Formosa Journal of Applied Sciences (FJAS)
Vol. 4, No.2 2025: 507-514

# Biological Identification of Freshwater Local Fish in Bali Province

Sang Ayu Made Putri Suryani[1*], Dewa Nyoman Sadguna[2], Gede Sudiarta[3], Kadek Johan Okan Adnyana[4]
Manajemen Sumber Daya Perairan, Universitas Warmadewa
**Corresponding Author:** Sang Ayu Made Putri Suryani
suryanip@rocketmail.com

A R T I C L E I N F O

*Keywords:* COI, Freshwater Fish, PCR, Phylogenetic Tree, Local Fish

*Received : 05, December*
*Revised : 05, January*
*Accepted: 07, February*



A B S T R A C T

This study aims to identify the molecular identification of ornamental fish in rivers in Bali using the COI gene DNA barcode. In this study, to determine the types of local fish by the PCR method of amplifying the COI gene followed by sequencing. The sequencing results showed that the length of the nucleotides produced was 615 bp to 699 bp. There are twelve species that have the potential to be ornamental fish, namely *Rasbora lateristriata, Barbodes binotatus, Xiphophorus helleri, Sicyopterus microcephalus, Puntigrus tetrazona, Moenkhausia santaefilomenae, Hyphessobrycon eques, Poecilia reticulata, Xiphophorus maculatus, Pethia conchonius & etc*. The nucleotide base sequences of the COI gene are uploaded on BLAST to ensure their veracity and proximity to other species. The observed species found the truth that local Balinese fish have a similarity of 70-99%.

DOI prefik: https://doi.org/10.55927/fjas.v4i2.18
ISSN-E: 2962-6447
https://journal.formosapublisher.org/index.php/fjas

## INTRODUCTION

DNA barcoding is a method of identifying species using short gene sequences from an organism's genome (Kress et al., 2015). DNA barcoding was first proposed by (Hebert et al., 2003). One of the benefits of DNA Barcoding is monitoring fishing and trading activities of species that are fishery commodities in relation to conservation efforts for protected species (Kurniasih, 2024). Applications of DNA barcoding include identifying various organisms to the species level (Waugh, 2007), solving problems regarding cryptic species (Trivedi et al., 2016), as well as identifying morphologically similar species (such as fish larvae). The gene that is commonly used for DNA barcoding in animal species is the Cytochrome Oxidase subunit 1 (CO1) gene (Kochzius et al., 2010). The CO1 gene is a gene in mitochondrial DNA that functions in the respiration process (Pierron et al., 2012), namely catalyzing the reduction of oxygen to water (Waugh, 2007). The size of the CO1 gene is relatively short, namely around 650 bp (Ward et al., 2005). The CO1 gene sequence has been standardized and is suitable for use as a DNA barcode at the species level for various animal groups (Kress et al., 2015). Genetic diversity originating from the nucleotide sequence of the COI gene becomes a characteristic for fish populations with the help of DNA barcoding techniques.

DNA barcoding uses the COI gene marker because it has the advantage of more accurate identification and does not require a long time to carry out so that efforts to develop local fish species can run well (Meyer et al., 2012). A very popular identification technique is DNA barcoding. DNA barcoding is one way to identify species quickly and accurately by using standardized gene regions as species markers (Hsiao et al., 2016). The source of DNA in eukaryotic animals comes from nuclear DNA and mitochondrial DNA (Slynko et al., 2018). Genes that are often used in DNA Barcoding techniques usually use conserved genes in mitochondria, one example is the Cytochrome Oxidase subunit I (COI) gene marker. The diversity of the COI gene nucleotide sequence can be the basis for an animal DNA barcoding system in determining environmental conditions (Goodwin et al., 2017). Conservation activities begin with the provision of various information such as bioecology, taxonomy including genetic information (Nuryanto & Solihin, 2006). This information is very necessary in order to determine the appropriate conservation method. This research aims to analyze the genetic diversity of local fish as a basis for managing and conserving local fish biological resources

