DNA Barcoding of Snapper Fish (*Lutjanus* spp.) from Kaimana and Fakfak, West Papua

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Abstract

Kaimana and Fakfak are two regions in West Papua which have high biodiversity. The total fish production in Kaimana and Fakfak are 10,039 tons.y^{-1} and 17,806 tons.y^{-1}, respectively. The snapper fish (Lutjanus spp.) is one of the important economic commodities in Kaimana and Fakfak regions. There has been a decrease in the number of exports of snapper fish in 2018 to 2019 from 4,742 tons to 4,290 tons due to overfishing and environmental pollution. This study employed DNA barcoding technology to identify the species of snapper fish collected from Kaimana and Fakfak. The DNA isolation was conducted by using genomic DNA mini kit (tissue) and the amplification of COI gene with Go Taq green master mix. Agarose gel electrophoresis was used to visualize the PCR product. A total of 16 sequences with length 654 base pairs of COI gene were identified as five species of Lutjanidae, which were Lutjanus decussatus, L. gibbus, L. quinquelineatus, L. malabaricus, and L. johnii. Homology analysis with BLAST NCBI and BOLD System showed that all samples have similarity of 99.08-100% and query cover of 93-100%. Relationship analysis using phylogenetic tree and genetic distances showed results of intraspecific close relatives (0.001-0.016) and interspecific distant relatives (>0.1000). The phylogenetic tree illustrated that all species of Lutjanidae are separated into monophyletic clades. DNA barcoding technology successfully identified the snapper fish collected from Kaimana and Fakfak.

Keywords: *Snapper, Lutjanus spp, DNA Barcoding, Phylogenetics, Identification*

Introduction

Kaimana and Fakfak are areas located in West Papua that are rich in biodiversity. The total fish production in Kaimana amounted to 10,039 tons.year^{-1} and Fakfak to 17,806 tons.year^{-1} (Sari *et al*., 2019). Kaimana waters has a long coastline 1,782.58 km with 1,003 fish species (Sjafrie and Setyastuty, 2020), while Fakfak Waters has a coastline length of 879 km with 330 fish species (Randa *et al*., 2024). There are many species found in Kaimana and Fakfak waters by fishermen, one of which is *Lutjanus* spp.

Snapper (*Lutjanus* spp.) is an important economical fish with the highest average selling price of IDR 55,558 (Giamurti *et al*., 2015). Snapper is a delicious and highly nutritious fish, making it economically desirable (Yusuf *et al*., 2024). Snapper fish live in depths of 10-50 m around coral reefs and are carnivorous fish that eat small fish and crabs (Martínez-Juárez *et al*., 2024).

There was a decrease in the number of exports from 2018 to 2019 from 4,742 tons to 4,290 tons (Rapi *et al*., 2022) due to overfishing and environmental pollution, this could threaten the snapper population. Status of snapper is in optimum, moderately exploited, and overfished conditions (Thahir and Lagoa, 2018). Conservation and aquaculture activities can be a solution to the problems (Akbar *et al*., 2014). However, determining the right conservation and cultivation techniques requires the right initial steps, such as obtaining genetic information. DNA barcoding using cytochrome oxidase sub unit I (COI) gene markers is a method that can identify up to the species level, population structure, and relationships between populations using nucleotide base sequences (Shao *et al*., 2025).The COI gene has a high mutation rate compared to other genes nuclear DNA, so it can be

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used to analyze population structure (Darmawan *et al*., 2024).

DNA barcoding has emerged as a crucial tool for accurate identification of snapper species (Lutjanidae), which often exhibit high morphological similarities that pose challenges for traditional taxonomic identification. Maharani *et al*. (2025) successfully employed the cytochrome oxidase subunit I (COI) gene to identify eight species of the Lutjanidae family in Nabire waters, revealing a fragment length of 620 base pairs (bp). Similarly, Fadli *et al*. (2024) used DNA barcoding to analyze 78 sequences belonging to 15 species of commercially important snappers from Aceh waters, providing a reliable reference library for fisheries management through precise species identification. Molecular identification is particularly valuable for reliably differentiating species with similar external morphological traits that are challenging to separate based solely on physical characteristics (Maharani *et al*., 2025). The COI gene has proven effective in revealing cryptic lineages within snapper populations, as demonstrated by Galal-Khallaf *et al*. (2024), who discovered unexpected cryptic lineages in the blackspot snapper *Lutjanus ehrenbergii* through DNA barcoding of Egyptian and Qatari common snappers.

