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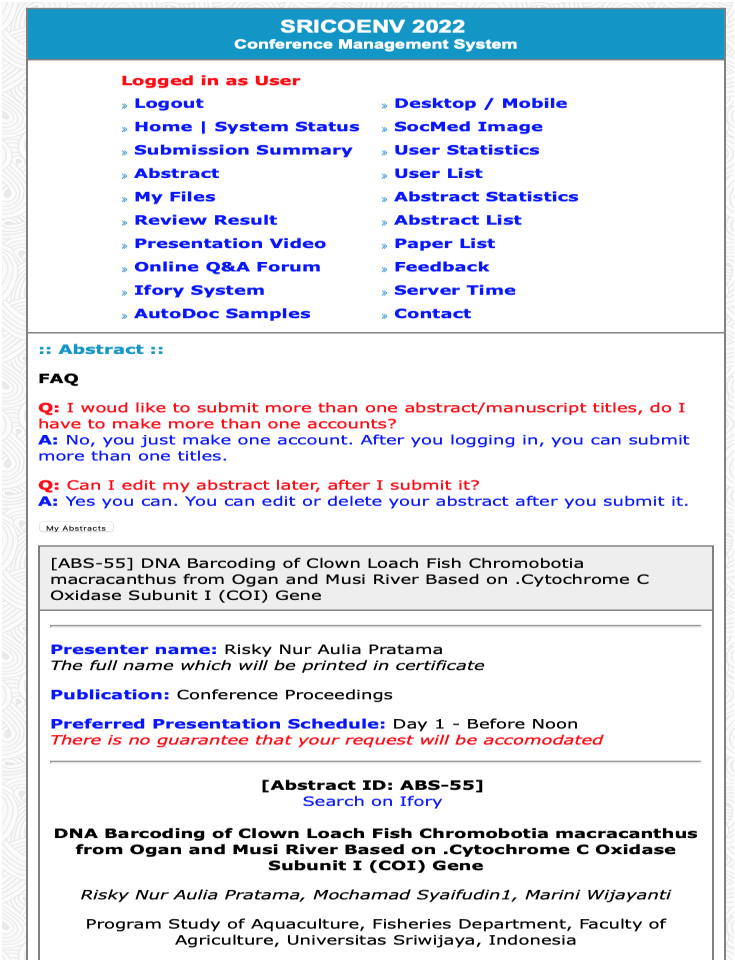
Penulis	: Risky Nur Aulia Pratama, Mochamad Syaifudin[1], Marini Wijayanti



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DNA Barcoding of Clown Loach Fish Chromobotia macracanthus from Ogan and Musi River Based on .Cytochrome C Oxidase Subunit I (COI) Gene

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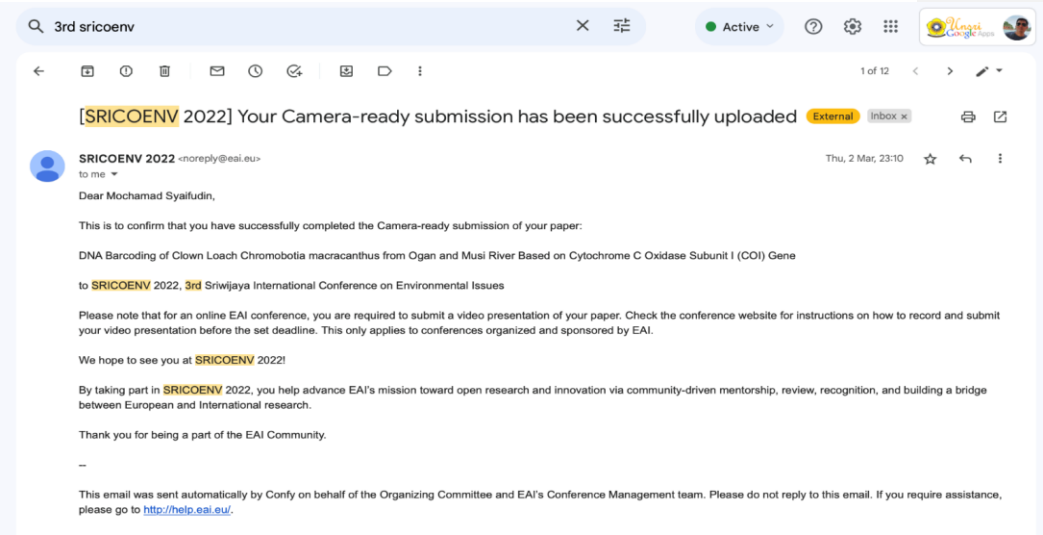