## LITERATURE REVIEW

The DNA barcoding method using the CO1 gene has been used in various fish species. DNA barcoding has the advantage of identifying species with a high level of accuracy compared to morphological observations (Madduppa et al., 2017; Saleky & Dailami, 2021). DNA barcoding based on Cytochrome Oxidase Subunit I (COI) gene markers is widely used in species identification and biodiversity studies (Thu et al., 2019). The COI gene is used as an effective DNA marker in fish species from different waters throughout the world (Thu et al., 2019) and has been applied to various types of fish in various regions such as

Australia, India, Asia Pacific and various regions in the world including Indonesia (Dailami et al., 2021; Ward et al., 2008).

Biodiversity is currently considered to be part of the evolutionary results of past climate and tectonic changes (Weigand & Macher, 2018). Sustainable management of barramundi fish resources requires monitoring and clarification processes related to population structure in nature through phylogenetic analysis and population genetic diversity (Nugraha & Munir, 2011). Genetic relationships between species within one population and between populations can be determined through phylogenetic reconstruction (Pramono et al., 2017; Saleky & Dailami, 2021).

## METHODOLOGY

### Sample Collection

Samples were collected from river waters in the East Bali region, namely rivers in the Karangasem region, namely the Telaga Waja river and the Tukad Daya River in the eastern Tamblang region. Fish samples were collected during the dry season from March to July 2023. Samples were collected using nets and stored in sample bottles containing 96% ethanol. Samples were analyzed at the Indonesian Biodiversity Laboratory for DNA analysis and for sequencing analysis carried out at the Jakarta Genetic Science.

### Laboratory Procedure

Tissue samples were taken (approximately 10 grams in size) then an extraction process was carried out to isolate DNA. The extraction method used is following the Chelex 10% protocol. The extraction results are then analyzed to the next stage, namely PCR (Polymerase Chain Reaction). The PCR process uses the BIONESIA laboratory protocol. The primers used in the amplification process for fish samples are FISH Forward (5'-TCA ACC AAC CAC AAA GAC ATT GGC AC-3') and FISH Reverse (5'-TAG ACT TCT GGG TGG CCA AAG AAT CA-3') ( Ward et al., 2005). The reaction mixture was then amplified using an Applied Biosystems™ 2720 Thermal Cycler machine. The PCR results were then visualized with electrophoresis on a 1% Agarose gel with Nucleic Acid Gel Stain (GelRed®) staining. Samples that were positive (illuminated DNA bands) were then subjected to a DNA reading (sequencing) process using the Sanger method and were detected at PT. Genetic Science Jakarta.

### Data analysis

The results of samples that have been sequenced are analyzed using a computer. The sequence data obtained was then edited and aligned using the ClustalW method in the MEGA 7 program. Each base arrangement was then checked manually and it was ensured that all data used was of good quality. Data that has poor sequencing results is then subjected to PCR and re-sequencing. The data was then matched with the database in the Genbank NCBI using the BLAST method on the NCBI website (https://blast.ncbi.nlm.nih.gov/Blast.cgi).

Each data is then recorded for the level of similarity and accuracy of the data. Apart from using the Basic Local Alignment Search Tool method, the data was then analyzed using a phylogenetic tree to see the relationship between samples as well as confirming the Basic Local Alignment Search Tool results in identification up to the species level. The kinship tree was created using the Neighbor-joining (NJ) method with bootstrap replication 1000 times in MEGA 7 software. Genetic distance values were analyzed using the p-distance method.

## RESEARCH RESULT AND DISSCUSION

### Polimerase Chain Reaction

Validation of the COI gene using Basic Local Alignment search Tools (BLAST) obtained a value of 100% (table 1). This shows that species identification using DNA barcoding is very suitable, thus strengthening that the taxonomically correct identification. Molecular identification using the COI gene sequence has been confirmed to be correct and close to other species based on uploading to BLAST on the NCBI website. The ability of DNA barcoding techniques to distinguish species can be seen by phylogenetic tree analysis which is carried out after determining the nucleotide sequence to show the closeness between example species.