The application of DNA barcoding in snapper research extends beyond mere species identification to understanding evolutionary relationships and genetic connectivity between populations. Sala *et al*. (2023) utilized the COI gene for molecular identification and phylogenetic reconstruction of nine red snapper species from Yapen Island waters in Papua, Indonesia, revealing four distinct clades with significant bootstrap values ranging from 98 to 99%. Their study highlighted that the greatest genetic distance was found between *Lutjanus fulvus* and *Pristipomoides multidens*, while the smallest genetic distance was observed between *Lutjanus vitta* and *Lutjanus ehrenbergii*. These molecular approaches provide essential information for developing conservation plans and sustainable management strategies for snapper fisheries (Sala *et al*., 2023). As Fadli *et al*. (2024) noted, DNA barcoding offers a complementary approach to address the limitations of traditional taxonomy by using the mitochondrial COI gene to rapidly and accurately identify species based on their unique genetic fingerprints, which is crucial for effective fisheries management and conservation efforts. There is still no research on DNA barcoding of snapper fish in the waters of Kaimana and Fakfak West Papua using COI gene markers, so it is necessary to conduct such research as a reference for the development of aquaculture and conservation of snapper fish gene markers, so it is necessary to conduct such research as a reference for the development of cultivation and preservation of snapper fish (*Lutjanus* spp.).

Materials and Methods

The samples used were 16 individuals, consisting of 8 samples from Kaimana and 8 samples from Fakfak. The pectoral fin was used as the tissue sample for genetic analysis (Macphee *et al*., 2025). The morphology of snapper in general is to have a long and wide body shape and a flat or oval body. The head of the snapper is convex or slightly concave; the shape of the tail is slightly concave at the rear limit of the tail with both ends slightly concave blunt. The lower gill cover of the snapper has strong spines and the upper part has a toothed lobe (Rapi *et al*., 2019). Samples were collected from Kaimana and Fakfak (Figure 1) waters using screw caps tubes containing 90% alcohol for genome isolation, amplification and sequencing were carried out at the Brainy Bee Molecular Laboratory, Malang.

Genomic DNA isolation

Genomic DNA isolation was carried out using a genomic DNA mini kit (tissue) with standard protocol provided by the manufacture and followed the methods that used by Dailami *et al.* (2025). Overall, the process consists of five stages which were tissue destruction and lysis, DNA binding, DNA washing, DNA purification, and ends with DNA elution. Snapper fish samples were cut into 30 mg pieces in a microtube containing 200 µL of GT solution. To degrade proteins, 10 µL of proteinase-K was added and incubated for 30 min at 60^{0}C. Then the sample was added with 200 µL of GBT solution and incubated again for 20 min at 60^{0}C. The sample solution was then added 200 µL of ethanol absolute and poured all the solution on the GS Column. The washing stage was carried out by adding 400 µL of W1 buffer and centrifuged for 30 sec at 7,000 rpm. The residue in the collection tube was discarded, and the collection tube was reinstalled on the GS Column, after which 600 µL of wash buffer was added and centrifuged for 30 sec at 7,000 rpm and residue was removed. The GS column was again centrifuged for 3 min at 7,000 rpm to dry the column matrix. The last step was added elution buffer that has been heated at 60^{0}C as much as 100 µL in the center of the matrix column and let stand for 5 min so that it is well absorbed. After 5 min, centrifugation was carried out for 30 sec at 7,000 rpm to remove the elution buffer. To obtain purified DNA, the isolate was then stored in the freezer until it was used in the next stage.