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DNA *Barcoding* of Clown Loach Fish *Chromobotia macracanthus* from Ogan and Musi River Based on Cytochrome C Oxidase Subunit I (COI) Gene

Risky Nur Aulia Pratama, Mochamad Syaifudin[1], Marini Wijayanti

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ABSTRACT. Clown loach *Chromobotia macracanthus*, or tiger botia is a type of ornamental fish which is categorized as the Cobtidae tribe. Tiger botia in Indonesia inhabit in Batanghari Jambi, and the Musi-Ogan watershed of South Sumatra. Genetic studies of tiger botia from Sumatra with DNA barcoding are needed as genetic conservation. This study aims to determine the nucleotide sequence of COI gene, genetic distance and construct phylogenetic of tiger botia. These research steps were DNA isolation, DNA amplification using PCR, and sequencing of the COI gene. The results indicated that the size of DNA band was 638 bp. The BLASTn showed that LK 2,3,4,5 and SK 1,2,3,5 had the highest similarity 98.80% with Indian *C. macracanthus*, except on LK 3 which had 98.11%. The genetic distance indicated 0.000 and the phylogenetics tree had a bootstrap value of 98% againts the same genus of *C. macracanthus*. The water quality parameters from 2 rivers i.e, temperature ranged from 29.8-31.1°C, pH 5.50-7.20, dissolved oxygen 5.47-6.76 mg L^{-1}, ammonia 0.0189-0.2115 mg L^{-1}, TDS 0.012- 0.172 mg L^{-1}, salinity 0.5 ppt, total alkalinity 8-18 ppm, water transparency 20-38.5 cm, and current velocity 2.9-10.2 cm s^{-1}.

Key words : Clown loach fish, COI gene, phylogenetic

ABSTRACT. The clown loach Chromobotia macracanthus, also known as the tiger loach, is a species of ornamental fish included in the tribe Cobtidae. The tiger loach is native to Indonesia in Batanghari Jambi and the Musi-Ogan watershed in southern Sumatra. Genetic studies of tiger loach from Sumatra with DNA barcoding are needed for genetic conservation. The objective of this study is to determine the nucleotide sequence of the COI gene, determine the genetic distance, and provide a phylogenetic representation of Tigerbotia. These research steps were DNA isolation, DNA amplification by PCR, and sequencing of the COI gene. The results showed that the DNA band size was 638 bp. BLASTn showed that LK 2,3,4,5 and SK 1,2,3,5 had the highest similarity of 98.80% with Indian C. macracanthus, except for LK 3, which had 98.11%. The genetic distance was 0.000 and the phylogenetic tree had a bootstrap value of 98% against the same genus of C. macracanthus. The water quality parameters of 2 rivers, i.e., temperature in the range of 29.8-31.1°C, pH 5.50-7.20, dissolved oxygen 5.47-6.76 mg L-1, ammonia 0.0189-0.2115 mg L-1, TDS 0.012- 0.172 mg L-1, salinity 0.5 ppt, total alkalinity 8-18 ppm, water transparency 20-38.5 cm and flow velocity 2.9-10.2 cm s-1.

Keywords: loach, COI gene, phylogenetic

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1. Introduction

The Musi River has several tributary branches that pass through several areas in South Sumatra, one of the tributaries is the Ogan watershed which is located in Ogan Ilir Regency, Indralaya district, and passes through several villages of Indralaya Mulya, Tanjung Seteko and Lubuk Keliat [9]. The potential for fish diversity in the freshwater of South Sumatra is very high. According to Iqbal [10] along the Musi river area and the East Coast of South Sumatra, 620 species of fish have been found and identified.

The Musi has several tributaries that flow through several areas in southern Sumatra. One of the tributaries is the Ogan watershed, which is located in Ogan Ilir district, Indralaya, and flows through several villages of Indralaya Mulya, Tanjung Seteko and Lubuk Keliat [9]. The potential for fish diversity in the freshwater of South Sumatra is very high. According to Iqbal [10], 620 fish species have been found and identified in the Musi River basin and the east coast of South Sumatra.