**Table 1. Barcode Results Using BLAST from Samples Using FISH F1/R1 Primers**

| Field ID | Lab ID | Species | bp | Gene | Accession Number | Query Cover (%) | Identity (%) |
|---|---|---|---|---|---|---|---|
| 1 | BIOSUB 209.001 | *Rasbora lateristriata* | 687 | COI | LC130775.1 | 95% | 100% |
| 2 | BIOSUB 209.002 | *Barbodes binotatus* | 694 | | MG699678.1 | 93% | 100% |
| 3 | BIOSUB 209.003 | *Barbodes binotatus* | 687 | | MG699648.1 | 94% | 100% |
| 4 | BIOSUB 209.004 | *Rasbora lateristriata* | 694 | | LC130755.1 | 94% | 100% |
| 5 | BIOSUB 209.005 | *Rasbora lateristriata* | 687 | | LC130755.1 | 95% | 100% |
| 6 | BIOSUB 209.006 | *Xiphophorus hellerii* | 694 | | KJ669651.1 | 94% | 100% |
| 7 | BIOSUB 209.007 | *Xiphophorus hellerii* | 687 | | KU693081.1 | 94% | 100% |
| 8 | BIOSUB 209.008 | *Sicyopterus microcephalus* | 692 | | KU693055.1 | 94% | 100% |

It can be seen that *Rasbora lateristriata* detected 687 to 694 bp of nucleotide bases, *Barbodes binotatus* detected 687 to 694 bp, *Xiphophorus hellerii* detected 687 to 694 bp while Sicyopterus *microcephalus* detected 692 bp (as in table 1 and figure 1). Kinship relationships using Neighbor-Joining and minimum evolution with 1000 bootstrap processing form 3 large clades with high boostrap values. The higher the boostrap value formed, the better the reconstruction of the phylogenetic tree. It was found that *Rasbora lateristriata* is close to *Barbodes binotatu*s because in the phylogenetic reconstruction it is formed in one clade and is not related to *Xiphophorus helleri* and *Sicyopterus microcephalus* (Figure 1). Phylogenetic reconstruction shows connectivity between species.