Polymerase Chain Reaction (PCR)

The PCR process was done by using Mini PCR thermal cycler following the laboratory protocol that had been used by Dailami *et al.* (2025). Total 16 DNA extracts were used in amplification process of the COI gene. The process was started by preparing the

master mix, which includes Nuclease free water 21 µL, Go Taq Green 25 µL, and forward and reverse primers 1 µL each. The total volume of master mix that was used per sample was 48 µL with addition of 2 µL extract DNA, giving the total reaction of 50 µL. One set Primers were used in the PCR process, which were Fish F1 5'-TCAACCAACCACAAAGACATTGGCAC-3' (forward) and Fish R1 5'-TAGACTTCTGGGTGGCCAAA GAATCA-3' (reverse) (Ward *et al*., 2005). The PCR program was performed using MiniPCR application with programming denaturation 95°C for 5 min, then 35 cycles denaturation at 95°C for 30 sec; annealing at 50°C for 30 sec, and extension at 72°C for 1 min.

Electrophoresis

PCR product visualization was performed with agarose gel electrophoresis following the methods used by Dailami *et al.* (2025). In detail, the gel was made using 30 ml TAE 1X and 2% agarose gel (0.6 g). Gel solution was homogenized and heated using a hot plate until homogeneous (clear), then cooled to a temperature of nail warm and given 1 µL Floro safe DNA stain. The gel was printed using a gel mold then wait until the gel hardened for about 10-15 min. The hardened gel was transferred into an electrophoresis tank containing a solution of TAE 1x buffer solution. Gel wells are filled with amplicons starting from the right side with markers as markers and followed by amplification results, then electrophoresis was set for 30 min, then running program. The voltage used for electrophoresis is 60 volts. The results of electrophoresis can be seen by turning on the blue light and documented using a digital camera.

Sequencing

Sequencing was carried out using the Sanger method. DNA samples were sent to Genetic Science Indonesia and continued to Apical Scientific in Malaysia. Sequencing results in the form of electropherograms of forward and reverse of each sample in the form of AB1 files.

Data analysis

Data of sequencing results in the form of electropherograms consist of forward and reverse sequences. MEGA XI was used for editing forward sequences and reverse sequences until consensus, base composition analysis, base variation, phylogenetic tree construction and genetic distance. Species identification was performed using the Basic Local Alignment Search Tool (BLASTn) and Barcode of Life Data System (BOLD System). Each was taken from the top 3 results based on the level of sequence similarity.

Analysis of phylogenetic trees was performed using the Hasegawa-Kishino-Yano+Gamma+ Invariant model (HKY+G+I), using the Maximum Likelihood (ML) method and using the adaptive bootstrap method with threshold 5% on MEGA XII (Kumar *et al.*, 2024). Intraspecific sequences were taken as much as 2-3 sequences with different water locations and interspecific sequences were taken based on one species each from the genus *Lutjanus* spp. The outgroup used was *Pristipomoides multidens*, obtained from the literature (Gold *et al*., 2011).

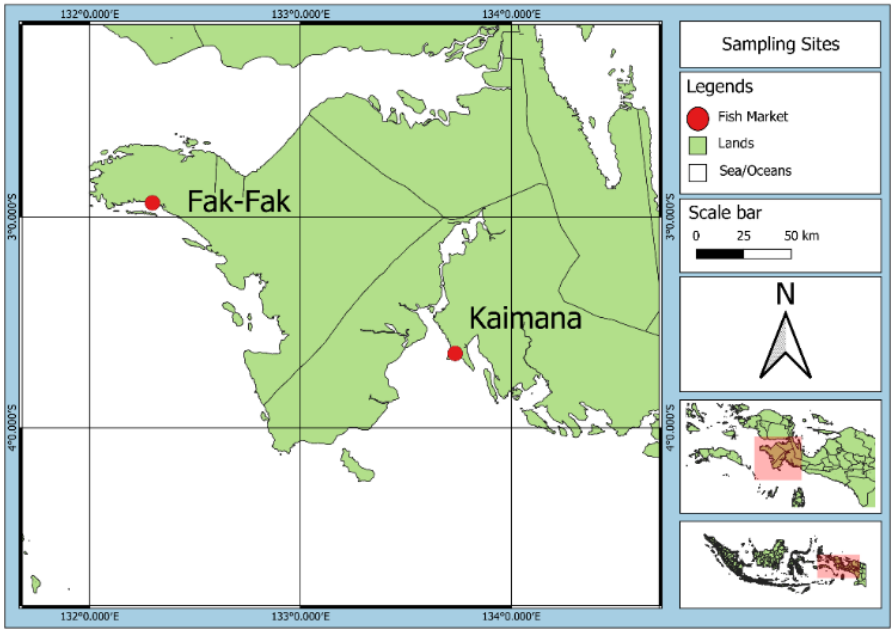

Figure 1. Map of snapper fish (*Lutjanus* spp.) sampling sites

Secondary data as comparison data taken from GenBank for intraspecific selected sequences of the same species from different waters and for interspecific all sequences with different species of the genus *Lutjanus* were taken, 33 sequences were obtained.