The high activity in the Musi river makes the fish go to the tributaries and swamps and occurs almost every season or every day [12]. The tributary area and lebak swamp are nursery ground for some fish such as baung asian redtail catfish, Channidae and others [11]. According to Robin [17] Musi and Ogan watersheds there are endemic fish from South Sumatra, namely clown fish (*Chromobotia macracanthus)*. The high demand from within and outside the country, makes fishing of tiger botia in the wild still common in the waters. Overfishing can lead to extinction and endanger the sustainability of the fish [7].

The high activity in the Musi River causes fish to migrate to the tributaries and marshes, which happens almost every season or every day [12]. The tributary area and Lebak swamp are nursery areas for some fishes such as Asian redtail catfish, Channidae and others [11]. According to Robin [17], there are endemic fishes from South Sumatra in Musi and Ogan watersheds, namely clownfish (Chromobotia macracanthus). The high demand from both domestic and foreign countries means that catching tiger botia in the wild is still common in the waters. Overfishing can lead to extinction and threaten the sustainability of the fish [7].

Genetic conservation efforts can be initiated by knowing the genetic characteristics of tiger botia by analyzing their mitochondrial DNA, where the mtDNA can provide biologically informed in identification, taxonomic classification, and determining the distribution of species populations [9] Commonly used species identification is by morphological approach and species characteristics [16]. However, this technique has the disadvantage of inaccurate results and overlaps some species characters of nearby taxa [15]. Therefore, identification at the molecular level is needed as an effort to determine the genetics of species and the conservation of genetic resources through the DNA b*arcoding* technique using the COI (Cytochrome C Oxidase gene Subunit I). This technique can be widely applied to all live stadia of fish from egg to adult fish [6]. Research on the DNA barcoding of tiger botia of Indian waters has been carried out by Panpromin [13], which resulted in a nucleotide length of DNA of 636 bp. In addition, it was also carried out on botia fish on the islands of Sumatra and Kalimantan, but using the cytochrome B mtDNA gene and the RAG 2 DNA nucleus, and differences in genetic characteristics and physiology were found between both populations [8]. It is necessary to use DNA Barcoding efforts aimed to know the percentage of similarity of tiger botia to the molecular level, to investigate genetic distance and phylogenetic with other species in *the Genebank* data center, and to know the chemical physics of tiger botia fish habitat waters in Sumatra.

Genetic conservation measures can be initiated by determining the genetic characteristics of Tigerbotia through the analysis of its mitochondrial DNA, where mtDNA can provide biological information for identification, taxonomic classification, and determining the distribution of species populations [9].The usual species identification is done through morphological approach and species characteristics [16]. However, this technique has the disadvantage of providing inaccurate results and overlapping some species characteristics of neighboring taxa [15]. Therefore, identification at the

molecular level is required to determine species genetics and conservation of genetic resources by the DNA barcoding technique using the cytochrome c oxidase subunit I (COI) gene. This technique can be widely applied to all living stages of fish, from eggs to adults [6]. Panpromin [13] studied DNA barcoding of tiger cichlids in Indian waters, resulting in a nucleotide length of DNA of 636 bp. In addition, it was also performed in Botia fish in Sumatra and Kalimantan islands, but using the cytochrome B mtDNA gene and the RAG 2 DNA core, and differences in genetic characteristics and physiology were found between the two populations [8]. It is necessary to use DNA barcoding to determine the percentage degree of similarity of Tigerbotia at the molecular level, to investigate the genetic distance and phylogenetic relationship with other species in the Genebank data center, and to know the chemical physics of Tigerbotia fish habitat waters in Sumatra.

2. Materials and Methods

2.1. Materials

This research was conducted at the Plant Physiology Laboratory, Program Study of Agronomy, Faculty of Agriculture, and the Biotechnology Laboratory at Faculty of Medicine, Universitas Sriwijaya frm October 2021 to January 2022. The materials used in this study were botia fish (*Chromobotia macracanthus*) from Ogan River (n=5), Lubuk Kelliat Village, Indralaya District, Ogan Ilir Regency (3°27'19,801"S - 104°42'7,819"E) and Musi River (n=5) at Serasan Jaya Village, Sekayu Subdistrict, Musi Banyuasin Regency, South Sumatra Province (2°53′39,069"S - 103°50′22,548"E.). The map of the sampling location is presented in Figure 1.

