demonstrates that the results of the meristic computation have roughly the same similarities from the three species. The three fish exhibit comparable pectoral and ventral fin rays despite they have varying morphometric proportions. There are an equal amount of dorsal and anal fin rays on wild tilapia numbers 8 and 16. The only similarity between wild tilapia numbers 16 and 17 is in the caudal fin radius.

3.3 DNA Extraction

Purified DNA is sought after during DNA extraction in place of other cell components. Using a DNA extraction kit called GeneAll Exgene Clinic SV small, 100p, DNA was isolated from the tilapia's muscle tissue and flesh. The wild tilapia numbers 8, 16 and 17 and the captive tilapia numbers 5, 13 and 20 that were employed in the extraction stage were coded in the following order: A, B, C, D, E, and F. Figure 3 illustrates how

Report	DNA 090622	Test Type	Nucleic Acid	Date/time	6/9/2022 1:24 PM	Page #	1

Sample ID	User ID	Date	Time	ng/ul	A260	A280	260/280	260/230	Constant	Cursor Pos.	Cursor abs.	340 raw
A	Default	6/9/2022	1:20 PM	169.06	3.381	1.654	2.04	2.00	50.00	230	1.687	-0.030
B	Default	6/9/2022	1:21 PM	311.80	6.236	3.037	2.05	2.16	50.00	230	2.886	-0.004
C	Default	6/9/2022	1:22 PM	187.03	3.741	1.881	1.99	2.05	50.00	230	1.821	-0.006
D	Default	6/9/2022	1:22 PM	98.96	1.979	1.000	1.98	1.85	50.00	230	1.069	-0.015
E	Default	6/9/2022	1:23 PM	29.55	0.591	0.310	1.91	1.12	50.00	230	0.527	-0.020
F	Default	6/9/2022	1:24 PM	70.02	1.400	0.715	1.96	1.68	50.00	230	0.833	-0.001

Fig. 3. Results of Measurement of Purity and Concentration of Fish DNA

the Nanodrop Spectrophotometer can be used to calculate the extract's absorbance at wavelengths A260 and A280 nm in order to determine the concentration and purity of the extract.

According to the findings of DNA amount measurements, the DNA concentration in the tilapia samples ranged from 29.55 ng/L to 311.80 ng/L, and the DNA purity was between 1.91 and 2.05. Each sample has a varied concentration of DNA because of variations in processing, the addition of mixed materials, and the incidence of DNA degradation, according to [16]. In contrast, DNA is regarded as being pure if the ratio falls between 1.8 and 2.0. If the A260/A280 ratio is less than 1.8, it may suggest the presence of protein impurities, whereas a ratio greater than 2.0 indicates the presence of RNA contamination [17]. Because the results for DNA purity of the tilapia samples still fall within the range of 1.8 to 2.0, it is still possible to use them for the subsequent stage of DNA amplification using the PCR technique. Additionally, the DNA samples A, B, and C concentrations, which exhibit a high value, need to be diluted.

3.4 Results of DNA Amplification with PCR Method

Electrophoresis allows for the immediate visualization of PCR data. All research samples produced a PCR result in the form of a 700 bp DNA band (base pair), which was acquired by amplification of the CO1 gene in tilapia samples at 540C for 1 minute. The existence of DNA bands that are plainly discernible in the 700 bp length range suggests that the sample has a decent concentration. DNA bands that collect and do not spread indicate the existence of a high protein contamination may be present if the A260/A280 ratio value is less concentration of isolate DNA [16] (Fig. 4).

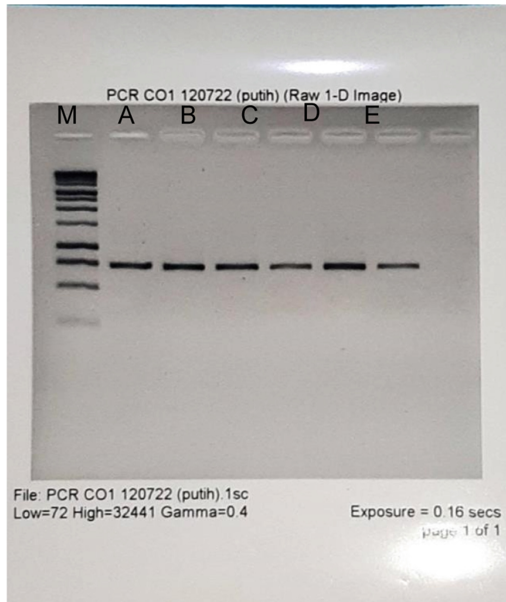

Fig. 4. PCR results of captive and wild tilapia DNA using CO1 Primer Information.

