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Molecular and Morphological Characteristics of *Redigobius tambujon* Collected from Tuweley River, Tolitoli, Central Sulawesi, Indonesia

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Abstract

The Tuweley River is an aquatic ecosystem rich in biodiversity. One of the fish species found in this river is *Redigobius*. Studies on this species' molecular and morphological aspects are very important to support conservation efforts and management of local aquatic resources. This research was conducted from September 2022 to March 2023. The specimen collection is in the Tuweley River, Tolitoli Regency, Central Sulawesi Province, Indonesia. Morphological character measurements were carried out at the Integrated Laboratory of Universitas Madako Tolitoli, while DNA (Deoxyribonucleic Acid) analysis was carried out at the Bionesia Laboratory, Bali. This study employs a quantitative descriptive approach to analyze the data. The data examined include morphological and molecular characteristics (qualitative) and quantitative data consisting of morphometric, meristic characters, and DNA analysis of *R. tambujon*. These data were tabulated in table form and analyzed descriptively to obtain a comprehensive overview of the species' characteristics. The study results showed that the fish collected in the Tuweley River were the *R. tambujon* species, based on DNA analysis and morphological character measurements.

INTRODUCTION

The Tuweley River in Tolitoli Regency, Central Sulawesi, is one of the aquatic ecosystems rich in biodiversity (Aliyas *et al*., 2023). One fish species found in this river is *Redigobius tambujon*, which belongs to the Gobiidae family. According to Larson (2010), *R. tambujon* is interesting to study because of its wide geographical distribution in the Indo-Pacific region and its potential as an indicator of the health of aquatic ecosystems. Studies on this species' molecular and morphological aspects are important to support the conservation and management of local aquatic resources.

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Morphological characters are a classic approach to identifying and classifying fish species. Fish morphology includes physical characteristics such as body shape, color patterns, fin size, and other morphometrics (Maulid and Nurilmala, 2015). This study will reveal how morphological variations of *R. tambujon* in the Tuweley River can distinguish local populations from other species or subpopulations. This information is important for understanding species' ecological adaptation to dynamic river habitats.

In addition to morphological approaches, molecular analysis offers genetic data that can provide a deeper picture of evolutionary relationships and population structure (Halisah *et al*., 2024). This study used techniques such as mitochondrial DNA analysis to identify genetic differences among *R. tambujon* individuals and detect the presence of genetic polymorphisms that reflect the genetic dynamics of populations in the Tuweley River ecosystem.

Research on the combination of morphology and molecular is very important in fish resource management. Molecular data can complement morphological data, often influenced by environmental factors such as food availability and air quality (Aisyah *et al*., 2022; Aisyah and Farhaby, 2021). Combining these two approaches can obtain a more comprehensive understanding of the taxonomic status, adaptation patterns, and appropriate conservation strategies for the *R. tambujon* species.

Consequently, this research will commence with a focused field investigation of the Tuweley River, encompassing the acquisition of precise morphological measurements and the collection of tissue specimens for subsequent molecular genetic analyses. The resultant data are anticipated to yield a substantial contribution to the ongoing conservation initiatives aimed at preserving the biodiversity within the Tolitoli region. Furthermore, this study will establish a critical foundation of baseline data, thereby facilitating and informing future investigations within the domain of aquatic biology.

METHODOLOGY

Ethical Approval

All research procedures conducted in this study were in accordance No animals were harmed or subjected to inappropriate treatment during this study. The test species, *R. tambujon*, received consistent and appropriate care for weight and length measurements throughout the observation period. All captured specimens were promptly returned to their natural habitat in good condition and without any morphological alterations, consistent with their initial state. The research procedures adhered to the ethical guidelines established by the Ethical Clearance Committee of the Faculty of Fisheries, Universitas Madako Tolitoli.

Place and Time

This research was conducted from September 2022 to March 2023, with the specimen collection location in the Tuweley River, Tolitoli Regency, Central Sulawesi, Indonesia. Morphological character measurements were carried out at the Integrated Laboratory of Universitas Madako Tolitoli, while Deoxyribonucleic Acid (DNA) analysis was carried out at the Bionesia Bali Laboratory.