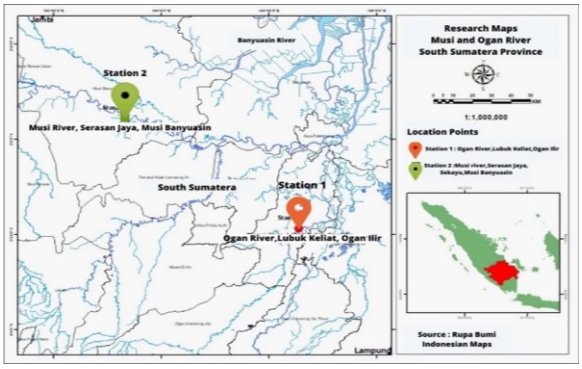

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Figure 1. sampling locations in the Ogan and Musi River

The fish samples were taken 5 individuals each river in a living condition using bubu and nets at juvenile stage with size of 8-15 cm. Water samples were taken three replications at each location. The fins clip were taken as much as 2 mm^2 and stored in a 96% ethanol solution, then labeled with code

SK for tiger botia from the Musi River and LK for tiger botia fish origin from Ogan River. Then, it stored at a temperature of -18 °C until DNA isolation is carried out.

Fish samples were collected using bubu and nets at the juvenile stage with a size of 8-15 cm from 5 individuals per river. Water samples were collected in three replicates at each site. From the fins, 2 mm2 were separated and preserved in a 96% ethanol solution. They were then labeled with the code SK for Tigerbotia from the Musi River and LK for Tigerbotia from the Ogan River. Then it was stored at a temperature of -18 °C until DNA isolation was performed.

2.2. DNA isolation

Fin samples were extracted following the method found in the manual *book* using a mini genome DNA isolation kit for animal tissue *(Geneaid Biotech Ltd)*. In general, DNA isolation consists of several stages, namely DNA sample preparation, cell lysis, RNAse treatment, DNA precipitation, DNA leaching and dissolution. Then the DNA sample is stored in a freezer (-18ºC).

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2.3. DNA amplification

PCR *(Polymerase Chain Reaction)* was conducted in a final volume of 50 µl. Each reaction contained 14 µl of aquabidest, 25 µl *of* MyTaq Polymerase red mix, 2 µl of Fish R2, 2 µl of Fish F2 and 7µl of DNA template. DNA amplification was carried out by stages: initiation cycle at 94°C for 2 minutes, denaturation at 94°C for 30 seconds, annealing or primary attachment at temperature 51°C for 1 minute, extension or elongation of 72°C for 1 minute in 35 cycles and final extension at 72°C for 10 minutes. Amplicon was visualized through gel electrophoresis.

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2.3. Electrophoresis

The 1% agarose gel is dissolved in a microwave-heated 1x TAE (*Tris-Acetate* EDTA) Buffer, then poured in a mold until it forms a well. DNA sample and a 1 kb marker in the pipette into the pipette well as much as 5 µl. Running electrophoresis for 30 minutes at a voltage of 100 volts, the electrophoresis agarose gel is soaked in diamond dye solution for 25 minutes. The DNA genome dan size was visualized with gel documentation.

Dissolve the 1% agarose gel in a microwave-heated 1x TAE (Tris-acetate-EDTA) buffer and then pour it into a mold until it forms a well. The DNA sample and a 1 kb marker are pipetted into the well (5 µl). Electrophoresis is performed for 30 minutes at a voltage of 100 volts,and the electrophoresis agarose gel is soaked in diamond dye solution for 25 minutes. The size of the DNA genome was visualized with the gel documentation.

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2.4. COI Gene Sequencing

Amplicon is labeled according to the joint research code of primers F2 (20 µl) and R2 (20 µl) on 0.5 ml tubes, then packaged and sent for sequencing in Singapore through the services of the Institute of Genetics Science in Jakarta.

The amplicon is labeled according to the common research code of primers F2 (20 µl) and

R2 (20 µl) in 0.5-ml tubes, then packaged and sent to Singapore for sequencing via the Institute of Genetics Science in Jakarta.

