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

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

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**Abstract.** The clown loach Chromobotia macracanthus, also known as the tiger botia, is a species of ornamental fish included in the tribe Cobtidae. The tiger botia is native to Indonesia in Batanghari Jambi, and the Musi-Ogan watershed in South Sumatra. Genetic studies of tiger botia from Sumatra with DNA barcoding are needed for genetic conservation. This study aims to determine the nucleotide sequence of COI gene, genetic distance and provide a 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. 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 phylogenetics tree had a bootstrap value of 98% againts 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 current velocity 2.9-10.2 cm s-1.

**Keywords:** tiger botia, COI gene, phylogenetic

## 1. Introduction

The Musi River has several tributaries that flow through several areas in South Sumatra, one of the tributaries is the Ogan watershed, which is located in Ogan Ilir Regency, 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 have been found and identified in the Musi River basin and the east coast of South Sumatra.

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 ground for some fish 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 clown

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fish (Chromobotia macracanthus). The high demand from both domestic and foreign countries, 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].

Genetic conservation measures can be initiated by determining the genetic characteristics of tiger botia through the analysis of its mitochondrial DNA, where the mtDNA can provide biological information for identification, taxonomic classification, and determining the distribution of species populations [9] Commonly, 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 the genetics of species and the conservation of genetic resources through the DNA barcoding technique using the COI (Cytochrome c oxidase subunit I) gene. This technique can be widely applied to all living stages 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 performed in loach 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 to determine the percentage of similarity of tiger botia to the molecular level, to investigate genetic distance and phylogenetic relationship with other species in the Genebank data center, and to know the chemical physics of tiger botia fish habitat waters in Sumatra.

## 2. Materials and Methods

### 2.1. Materials

This research was conducted in the Plant Physiology Laboratory, Program Study of Agronomy, Faculty of Agriculture, and the Biotechnology Laboratory, Faculty of Medicine, Universitas Sriwijaya, from October 2021 to January 2022. The materials used in this study were clown loach (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) in 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 site is presented in Figure 1.

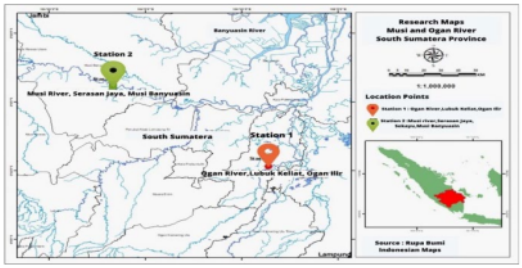


**Figure 1.** Sampling site in the Ogan and Musi River

Fish samples were collected using bubu and nets at juvenile stage with size of 8-15 cm, 5 individuals each river. Water samples were taken in three replicates at each site. The fin clips were taken as much as 2 mm2 and preserved in a 96% ethanol solution. They were then labeled with code SK for tiger botia from the Musi River and LK for tiger botia from Ogan River. Then, it was stored in a temperature of -18 °C until DNA isolation was carried out.

### 2.2. DNA isolation

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

### 2.3. DNA amplification

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

### 2.4. Electrophoresis

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

### 2.4. COI Gene Sequencing

The amplicon was labeled according to the common research code of primers F2 (20 µl) and R2 (20 µl) on 0.5 ml tubes, then packaged and sent to Singapore for sequencing in Singapore via the Institute of Genetics Science in Jakarta.

### 2.5. Water Quality

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 current velocity (cm s-1).

### 2.6. Data Analysis

The COI sequence was saved in the 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) GenBank and barcode of life. Furthermore, phylogenetic trees of tiger botia were constructed using the Neighbor Joining (NJ) method and genetic distances were analyzed using the Pairwaise Distance method. Water quality data were analyzed descriptively and supported by relevant literature.