Research Materials

This study employed a comprehensive suite of tools for both morphological and molecular analyses. Morphological assessments utilized standard ichthyological equipment, including snorkels and nets (20x20 cm) for specimen collection, jars for preservation, calipers (0.1 mm precision) for precise measurements, a digital camera for documentation, and an electronic balance (0.001 g resolution) for accurate weight determination. Environmental parameters were measured using a pH meter (Noyafa EZ9908, China) with an accuracy of 0.1 pH units, a thermometer (Jiawu DO9100, China) with an accuracy of 0.1°C, a DO

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meter (Jiawu DO9100, China) with an accuracy of 0.01 mg/L, a digital scale (Constant, China) with an accuracy of 0.01 g. Molecular analyses, aimed at DNA extraction and amplification, required a range of laboratory equipment. This included protective gear (gloves), labeling materials (license plates), dissection trays, various tubes (microtubes, PCR tube strips), a Bunsen burner for sterilization, micropipettes with corresponding tips for precise liquid handling, beakers, tweezers, a vortex mixer for homogenization, a heating block for controlled temperature incubation, a transilluminator for visualizing DNA, a centrifuge for sample separation, a thermal cycler for PCR amplification, an analytical balance for reagent preparation, an electrophoresis apparatus for DNA fragment separation, and microwave for agarose gel preparation.

The materials utilized in this study were carefully selected to support both morphological and molecular investigations. For morphological assessments, 70% ethanol was used as a fixative and preservative for the collected fish specimens. DNA analyses required a range of reagents, including 10% Chelex solution for DNA extraction, double-distilled water (ddH_2O) for solution preparation, 10x PCR buffer for optimal PCR conditions, magnesium chloride ($MgCl_2$) as a cofactor for Taq polymerase, CRK, and CRE primers for specific DNA target amplification, PE Amplitaq DNA polymerase for PCR, dNTPs (deoxynucleotide triphosphates) as building blocks for DNA synthesis, agarose powder for gel electrophoresis, a low mass ladder as a DNA size standard, loading dye to facilitate sample loading, and Biotium stain for DNA visualization.

Research Design

This study employs a quantitative descriptive approach to analyze the data. The data examined include morphological and molecular characteristics (qualitative) and quantitative data consisting of morphometric, meristic characters, and DNA analysis of *R. tambujon*. These data were tabulated in table form and analyzed descriptively to obtain a comprehensive overview of the species' characteristics.

Work Procedure

Fish collection

A purposive sampling method was used in the Tuweley River, Tolitoli. Specimens were caught using a 20 x 20 cm net. The caught fish were then taken to the laboratory, where they were photographed for documentation, prepared for DNA analysis, observed and measured for morphological characters, and labeled. For DNA testing, fish were prepared and preserved using 70% alcohol.

DNA analysis

Species identification was validated through two approaches: morphological observation and DNA analysis. A tissue sample of approximately 10 grams was extracted to isolate genomic DNA for DNA analysis. DNA extraction was performed using the 10% Chelex method, followed by a Polymerase Chain Reaction (PCR) to amplify the target DNA fragment. The PCR reaction was carried out following laboratory protocols and using specific primers, namely FISH F1 (5'-TCA ACC AAC CAC AAA GAC ATT GGC AC-3') and FISH R1 (5'-TAG ACT TCT GGG TGG CCA AAG AAT CA-3') (Ward *et al*., 2005), for samples coded BIOSUB188.001. Each PCR reaction had a total volume of 25 μl, consisting of 2 μl of extracted DNA template, 1.25 μl of each primer with a concentration of 10 mM, 9 μl of deionized water (ddH_2O), and 12.5 μl of Ready Mix (a ready-to-use PCR reagent mixture). DNA amplification was carried out using an Applied Biosystems™ 2720 Thermal Cycler machine with the following thermal conditions: initial denaturation at 94 °C for 3 minutes, followed by 38 cycles consisting of denaturation at 94 °C for 30 seconds, annealing at 50 °C for 30 seconds, and extension at 72 °C for 60 seconds. The last stage was a final extension at 72 °C for 2 minutes. PCR products were visualized by

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1% agarose gel electrophoresis, stained with Nucleic Acid Gel Stain (GelRed®). Samples that show DNA bands (positive) then proceed to the sequencing stage to determine the DNA base sequence.