2.5. Water Quality

Water quality measurements were carried out included measurements of temperature (ºC), pH, dissolved oxygen (mg $^{L-1}$) brightness (cm), ammonia (mg $^{L-1}$), total alkalinity (mg $^{L-1}$), total dissolved solids (TDS), salinity (ppt), and current velocity (cm $^{s-1}$).

Water quality measurements included temperature (ºC), pH, dissolved oxygen (mg L-1), brightness (cm), ammonia (mg L-1), total alkalinity (mg L-1), total dissolved solids (TDS), salinity (ppt), and flow velocity (cm s-1).

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2.6. Data Analysis

The COI sequence obtained in the fasta format, then they were aligned using MEGA 11.0 software. The sequences were analyze BLAST (Basic Local Alignment Search Tool) which useful for determining the homology of a DNA sequence or amino acids with data contained in the NCBI (National Center for Biotechnology Information) Genbank and barcode of life. Furthermore, the phylogenetic trees of tiger botia were constructed using the Neighbor Joining (NJ) method and genetic distances analyzed using the Pairwaise Distance method. Water quality data was analyzed descriptively and supported by supporting literature.

The COI sequence was obtained in Fasta format, then aligned with MEGA 11.0 software. The sequences were analyzed BLAST (Basic Local Alignment Search Tool), which is useful for determining the homology of a DNA sequence or amino acids with data contained in the NCBI (National Center for Biotechnology Information) genebank and barcode of life. In addition, phylogenetic trees of Tigerbotia were constructed using the Neighbor Joining method (NJ) and genetic distances were analyzed using the Pairwaise Distance method. Water quality data were analyzed descriptively and supported by relevant literature.

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3.Results and Discussion

3.1. DNA Electrophoresis and Genetic Distance

DNA bands that were successfully amplified can be visualized in Figure 2.

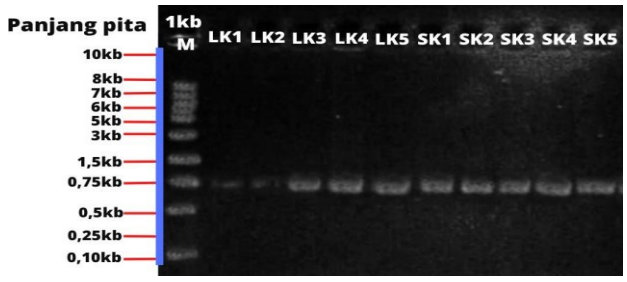

Figure 2. DNA bands of tiger botia from Ogan and Musi Banyuasin Rivers
LK 1-5: DNA band of tiger botia from the Ogan River. SK 1-5: DNA bands of tiger botia from the Musi River.

Fish DNA band of *C. macracanthus* from the Ogan and Musi River was 638 bp with an annealing temperature of PCR was 51°C. FishF2 and FishR2 primers can amplify DNA products ranging from 650 -750 bp, with the melting temperature *(Tm)* points of FishF2 and FishR2 being 54.5°C and 59.6°C [21]. The genetic distance between tiger botia to BLASTn species can be seen in Table 1.

The fish DNA band of C. macracanthus from the Ogan and Musi rivers was 638 bp at an annealing PCR temperature of 51°C.The primers FishF2 and FishR2 can amplify DNA products in the range of 650-750 bp, and the melting temperature (Tm) of FishF2 and FishR2 is 54.5°C and 59.6°C, respectively [21]. The genetic distance between Tigerbotia and BLASTn species is shown in Table 1.