Results and Discussion

Electrophoresis results

The PCR results tested using electrophoresis succeeded in obtaining a bright, firm DNA band, and there was no smear under the band. The length of the DNA band obtained is between 600-700 bp ladder, which is also found in mimi animals (Meilana *et al*., 2016), catfish (Nurilmala *et al*., 2022), and hickey (Fahmi *et al*., 2020). This shows that the COI gene fragment obtained in this study has a length that is suitable for the genetic identification process with DNA barcoding (Dailami *et al*., 2021). The results of electrophoresis can be seen in Figure 2.

The quality of DNA extraction is a critical factor in successful molecular identification of fish species. According to Lutz *et al*. (2023), the method of DNA extraction significantly affects the quantity and purity of DNA obtained, which directly impacts the success of PCR amplification and sequencing. In their comparative study of three extraction methods (saline solution, phenol-chloroform, and commercial kit) for snapper (*Lutjanus purpureus*) tissues, they found that saline-based protocols and commercial kits yielded the highest DNA integrity and concentration values. This aligns with the findings of this study, distinct bands were observed in the electrophoresis results, indicating high-quality DNA extraction.

The amplification of the COI gene region in the present study produced fragments between 600-700 bp, which is consistent with the standard DNA barcode fragment length used for fish species identification. Maharani *et al*. (2025) successfully employed the COI gene for molecular identification of red snapper species in Nabire, revealing a fragment length of 620 base pairs. Similarly, Sala *et al*. (2023) utilized the COI gene for molecular identification and phylogenetic reconstruction of nine red snapper species from Yapen Island waters in Papua, Indonesia. The consistency in fragment length across different studies validates the reliability of the COI gene as a molecular marker for species identification in the Lutjanidae family.

The absence of smearing in our electrophoresis results indicate minimal DNA degradation and contamination, which is essential for accurate sequencing and species identification. Galal-Khallaf *et al*. (2024) emphasized the importance of high-quality DNA extraction in revealing cryptic lineages within snapper populations, as demonstrated in their discovery of unexpected cryptic lineages in the blackspot snapper *L. ehrenbergii* through DNA barcoding. The quality of DNA extraction directly influences the resolution of genetic analyses and the ability to detect subtle genetic variations between closely related species or populations.

COI gene sequences

The COI gene sequences yielded electropherograms with clear, distinguishable peaks. The length of the base pairs obtained was 654 bp with average base composition of 28.74%, cytosine base 28.10%, adenine base 24.60%, and guanine 18.57% of the 16 samples analyzed. The nucleotide compositions vary among five species. This nucleotide composition analysis reveals an AT-rich pattern (thymine + adenine = 53.34%) in the COI gene region of the studied species, which is consistent with patterns observed in other fish species. The guanine content (18.57%) is notably lower than the other nucleotides, which is a common characteristic in the COI gene region of many fish species. Pranata *et al*. (2024) reported similar AT-rich compositions in their DNA barcoding study of red snapper (*L. gibbus*) with AT content 54.9% and GC content 45.1%. This results also found in eight species of red snapper that studied by Maharani *et al.* (2025) from Nabire, which shows the AT content range from 51.9%-55.5%.