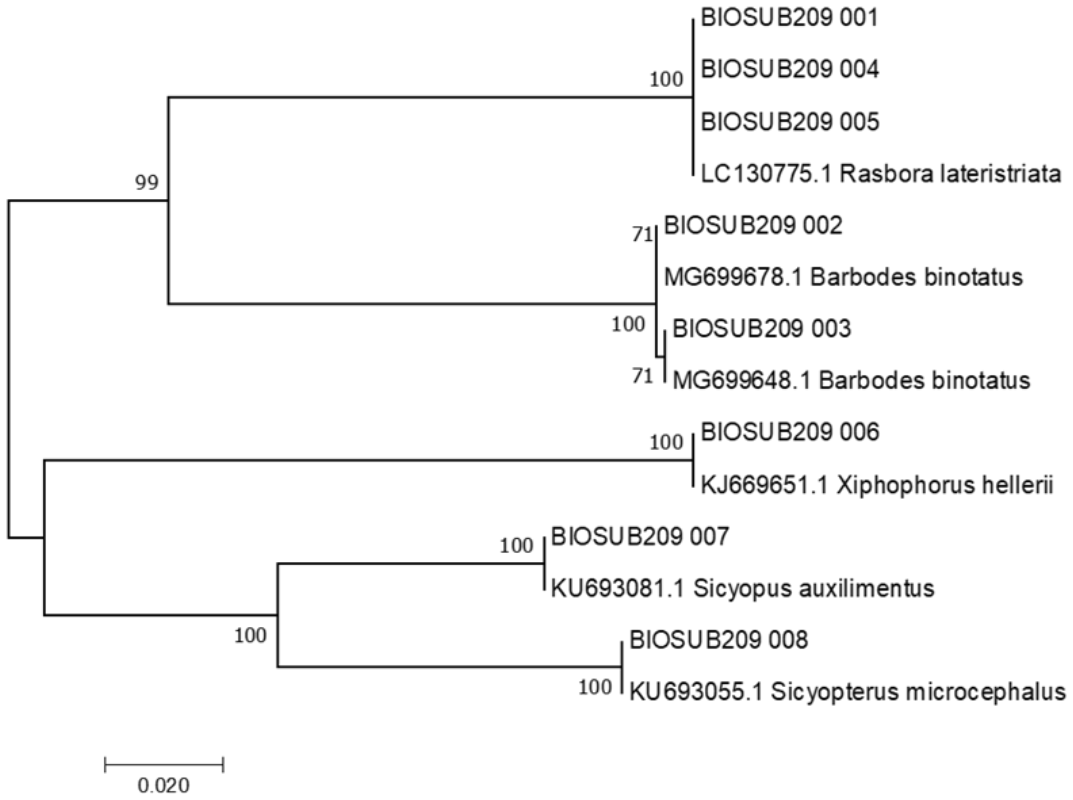


**Figure 1. Phylogenetic Results of Local Fish from East Bali using FISH F1 / FISH RI Primers**

## CONCLUSIONS AND RECOMMENDATIONS

DNA barcoding analysis obtained from local fish species, *Rasbora lateristriata* detected 687 to 694 bp of nucleotide bases, *Barbodes binotatus* detected 687 to 694 bp, *Xiphophorus hellerii* detected 687 to 694 bp while *Sicyopterus microcephalus* detected 692 bp. Kinship relationships using Neighbor-Joining and minimum evolution with bootstrap processing form 3 clades with a value of 100. Where *Rasbora lateristriata* is close to *Barbodes binotatus* and is not related to *Xiphophorus helleri* and *Sicyopterus microcephalus*.

**ADVANCED RESEARCH**

Still conduct further research to find out more about the Biological Identification of Freshwater Local Fish in Bali Province.

**ACKNOWLEDGMENT**

Thank you to the honorable Head of DPPM (Direktorat Penelitian dan Pengabdian Kepada Masyarakat) Warmadewa University who has already funded this research.

**REFERENCES**

Dailami, M., Rahmawati, A., Saleky, D., & Toha, A. H. A. (2021). DNA barcoding of tilapia fish from Merauke, Papua and Malang, East Java-Indonesia. Aquaculture, Aquarium, Conservation & Legislation, 14(2), 849–858.

Goodwin, K. D., Thompson, L. R., Duarte, B., Kahlke, T., Thompson, A. R., Marques, J. C., & Caçador, I. (2017). DNA sequencing as a tool to monitor marine ecological status. Frontiers in Marine Science, 4, 107.

Hebert, P. D. N., Cywinska, A., Ball, S. L., & DeWaard, J. R. (2003). Biological identifications through DNA barcodes. Proceedings of the Royal Society of London. Series B: Biological Sciences, 270(1512), 313–321.

Hsiao, S.-T., Chuang, S.-C., Chen, K.-S., Ho, P.-H., Wu, C.-L., & Chen, C. A. (2016). DNA barcoding reveals that the common cupped oyster in Taiwan is the Portuguese oyster Crassostrea angulata (Ostreoida; Ostreidae), not C. gigas. Scientific Reports, 6(1), 34057.

Kochzius, M., Seidel, C., Antoniou, A., Botla, S. K., Campo, D., Cariani, A., Vazquez, E. G., Hauschild, J., Hervet, C., & Hjörleifsdottir, S. (2010). Identifying fishes through DNA barcodes and microarrays. PloS One, 5(9), e12620.

Kress, W. J., García-Robledo, C., Uriarte, M., & Erickson, D. L. (2015). DNA barcodes for ecology, evolution, and conservation. Trends in Ecology & Evolution, 30(1), 25–35.