Table 1. The genetic distance of tiger botia

No	Species	Genetic distances																					
1	4394345_LK3_ *C.macracanthus* _LUBUK_KELIAT																						
2	4394347_LK4_ *C.macracanthus* _LUBUK_KELIAT	0,004																					
3	4394349_Lk5_ *C.macracanthus* _LUBUK_KELIAT	0,004	0,000																				
4	4394351_LK1_ *C.macracanthus* _LUBUK_KELIAT	0,004	0,000	0,000																			
5	4394353_SK1_ *C.macracanthus* _SEKAYU	0,004	0,000	0,000	0,000																		
6	4394355_SK2_ *C.macracanthus* _SEKAYU	0,004	0,000	0,000	0,000	0,000																	
7	4394357_SK3_ *C.macracanthus* _SEKAYU	0,004	0,000	0,000	0,000	0,000	0,000																
8	4394359_SK5_ *C.macracanthus* _SEKAYU	0,004	0,000	0,000	0,000	0,000	0,000	0,000															
9	KF738204.1_*C. macracanthus*_India	0,004	0,000	0,000	0,000	0,000	0,000	0,000	0,000														
10	KU568785.1_*C.macracanthus*_South Africa	0,078	0,073	0,073	0,073	0,073	0,073	0,073	0,073	0,073													
11	MG018973_*B. striata*_India	0,178	0,181	0,181	0,181	0,181	0,181	0,181	0,181	0,181	0,181												
12	JQ667507_*B. almorhae*_India	0,198	0,199	0,199	0,199	0199	0,199	0,199	0,199	0,199	0,186	0,118											
13	MK959524.1_*B.rostrata*_India	0,177	0,180	0,180	0,180	0,180	0,180	0,180	0,180	0,180	0,172	0,078	0,095										
14	KT781503_*B. dario*_India_(Bengal)	0,194	0,195	0,195	0,195	0,195	0,195	0,195	0,195	0,195	0,182	0,115	0,002	0,092									
15	MG238207.1_*T. brevicaudo*_China	0,256	0,251	0,251	0,251	0,251	0,251	0,251	0,251	0,251	0,265	0,249	0,265	0,266	0,260								
16	KU558013.1_*T. nujiangsea*_Tibet_(China)	0,248	0,244	0,244	0,244	0,244	0,244	0,244	0,244	0,244	0,251	0,225	0,254	0,244	0,249	0,026							
17	MW652525_*C. aeruginos*_ South Korea	0,218	0,214	0,214	0,214	0,214	0,214	0,214	0,214	0,214	0,210	0,267	0,236	0,257	0,240	0,266	0,238						
18	MG238133_*N. longistratus*_China	0,215	0,214	0,214	0,214	0,214	0,214	0,214	0,214	0,214	0,218	0,246	0,256	0,223	0,251	0,193	0,190	0,217					
19	MN342424.1_*Microrasbora*_Myanmar	0,233	0,229	0,229	0,229	0,229	0,229	0,229	0,229	0,229	0,245	0,249	0,248	0,262	0,252	0,251	0,240	0,214	0,256				
20	OK021579.1_*C.chaguntio*_Bangladesh	0,232	0,228	0,228	0,228	0,228	0,228	0,228	0,228	0,228	0,239	0,300	0,279	0,300	0,284	0,288	0,262	0,230	0,275	0,225			
21	MT738912_*S. saranz*_Sri Lanka	0,229	0,221	0,221	0,221	0,221	0,221	0,221	0,221	0,221	0,219	0,253	0,232	0,244	0,236	0,255	0,234	0,185	0,235	0,240	0,233		
22	KM438538.1_*O.niloticus*_Stirling Collection	0,368	0,374	0,374	0,374	0,374	0,374	0,374	0,374	0,374	0,378	0,325	0,364	0,345	0,358	0,348	0,319	0,358	0,298	0,329	0,288	0,376	
23	KP856792.1_*O. niloticus*_Indonesia	0,368	0,374	0,374	0,374	0,374	0,374	0,374	0,374	0,374	0,374	0,325	0,364	0,345	0,358	0,348	0,319	0,358	0,298	0,333	0,288	0,372	0,002

Table 1. denoted that samples of LK 1,2,4 and SK 1,2,3,5 had a genetic distance of 0.000 except LK 3 of 0.004 against *C. macracanthus* (India) while for *C macracanthus* (South Africa) was 0.078 (LK 3) and 0.073 (LK 1, 2, 4 and SK 1,2,3,4). According to Boonkusol and Tongbai [2] an individual was classified to be one species or has a close kinship if it has a genetic distance value of 0.000-0.034. Differences in the genetic distance of the same species in one genus can occur due to the presence of geneflows and geographical isolation, as a pattern of development and evolution of the arrangement genetic material [14].