Information
M; DNA Ladder marker 1kb
A: Fish sample number 5 **B :** Fish sample number 13 **C :** Fish sample number 20 **D**: Fish sample number 8 **E** : Fish sample number 16 **F** : Fish sample number 17

3.5 Analysis of DNA Sequencing Results

The electroferograms with AB1 format that represent the sequencing findings sent by 1st BASE DNA Sequencing are peaks. The forward and reverse sequences of the sent electropherogram are depicted in Fig. 5.

Information such as species descriptions and parameters for identification standards like Query Cover, Identity, and Accessions are displayed in the analysis results using BLAST. The degree of resemblance between the nucleotide length of the query (sample) and the database on GenBank is known as query cover. The nucleotide length and sample sequence are more comparable to the GenBank database than other databases, as shown by the query cover result, which is nearly 100%. Identity is the highest percentage match between the database sequence and the query sequence (sample). In GenBank, accession is a code [18] (Table 6).

The three fish samples taken from floating net cages (KJA) in Ranu Klakah were found to include two different species, namely *Oreochromis aureus x Oreochromis niloticus* and *Oreochromis niloticus*, based on the findings of BLAST identification. With the same query cover value of 100% and the identity value or percentage of similarity

Fig. 5. Electroferogram results from the forward primer of sample A

Table 6. Results of tilapia species identification using BLAST

Sample	Spesies	Query Cover	Per Ident	Accession
A	*Oreochromis aureus x Oreochromis niloticus*	100%	100.00%	DQ856613.1
B	*Oreochromis aureus x Oreochromis niloticus*	100%	99.85%	DQ856613.1
C	*Oreochromis niloticus*	100%	99.56%	MK130702.1
D	*Oreochromis niloticus*	100	99.27%	MK130702.1
E	*Oreochromis niloticus*	99	98.99%	MH515210.1
F	*Oreochromis niloticus*	98	99.13%	MH515233.1

between samples A and B, which are not far apart, namely 100% and 99.85%, fish numbers 5 with sample code A and fish numbers 13 with sample code B belong to the species *Oreochromis aureus x Oreochromis niloticus.*

Due to their identical nucleotide sequences, samples A and B are regarded as belonging to the same species as the comparison species. Sample C was correctly identified as the *Oreochromis niloticus* species based on the accession number on GenBank with a query cover of 100% and an identity value of 99.56%, in contrast to the findings of the identification of samples A and B.

Tilapia samples A and B, numbers 5 and 13, were later determined to be the species *Oreochromis aureus x Oreochromis niloticus*, also known as Srikandi fish. The Nirwana black tilapia (*Oreochromis niloticus*) and male blue tilapia were crossed to create the Srikandi tilapia (*Oreochromis aureus x Oreochromis niloticus*) (*Oreochromis aureus*). Srikandi tilapia, a superior fish with a high salinity tolerance of up to 30 g/L, was released in Indonesia in 2012 by Decree of the Minister of Maritime Affairs and Fisheries Number KEP.09/MEN/2012 [19]. It is believed that the distribution of seeds that fish farmers in

Ranu Klakah received led to the establishment of this species in the KJA Ranu Klakah. It is known that different fish breeders in multiple districts, including Probolinggo, Jember, Situbondo, and Kediri, provided the seeds disseminated in the KJA. Therefore, it cannot be determined whether the seeds on hand are of the same species. The tilapia number 20 that was identified as Sample C was of the species *Oreochromis niloticus*, which was in opposition to the results of the identification of samples A and B. The difference in the fishes' body colors reveals the physical trait that separates these two species. The Srikandi tilapia (*Oreochromis niloticus x Oreochromis aureus*) in tilapia numbers 5 and 13 have a darker blackish body color than the tilapia in tilapia number 20, which is tilapia (*Oreochromis niloticus*). This is consistent with the findings of fish morphological identification by Apriani, *et. al,* (2021) [15], which indicate that tilapia (*Oreochromis niloticus* is a species that has a bright body color and is not too dark).

The wild tilapia samples chosen, however, were all of the same species: *Oreochromis niloticus*. Other data are still inconsistent, such as Accession's varying counts among the three samples. The similarity in the three samples does not approach 100% at 99.27%, 98.99%, and 99.13% percentage points. The cover query's values are consecutively 100%, 99%, and 98%. While the percentage similarity (% identity) shows the percentage of the same number of nucleotides between samples and comparison sequences, the query cover description provides the similarity of nucleotide lengths between samples and comparison sequences [20]. The findings are considered to be the most comparable when the Query Coverage value and the Ident value in each database are close to 100%. The nucleotide lengths of the query (sample) and the GenBank database are compared, and the query cover shows the degree of alignment (% similarity). Identity is the degree to which the query sequence matches the sequence in the GenBank database [21]. Despite belonging to the same species, the three samples exhibit various morphological traits. The color of the fish's body or fins can be used to identify the morphological features of wild tilapia. Likewise with the shape of the fish, there is one that has a sharper head than other fish.