Samples that underwent sequencing yielded sequence data in AB1 file format. The sequence data were then computationally analyzed. The analysis process included editing and alignment of sequences using the MUSCLE algorithm implemented in MEGA X software. Each aligned base residue was manually inspected to ensure data quality. Low-quality sequences were identified, and the corresponding samples underwent PCR and re-sequencing. Sequence identification was performed by comparing the obtained data with the GenBank NCBI database using the Basic Local Alignment Search Tool (BLAST) algorithm available on the NCBI website (https://blast.ncbi.nlm.nih.gov/Blast.cgi). The similarity and accuracy scores of the BLAST results were recorded for each sequence."

Observation of Morphological Characters

Morphological characterization was carried out through morphometric measurements and meristic calculations (Corpuz *et al*., 2013). Morphometric measurements related to body dimensions and proportions were carried out with precision using digital calipers with an accuracy of 0.1 mm to measure linear parameters such as total length, standard length, body height, and eye diameter. All the measurements of different morphometric characters were determined as percentages of TL. Individual body mass was measured using a digital scale with an accuracy of up to 0.001 g.

Meristic data, including the number of separate elements such as fin rays (spines and soft), scales on the lateral line, and gills, were obtained by directly counting each specimen. Morphometric and meristic data collection was carried out carefully to minimize measurement errors and accurately represent morphological variation in the studied population. The method for measuring morphometrics and meristics is shown in Figures 1, 2, and 3.

Habitat Observation

Habitat observations include water quality parameters (temperature, pH, and DO) and river water substrates. Water pH is measured using a pH meter by inserting the device into the river body, temperature is measured with a thermometer, and dissolved oxygen is measured using a DO meter. Observations of the riverbed substrate are carried out visually by observing the condition of the riverbed (Bhagawati *et al*., 2017).

Data Analysis

The observed morphological characters include morphometric and meristic characters. This study measured various morphometric parameters to identify and analyze the morphological characteristics of the specimens. Figures 1, 2, and 3 explain the measurement methods in detail. These three figures visualize the complete morphometric measurements and support the understanding of the morphological characteristics of the specimens.

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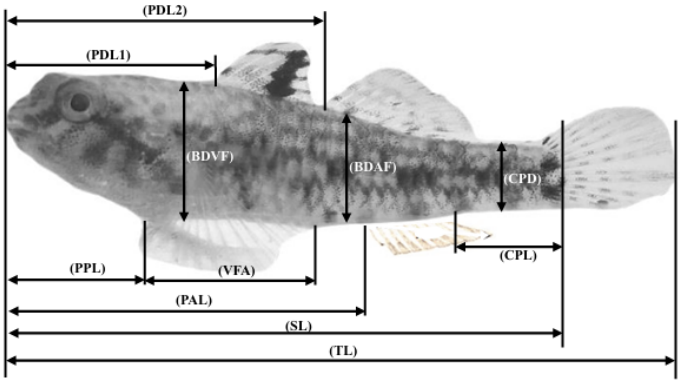

Figure 1. Total length (TL), standard length (SL), predorsal length (PDL1), 2nd dorsal fin origin (PDL2), pre-pelvic length (PPL), ventral fin to the anus (VFA), preanal length (PAL), caudal peduncle length (CPL), caudal peduncle depth (CPD), body depth at anal-fin origin (BDAF), body depth at pelvic fin origin (BDVF)

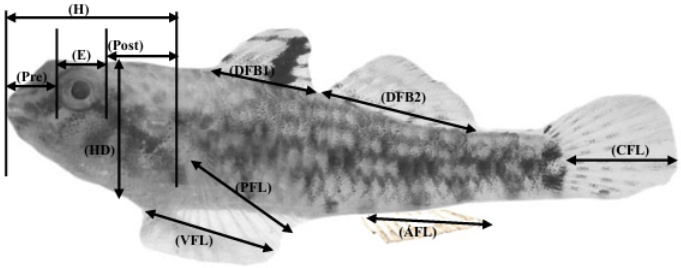

Figure 2. Dorsal fin base (DFB1), 2nd dorsal fin base (DFB2), pectoral fin length (PFL), anal fin base (AFB), ventral/pelvic fin length (VFL), dorsal fin length (DFL), anal fin length (AFL), caudal fin length (CFL), head length (H), head depth (HD), eye diameter (E), pre-orbital length (Pre), and post-orbital length (Post).