Kurniasih, T. (2024). Comparative analysis of morphometric characters and genetic diversity of superior Nile tilapia, Tilapia niloticus, populations in Indonesia. AACL Bioflux, 17(6).

Madduppa, H., Subhan, B., ANGGRAINI, N. P., FADILLAH, R., & TARMAN, K. (2017). DNA barcoding reveals vulnerable and not evaluated species of sea cucumbers (Holothuroidea and Stichopodidae) from Kepulauan Seribu reefs, Indonesia. Biodiversitas Journal of Biological Diversity, 18(3), 893–898.

Meyer, M., Kircher, M., Gansauge, M.-T., Li, H., Racimo, F., Mallick, S., Schraiber, J. G., Jay, F., Prüfer, K., & De Filippo, C. (2012). A high-coverage genome sequence from an archaic Denisovan individual. Science, 338(6104), 222–226.

Nugraha, M. P., & Munir, R. (2011). Pengembangan Aplikasi QR Code Generator dan QR Code Reader dari Data Berbentuk Image. Informatics National Conference, 148–149.

Nuryanto, A., & Solihin, D. D. (2006). Variasi sekuens gen mitokondrial sitokrom c oksidase I dari siput lola (Trochus niloticus). Majalah Ilmiah Biologi BIOSFERA: A Scientific Journal, 23(1), 31–37.

Pierron, G., Tirode, F., Lucchesi, C., Reynaud, S., Ballet, S., Cohen-Gogo, S., Perrin, V., Coindre, J.-M., & Delattre, O. (2012). A new subtype of bone sarcoma defined by BCOR-CCNB3 gene fusion. Nature Genetics, 44(4), 461–466.

Pramono, T. B., Arfiati, D. A., Widodo, M. S., & Yanuhar, U. Y. (2017). Identifikasi ikan Genus Mystus dengan pendekatan genetik. Jurnal Sumberdaya Akuatik Indopasifik, 1(2), 123–132.

Saleky, D., & Dailami, M. (2021). Konservasi genetik ikan kakap putih (Lates calcarifer, Bloch, 1790) melalui pendekatan DNA barcoding dan analisis filogenetik di sungai Kumbe Merauke Papua. Jurnal Kelautan Tropis, 24(2), 141–150.

Slynko, Y. V, Slynko, E. E., Pirkova, A. V, Ladygina, L. V, & Ryabushko, V. I. (2018). Mitochondrial DNA barcoding of the pacific oyster Crassostrea gigas (Thunberg, 1793)(Mollusca: Bivalvia: Ostreidae), cultivated in the Black Sea. Russian Journal of Genetics, 54, 1445–1451.

Thu, P. T., Huang, W.-C., Chou, T.-K., Van Quan, N., Van Chien, P., Li, F., Shao, K.-T., & Liao, T.-Y. (2019). DNA barcoding of coastal ray-finned fishes in Vietnam. PloS One, 14(9), e0222631.

Trivedi, N. S., Mandavgane, S. A., Mehetre, S., & Kulkarni, B. D. (2016). Characterization and valorization of biomass ashes. Environmental Science and Pollution Research, 23, 20243–20256.

Ward, R. D., Holmes, B. H., White, W. T., & Last, P. R. (2008). DNA barcoding Australasian chondrichthyans: results and potential uses in conservation. Marine and Freshwater Research, 59(1), 57–71.

Ward, R. D., Zemlak, T. S., Innes, B. H., Last, P. R., & Hebert, P. D. N. (2005). DNA barcoding Australia's fish species. Philosophical Transactions of the Royal Society B: Biological Sciences, 360(1462), 1847–1857.

Waugh, J. (2007). DNA barcoding in animal species: progress, potential and pitfalls. BioEssays, 29(2), 188–197.

Weigand, A. M., & Macher, J.-N. (2018). A DNA metabarcoding protocol for hyporheic freshwater meiofauna: Evaluating highly degenerate COI primers and replication strategy. Metabarcoding and Metagenomics, 2, e26869.