## 3. Results and Discussion

### 3.1. DNA Electrophoresis and Genetic Distance

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

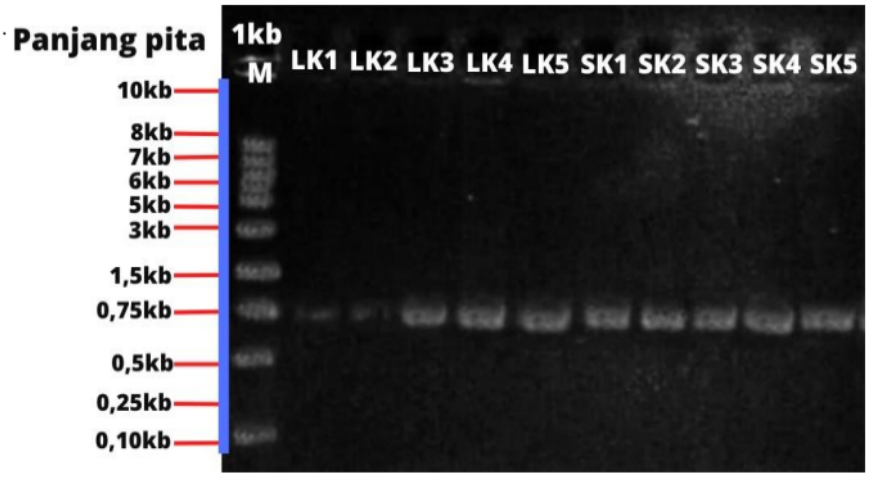


**Figure 2.** DNA bands of tiger botia from Ogan and Musi Banyuasin Rivers
LK 1-5: DNA band of tiger botia from the Ogan River. SK 1-5: DNA bands of tiger botia from the Musi River.

The fish DNA band of tiger botia from the Ogan and Musi River was 638 bp with an annealing temperature of PCR was 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 was 54.5°C and 59.6°C [21]. The genetic distance between tiger botia species was 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_LK2_ *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. brevicauda* 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 | MW852525_ *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. longistriatus* 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.chagunio* 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. zarana* 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 a genetic distances from non-genus loach 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 was 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 level of kinship and diversity of species in a community [21].

### 3.2. Phylogenetic Construction of Tiger Botia

The phylogenetic construction was showed in Figure 3. Five clusters were formed, two clusters belonging to botiidae family and three clusters non-botiidae, including the C. aeroginos (3), Trylophisa (4), and O. niloticus (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.

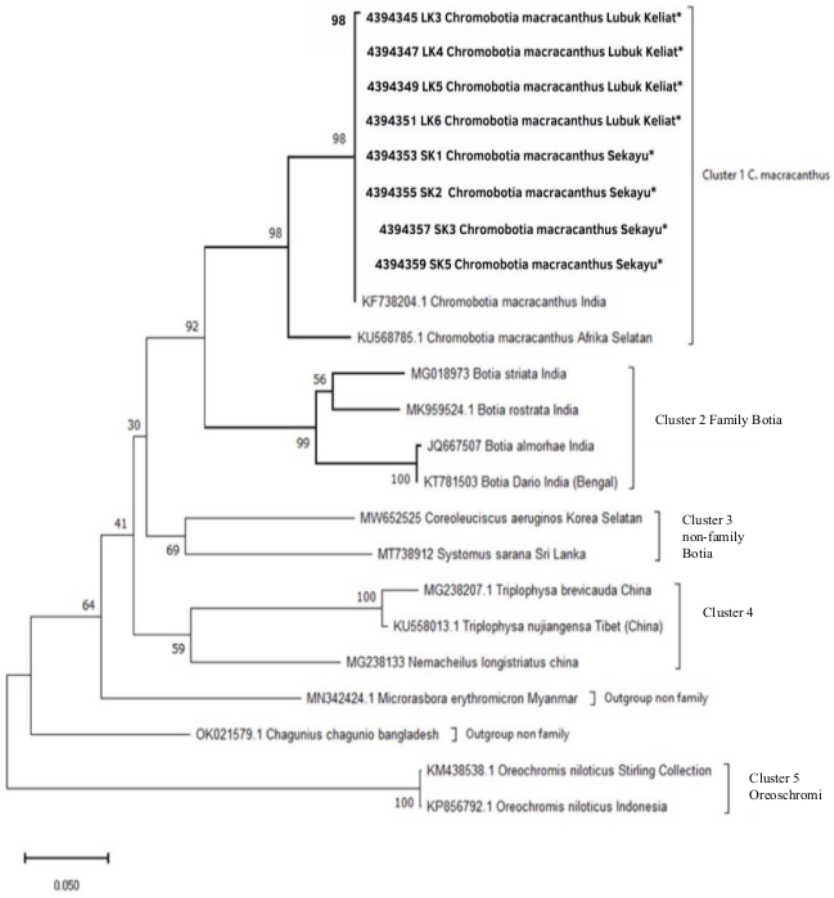


**Figure 3.** Phylogenetic tree of tiger botia from Ogan and Musi Rivers
* : the research sample

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 kinship 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 (subcluster 1), with B. almorhae and B. dario (subcluster 2) with a BV value of 99. According to Alotaibi [1] the higher degree of kinship based on BV in a species, suggests that the species has many morphological similarities, and other physical characteristics [4].

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

### 3.3. Water Quality Parameters

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 current velocity 2.9-10.2 cm s-1, which are in accordance with 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 [4].

## 4. Conclusions

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.

## 5. Acknowledgements

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