C. macracanthus from Ogan and Musi River had genetic distances to non-genus botia fish which include *B. striata* (India) 0.181, *B. almorhae* (India) 0.199, *B. rostrata* (India) 0.180*, B. dario* (India) 0.195*, T. brevicuda* (China) 0.251, *T. nujiangsea* (China) 0.244, and *C. aeruginos* (South Korea) 0.214, and *N. longistratus* (China) 0.214. While the genetic distance for non-family species

consisting of *microrasbora* (Myanmar) is 0.229, *C. chagunio* (Bangladesh) 0.228, *S. means* (Sri Lanka) 0.221, *O. niloticus* (Stirling) 0.374 and *O. niloticus* (Indonesia) 0.374 against *C. macracanthus* origin of the Ogan and the Musi River. The value of genetic distance between one species and another largely determines the level of kinship and diversity of species in a community [21].

C. macracanthus from the Ogan and Musi rivers had a genetic distance from non-family fishes B. striata (India) 0.181, B. almorhae (India) 0.199, B. rostrata (India) 0.180, B. dario (India) 0.195, T. brevicuda (China) 0.251, T. nujiangsea (China) 0.244, and C. aeruginos (South Korea) 0.214, and N. longistratus (China) 0.214. In the nonfamilial species Microrasbora (Myanmar), the genetic distance is 0.229, C. chagunio (Bangladesh) 0.228, S. means (Sri Lanka) 0.221, O. niloticus (Stirling) 0.374, and O. niloticus (Indonesia) 0.374 compared to C. macracanthus from the Ogan and Musi. The value of genetic distance between one species and another largely determines the degree of relatedness and diversity of species in a community [21].

3.2. Phylogenetic Construction of Botia Fish

The phylogenetic construction was showed in Figure 3. Five clusters were constructed, two clusters are botiidae family and three non-botiidae clusters, including the *C.aeroginos* (3), *Trylophisa* (4), and *O.vniloticus* (4), and the three species that make up the outgroup are *C. chagunio, N. longistratus* and *Microrasbora.* The bootstrap value (BV) values between chromobotia species was approximately 92,00.

The phylogenetic construction is shown in Figure 3. Five clusters were formed, two clusters belonging to the Botiidae family and three clusters not belonging to the Botiidae, including C. aeroginos (3), Trylophisa (4), and O. vniloticus (4), and the three species forming the outgroup are C. chagunio, N. longistratus, and Microrasbora. The bootstrap value (BV) between Chromobotia species was about 92.00.

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The subcluster of species with the highest BV ratio was found in *B. dario* (India) and *B almorhae* (India) with a value of BV 100, *T. nujiangsea* (China) *and T brevicauda* (China) which had a BV value of 100, as well as *O. niloticus* (Striling and Indonesia). BV defines the degree of phylogenetic topology confidence of a species with another species, based on the repeat of the nucleotide sequence [20]. The higher BV value between species signifies in one branch of the line (monophyletic), as well as forming one large cluster that indicated the presence of kinship and relationships between the species of *macracanthus* (cluster *1*) and botia species (Cluster *2*) are still in the same family with a BV value of 92. In *Cluster* two, there were two sub-clusters filled by *B. striata* and *B. rostrata (*subcluster 1), with *B. almorhae* and *B. dario* (subcluster 2) which has a BV value of 99. According to Alotaibi [1] the higher the level of kinship based on BV in a species, suggests that the species has many morphological similarities, and other physical characteristics [4].

The subset of species with the highest BV ratio was found in B. dario (India) and B almorhae (India) with a value of BV 100, T. nujiangsea (China) and T brevicauda (China), which had a BV value of 100, and O. niloticus (Striling and Indonesia). BV defines the degree of phylogenetic topology confidence of a species with another species based on nucleotide sequence repeat [20]. The higher BV value between species means that they are in one branch of the lineage (monophyletic) and form a large cluster, which indicates the presence of relationship and relationships between the species of Macracanthus (cluster 1) and Botia species (cluster 2), which still belong to the same family with a BV value of 92. In cluster two, there were two sub-clusters filled by B. striata and B. rostrata (sub-cluster 1) with B. almorhae and B. dario (sub-cluster 2) with a BV value of 99. According to Alotaibi [1], a higher degree of relatedness based on BV in a species indicates that the species shares many morphological similarities and other physical characteristics [4].

Comparison between *botia* and non-botia family clusters resulted in low BV values. Species that have a low kinship level and BV value, will have little similarity morphologically, and the acid-base arrangement in the DNA of the species so that the chances of changing the cluster array are potentially higher [2].