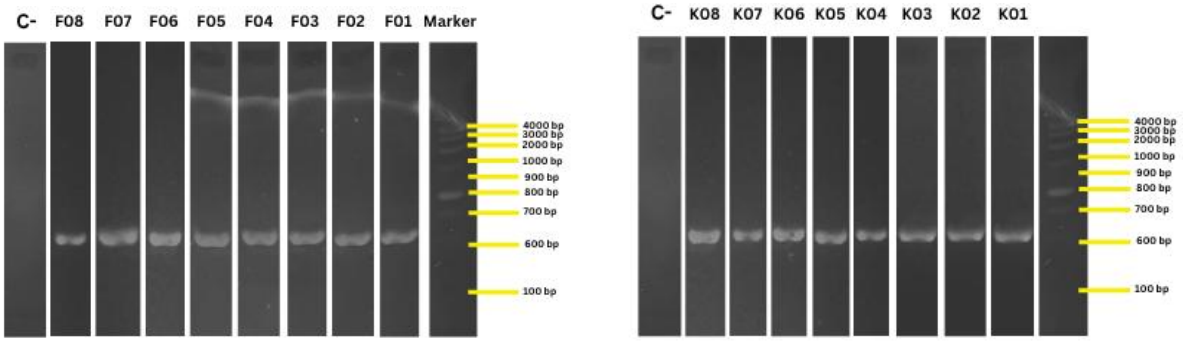

Figure 2. Electrophoresis results of snapper fish samples from Kaimana and Fakfak.
F01-F08= Sample code Fakfak; K01-K08= Sample code Kaimana; C-= Negative control

The higher proportion of AT content is characteristic of mitochondrial DNA in many vertebrates, including fish, and can influence the stability and function of the gene region. The variation in nucleotide composition among the five species indicates species-specific patterns that can serve as molecular signatures for identification. Maharani *et al*. (2025) demonstrated that nucleotide composition analysis of the COI gene was effective in differentiating eight species of the Lutjanidae family in Nabire waters, with each species showing distinct nucleotide patterns. These compositional differences contribute to the genetic distances observed between species and can be used as additional markers for species delineation. Sala *et al*. (2023) observed similar patterns in their phylogenetic study of red snappers in Yapen Island waters, where guanine was consistently the least abundant nucleotide. This bias in nucleotide composition can be attributed to evolutionary pressures and functional constraints on the COI gene.

Species identification

The results of identification using BLASTn and BOLD System obtained 5 species in each location. The species that appeared during identification were *L. decussatus*, *L. malabaricus*, *L. gibbus*, *L. johnii*, and *L. quinquelineatus*. A total of 16 samples collected from Kaimana and Fakfak represents from 5 species with a similarity rate of 99.08-100% and query cover of 93-100%. The higher of similarity, indicates the higher the similarity between the sample sequences with those in GenBank (Limmon *et al*., 2024). The high query cover percentages (93-100%) observed in our BLAST results indicate that most of the sequence length matched with reference sequences in the database, further validating the reliability of our identifications. The identification results are provided in Table 1 and 2.

DNA barcoding has emerged as a powerful tool for accurate species identification, particularly for morphologically similar species like those in the Lutjanidae family. The high similarity rates (99.08-100%) observed in our study indicate robust and reliable species identification, which is crucial for fisheries management and conservation efforts. This finding is consistent with research by Andriyono *et al*. (2022), who identified four different *Lutjanus* species (*L. gibbus, L. rufolineatus, L. bengalensis,* and *L. erythropterus*) from Sendang Biru, Malang, using the COI gene marker with similarly high sequence similarities (96.72-100%). The presence of *L. gibbus* in both studies demonstrates the wide distribution of this species across Indonesian waters. The slightly lower similarity percentage for *L. gibbus* (96.72%) reported by Andriyono *et al*. (2022) compared to our findings (>99%) may suggest regional genetic variations within this species across different parts of Indonesia. Additionally, both studies highlight the effectiveness of DNA barcoding for accurate identification of Lutjanidae species, which can be challenging to distinguish based solely on morphological characteristics due to their similar external features.

The five species identified in both Kaimana and Fakfak waters represent important components of the local fisheries economy. *Lutjanus malabaricus* and *L. gibbus* are particularly significant commercial species in the Indo-Pacific region. Maharani *et al*. (2025) also identified *L. gibbus* among the eight Lutjanidae species in Nabire waters, highlighting the widespread distribution of this species throughout Papua's waters. The consistent identification of these species across different studies in the region provides valuable data for monitoring population dynamics and implementing sustainable fishing practices.