Three clusters were present for each species in the phylogenetic tree (Fig. 6) that CO1 successfully created from DNA samples. Samples A and B, which were of the *Oreochromis niloticus x Oreochromis aureus* species and had a similarity value of 98%, made up the first cluster. This result demonstrates that while the species that have been named are the same species, their percent identities vary. The variation in the fraction that is included in the tiniest is thought to represent a minor genetic variation. Samples C and D, which are *Oreochromis niloticus* species with a similarity value of 100%, make up the second cluster. This implies that both species are the same. Then, it has an 82% similarity when examined between sample clusters A-B and sample cluster C-D. Less than 97% percent similarity suggests separate species [21]. While the similarity value for the comparison between the sample clusters A–D and E–F is 97%. There is no similarity value between the clusters A–F and the outgroup clusters, nevertheless. This demonstrates that although though the outgroup species, *Oreochromis mosambicus*, is still in the same genus, it has a distant relationship with the species group, *Oreochromis niloticus*.

The outcomes of this identification are consistent with those of the BLAST analysis used to identify the species. The tilapia identified and sold in Ranu Klakah, Lumajang are

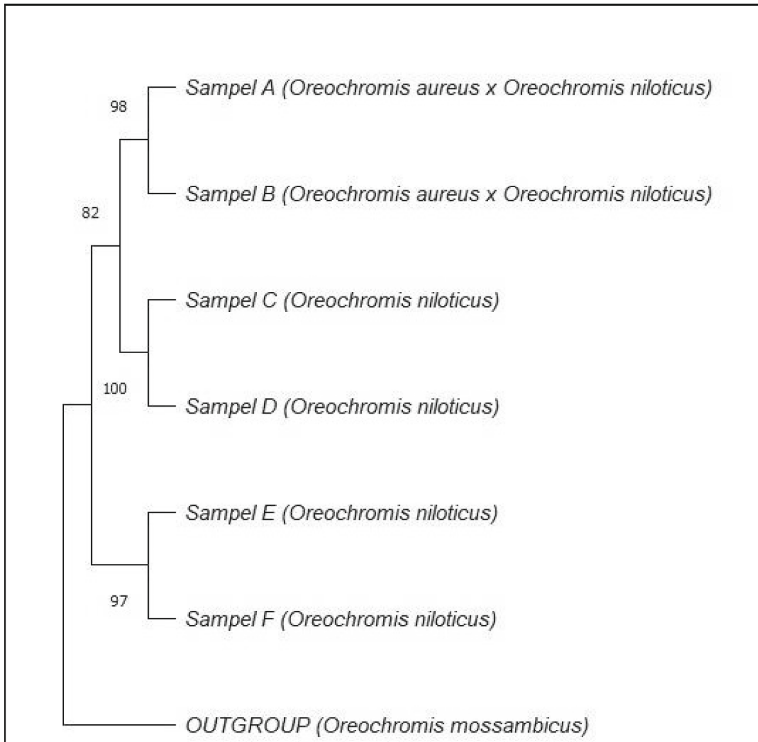

Fig. 6. Phylogenetic tree using Maximum Parsimony Method, Bootstrap 1000x

therefore *Oreochromis niloticus* (black tilapia) and *Oreochromis niloticus x Oreochromis aureus* (Srikandi tilapia).

4 Conclusion

Using molecular identification, it was determined that the tilapia in the floating net cage (KJA) belonged to two distinct species, namely *Oreochromis aureus x Oreochromis niloticus*, also known as Srikandi Tilapia, and *Oreochromis niloticus* species, which had a homology value of 99–100% with species in GenBank. The body color of the Ore-och*romis aureus x Oreochromisniloticus* species, which is darker than the *Oreochromis niloticus* species, distinguishes the two species.

The results of tilapia's molecular identification in the wild, however, revealed that it still belonged to the same species, *Oreochromis niloticus*. These results were acquired using BLAST analysis, and a similarity score of 98.99 to 99.27% was used to compare them to the NCBI GenBank. Despite belonging to the same species, the three fish samples had different fish bodies. These variations include the presence of a green hue on the fish's head and body and a red hue on the tip of its fin.

The findings of water quality measurements are still quite good in terms of biota survival in the waters. Waters' pH value is 7, DO level is between 11.8 mg/L and 13.3

mg/L, BOD level is between 1.8 mg/L and 3.1 mg/L, ammonia concentration is less than 0.15, nitrate concentration is 0 mg/L, and nitrate in waters ranges from 25 mg/L to 50 mg/L, according to observations of temperature parameters.

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