Comparison between Botia and non-Botia family clusters revealed low BV values. Species that have low relatedness and BV value have low similarity morphologically, and acid-base arrangement in the DNA of the species, so the chances of changing the cluster arrangement are potentially higher [2].

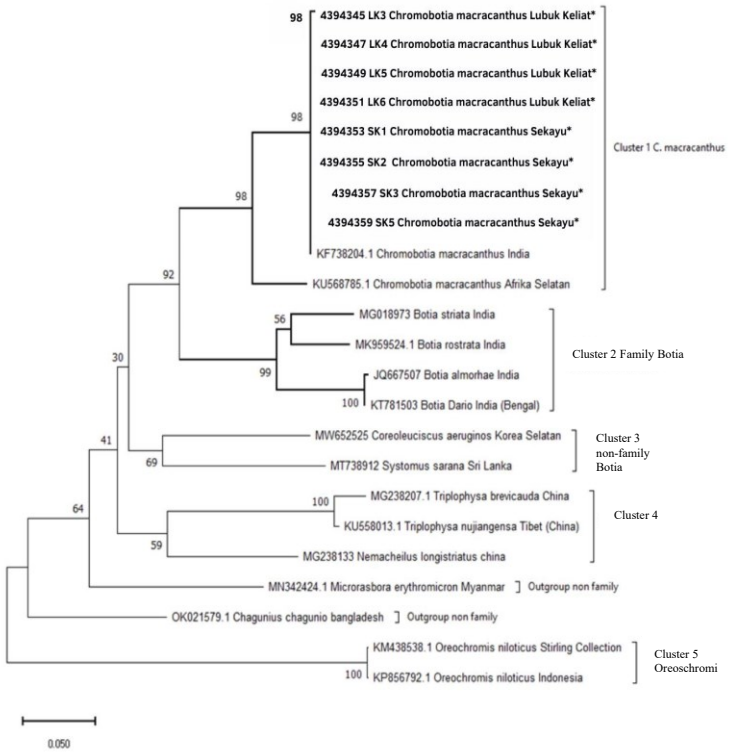

Figure 3. Phylogenetic tree of botia fish from Ogan and Musi Rivers
* : the research sample

3.3. Water Quality Parameters

The results of water quality parameters from two rivers include temperatures of 29.8-31.1 °C, pH 5.50-7.20, DO 5.47-6.76 mg L^{-1}, ammonia 0.0189-0,2115 mg L^{-1}, TDS 0.012-0.172 mg L^{-1}, salinity 0.5 ppt, total alkalinity 8—18 ppm, brightness 20.00-38.5 cm, and current velocity 2.9-10.2 cm s^{-1} which has been in accordance with the feasibility standards of the national water quality

standard government No. 22. 2021 [7]. Water quality is an external factor that can influence the development or evolution of species starting from the gene level [6]. Changes in water quality parameters that occur in a slow period can stimulate genetic mutations and gene expression, namely by changing the arrangement of nucleotides and genetic constituent materials so that it can affect the synthesis and mechanism of action of cells as part of environmental adjustment [4].

Water quality parameter results from two rivers include temperatures 29.8-31.1 °C, pH 5.50-7.20, DO 5.47-6.76 mg L-1, ammonia 0.0189-0.2115 mg L-1, TDS 0.012-0.172 mg L-1, salinity 0.5 ppt, total alkalinity 8-18 ppm, brightness 20.00-38.5 cm and flow velocity 2.9-10.2 cm s-1, which are in accordance with the feasibility standards of the national water quality standard government No. 22. 2021 [7]. Water quality is an external factor that can affect the development or evolution of species starting from the gene level [6]. Changes in water quality parameters that occur in a slow period of time can stimulate genetic mutations and gene expression, through changing the arrangement of nucleotides and genetic components, so that they can affect the synthesis and mechanism of action of cells as part of environmental adaptation [4].

4. Conclusions

Tiger botia from the Ogan and Musi Rivers had a DNA band length of 638 bp. The phylogenetic produced five clusters, two family clusters of *botiidae*, and three non-family clusters of *botiidae* with a BV value of 98 for fellow genus *C. macracanthus*.

Tiger botia from the Ogan and Musi rivers had a DNA band length of 638 bp. Phylogenetic analysis revealed five clusters, two family clusters of Botiidae and three non-family clusters of Botiidae with a BV value of 98 for the sister genus C. macracanthus.

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