Phylogenetic

The phylogenetic tree was reconstructed with 638 base pairs of COI fragment gene from 46 sequences that consists of 16 sequences of samples from Kaimana and Fakfak, 1 outgroup, and 29 sequences that represent each species of Lutjanidae available in GenBank. Among the family Lutjanidae (46 sequences), there are 430 conserved sites, 208 variable sites with 193 parsimony informative sites, and 15 singleton sites. This result is similar to the study by Halim *et al.* (2022) by analyzing 84 individuals of five species of red snapper from Malaysia, this study shows that there are 327 conserved sites, 168 parsimony informative sites and 17 synonymous sites. The slightly higher number of parsimony informative sites in our study because the number of species that used in our dataset is more than the study by Halim *et al.* (2022).

The phylogenetic tree analysis confirmed the identification results from BLASTn and BOLD system analyses, as all samples formed a monophyletic clade with sequences of the same species (Table 2). The phylogenetic tree is provided in Figure 3. The highlighted part is the research sample. Based on the dendrogram on the phylogenetic tree of the sample *L. quinquelineatus* is in the same clade as *L. quinquelineatus* from GenBank. The same thing also occurred in the samples of *L. decussatus*, *L. johnii, L. gibbus*, and *L. malabaricus*. The bootstrap value obtained is 100 at branching of each sample species. Bootstrap values of 70-100% are good enough to support the clades, while bootstrap <50% is considered as low support the clades (Holmes, 2003).

The branching phenomenon in the sequence of *L. quinquelineatus* Kaimana sample K07 with the

sequence of *L. quinquelineaus* Philippine and Ambon waters show that there are sequence similarities. Branching in the sequences of *L. decussatus* samples F02 and F03 with Philippine *L. decussatus* also showed sequence similarity. This phenomenon can occur due to gene flow (Saleky and Dailami, 2021), so that between *L. quinquelineatus* in the waters of Kaimana with *L. quinquelineatus* is still in the same gene, as well as *L. decussatus* in Fakfak waters with *L. quinquelineatus* in the Philippines. Gene flow occurs

Table 1. Genetic Identification of samples of snapper fish using BLASTn, all samples are successfully identified based on COI gene, with identity 99-100%. Id sample with code F is from Fakfak and K is from Kaimana.