Meristic measurements are carried out by recording the number or specific patterns on the body parts of the fish as a parameter for species identification. The recorded meristic data include the number of fin rays. Figure 4 shows the meristic measurement method characteristic of the species observed. The results of these measurements are essential to support the process of fish identification and classification in taxonomic studies.

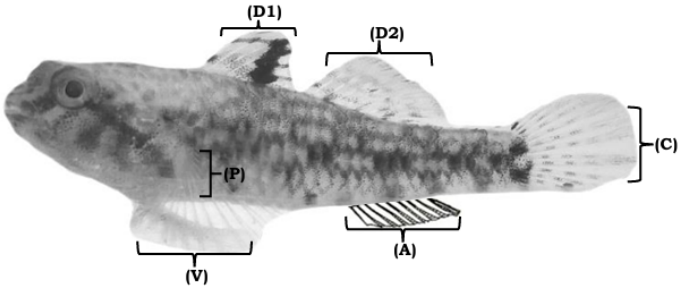

Figure 3. Dorsal 1 fin (D1); dorsal 2 fin (D2); caudal fin (C); anal fin (A); pectoral fin (P); ventral fin (V).

RESULTS AND DISCUSSIONS

In Indonesia, especially on Sulawesi Island, various species of *Redigobius* have been found, such as *Redigobius penango* and *Redigobius isognathus*, which are widely distributed in the fresh waters of Southeast Sulawesi (Popta, 1912; Tjakrawidjaja, 2006). Research has also recorded the

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presence of *Redigobius* sp. in the river waters of Central Sulawesi (Gani *et al*., 2019). The latest discovery identified Redigobius fotuno as an endemic species on Muna Island, Southeast Sulawesi (Kobayashi *et al*., 2024).

However, research on the *R. tambujon* species is still limited, especially on Sulawesi Island. According to Larson (2010), information on the presence of *R. tambujon* in Indonesia reports its discovery in Riau, Aru Island, Halmahera, Bogor, and Papua.

The results of the DNA analysis showed that the average length of the sample nucleotide sequence was 655 bp. Basic Local Alignment Search Tool (BLAST) process revealed that the sample DNA sequence was similar to the *R. tambujon* species by 97.52% (Table 1 and Figure 5). This identification provides important information in understanding fish biodiversity in the Tuweley River and supports further research on the ecology and conservation of the species.

Figure 5. *R. tambujon* in Tuweley river.

Table 1. Results of DNA barcode analysis.

Species	bp	Gene	Accession number	Identity (%)
Redigibius tambujon	655	COI	BOLD:AAY0617	97.52

Morphological Characters

R. tambujon was collected from the Tuweley River, and in addition to being identified molecularly, it was also identified through a morphological approach on 31 individuals. Morphometric (Table 2) and meristic (Table 3) analyses were conducted to validate species identification, support conservation efforts, and understand its ecology.

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Table 2. Morphometric characters and Percentage ratio of various morphometric characters with TL.

Number	Observed characters	Range (mm)	Average (mm)	Standard Deviation	Percentages
1	Weight	0,21-5,79	1,00	1,07	
2	Total length	28-53	39,97	6,32	
3	Standard length	20-44	33,16	6,08	82,97%
4	Predorsal length	8-14	10,94	1,71	27,36%
5	2nd dorsal fin origin	8-21	13,52	2,72	33,82%
6	Prepelvic length	6-16	9,65	2,33	24,13%
7	Ventral fin to anus	3-12	6,45	1,80	16,14%
8	Preanal length	7-22	11,87	3,28	29,70%
9	Caudal peduncle length	6-13	8,90	2,01	22,27%
10	Caudal peduncle depth	3-6	4,32	0,65	10,82%
11	Body depth at anal-fin origin	5-9	6,58	0,89	16,46%
12	Body depth at pelvic fin origin	3-8	5,23	1,06	13,08%
13	Head length	5-12	6,77	1,33	16,95%
14	Head depth	3-6	4,19	0,83	10,48%
15	Eye diameter	1-5	3,45	0,93	8,64%
16	Pre-orbital length	2-4	3,16	0,64	7,91%
17	Post-orbital length	3-5	4,29	0,69	10,73%
18	Dorsal fin base	3-8	6,16	1,46	15,42%
19	2nd dorsal fin base	4-8	6,68	0,98	16,71%
20	Pectoral fin length	5-9	7,19	1,05	18,00%
21	Ventral/pelvic fin length	4-9	6,29	1,13	15,74%
22	Anal fin length	3-9	5,00	1,21	12,51%
23	Caudal fin length	3-9	7,16	1,34	17,92%

The results of the morphometric analysis showed significant variations in several parameters, such as total length (average 39 mm, range 28–53 mm), standard length (average 33.16 mm, range 20-44), and other parameters (Table 2). These morphological variations are thought to reflect ecological adaptations to the environment, such as the ability to swim against the current or avoid predators, in line with studies that emphasize the influence of environmental factors, such as food availability, current, dissolved oxygen, and pH, on morphometric variations (Cabuga *et al*., 2024; Larson, 2010).

R. tambujon collected in the Tuweley River showed distinctive morphological characteristics and ecological adaptations. Meristic data showed the number of fin rays as follows: first dorsal fin (D1) VI-VIII, second dorsal fin (D2) 6-9, pectoral fins (P) 15-18, pelvic fins (V) 5-9, caudal fin (C) 8-13, and anal fins (A) 6-7. The body is brownish with black patterns. The first dorsal fin is transparent with black patterns and orange tips, while the second dorsal fin and caudal fin are transparent with a few black patterns. The pectoral and ventral fins are completely transparent. This coloration pattern is important for species identification and indicates adaptation to its habitat. The morphological characteristics of *R. tambujon* are similar to those reported by Larson (2010) in the Andaman Islands and the Philippines.

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Table 3. Meristic of *R. tambujon*.

Number	Types of fins	Range	Average	Standard Deviation
1	Dorsal 1	VI-VIII	VII	0,60
2	Dorsal 2	6-9	7,74	1,09
3	Pectoral	15-18	17,13	0,88
4	Ventral	5-9	7,65	0,95
5	Caudal	8-13	11,10	1,16
6	Anal	6-7	6,39	0,50

Environmental Parameters

Water quality measurements in the Tuweley River, the habitat of *R. tambujon*, were carried out in situ with results showing water temperature of 27–28°C, pH 7.3–7.7, and dissolved oxygen (DO) 6.6–7.2 mg/L (Table 4). According to Pusey *et al*. (2004), *Redigobius* species generally inhabit shallow rivers with clear water and rocky or sandy substrates, where habitat temperatures range from 11–27°C depending on location and season, and slightly acidic to neutral pH (6.5–7.5). High DO concentrations, namely 4.8–9.2 mg/L, are essential for the survival of this species, supporting metabolic needs, activity, and reproduction.

Table 4. Environmental parameter data

Number	Parameter	Unit	Range
1	Temperature	°C	27-28
2	pH	-	7,3-7,7
3	Dissolved oxygen	Mg/l	6,6-7,2
4	Substrate	-	sand, gravel, rocky

Habitat surveys of *R. tambujon* indicate that the species inhabits rocky, gravelly, and sandy substrates in freshwater habitats. Larson (2010) supports this finding by reporting the presence of *R. tambujon* in various locations, including the Andaman Islands, Micronesia (Palau), and Indonesia (Sumatra, Java, Aru Islands, Halmahera, and West Papua). The species is generally found in flowing waters, both upstream and downstream, with a preference for solid rocky, gravelly, and sandy substrates.

CONCLUSION

Based on the research results on Redigobius fish collected in the Tuweley River, through DNA analysis and measurement of morphological characteristics, it was concluded that the fish species collected in the river was *R. tambujon*.

CONFLICT OF INTEREST

No conflict of interest among all authors upon writing and publishing the manuscript.

AUTHOR CONTRIBUTION

The contributions of each author are as follows: Suardi Laheng analyzed data, drafted the manuscript, and revised it; Dwi Utami Putri and Ika Wahyuni Putri collected data; Aliyas and Moh. Paisal followed the conception and design experiments.

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