ID	Identification using BLAST (3 highest)	*Query Cover* (%)	*Identity* (%)	*Accession Number*
F01	1. *Lutjanus decussatus*	100%	100%	OQ299548.1
	2. *Lutjanus decussatus*	100%	100%	KU682547.1
	3. *Lutjanus decussatus*	100%	100%	OQ386044.1
F02	1. *Lutjanus decussatus*	100%	100%	OQ387088.1
	2. *Lutjanus decussatus*	100%	100%	OR524464.1
	3. *Lutjanus decussatus*	100%	100%	OQ386916.1
F03	1. *Lutjanus decussatus*	100%	100%	OQ387088.1
	2. *Lutjanus decussatus*	100%	100%	OR524464.1
	3. *Lutjanus decussatus*	100%	100%	OQ386916.1
F04	1. *Lutjanus malabaricus*	100%	100%	OR758620.1
	2. *Lutjanus malabaricus*	100%	100%	PQ804621.1
	3. *Lutjanus malabaricus*	100%	100%	OR524468.1
F05	1. *Lutjanus gibbus*	100%	99.69%	MW034069.1
	2. *Lutjanus gibbus*	100%	99.69%	MW034066.1
	3. *Lutjanus gibbus*	100%	99.69%	MW034065.1
F06	1. *Lutjanus johnii*	98%	100%	EF609396.1
	2. *Lutjanus johnii*	99%	99.23%	JN311954.1
	3. *Lutjanus johnii*	99%	99.23%	KC970397.1
F07	1. *Lutjanus quinquelineatus*	100%	100%	PQ812460.1
	2. *Lutjanus quinquelineatus*	100%	100%	GU674055.1
	3. *Lutjanus quinquelineatus*	100%	100%	JF952789.1
F08	1. *Lutjanus johnii*	98%	100%	EF609396.1
	2. *Lutjanus johnii*	99%	99.23%	JN311954.1
	3. *Lutjanus johnii*	99%	99.23%	KC970397.1
K01	1. *Lutjanus malabaricus*	100%	100%	OR758620.1
	2. Lutjanus erythropterus	100%	100%	OR524468.1
	3. *Lutjanus malabaricus*	93%	100%	OP185225.1
K02	1. *Lutjanus gibbus*	100%	100%	PQ860844.1
	2. *Lutjanus gibbus*	100%	100%	OQ387116.1
	3. *Lutjanus gibbus*	100%	100%	MW034070.1
K03	1. *Lutjanus gibbus*	100%	100%	PQ860844.1
	2. *Lutjanus gibbus*	100%	100%	OQ387116.1
	3. *Lutjanus gibbus*	100%	100%	MW034070.1
K04	1. *Lutjanus gibbus*	100%	99.84%	MN870401.1
	2. *Lutjanus gibbus*	99%	99.84%	PV050704.1
	3. *Lutjanus gibbus*	100%	99.69%	MW034069.1
K05	1. *Lutjanus decussatus*	100%	100%	OQ299548.1
	2. *Lutjanus decussatus*	100%	100%	KU682547.1
	3. *Lutjanus decussatus*	100%	100%	OQ386044.1
K06	1. *Lutjanus quinquelineatus*	100%	100%	GU674055.1
	2. *Lutjanus quinquelineatus*	100%	100%	MN870489.1
	3. *Lutjanus quinquelineatus*	100%	100%	JF952789.1
K07	1. *Lutjanus quinquelineatus*	100%	100%	EF609399.1
	2. *Lutjanus quinquelineatus*	100%	100%	OQ385849.1
	3. *Lutjanus quinquelineatus*	100%	100%	MN870378.1
K08	1. *Lutjanus johnii*	100%	99.69%	NC_024572.1
	2. *Lutjanus johnii*	100%	99.08%	OQ387612.1
	3. *Lutjanus johnii*	100%	99.08%	OQ386844.1

Table 2. Genetic identification of samples by using BOLD system, all samples are successfully identified with BOLD system with similarity 99-100%. Id sample with code F is from Fakfak and K is from Kaimana.

ID Sampel	Species identification using BOLD *system* (3 highest)	*Similarity* (%)	PID	BIN
F01	1. *Lutjanus decussatus*	100%	ZOSKT706-16	BOLD:AAF0336
	2. *Lutjanus decussatus*	100%	BIFZC119-17	BOLD:AAF0336
	3. *Lutjanus decussatus*	100%	FADLI117-17	BOLD:AAF0336
F02	1. *Lutjanus decussatus*	100%	MINDA433-23	BOLD:AAF0336
	2. *Lutjanus decussatus*	100%	PHILA049-13	BOLD:AAF0336
	3. *Lutjanus decussatus*	99.85%	BIFZC119-17	BOLD:AAF0336
F03	1. *Lutjanus decussatus*	100%	MINDA433-23	BOLD:AAF0336
	2. *Lutjanus decussatus*	100%	PHILA049-13	BOLD:AAF0336
	3. *Lutjanus decussatus*	99.85%	BIFZC119-17	BOLD:AAF0336
F04	1. *Lutjanus malabaricus*	100%	FOAH623-08	BOLD:AAA7595
	2. *Lutjanus malabaricus*	100%	FOAC620-05	BOLD:AAA7595
	3. *Lutjanus malabaricus*	100%	FOAC616-05	BOLD:AAA7595
F05	1. *Lutjanus gibbus*	100%	MBFA937-07	BOLD:AAB3276
	2. *Lutjanus gibbus*	99.69%	MINDA173-23	BOLD:AAB3276
	3. *Lutjanus gibbus*	99.69%	SBF457-11	BOLD:AAB3276
F06	1. *Lutjanus johnii*	100%	FOAC011-05	BOLD:AAC7492
	2. *Lutjanus johnii*	100%	FOAC013-05	BOLD:AAC7492
	3. *Lutjanus johnii*	100%	FOAC012-05	BOLD:AAC7492
F07	1. *Lutjanus quinquelineatus*	100%	ANGBF38839-19	BOLD:AAC0628
	2. *Lutjanus quinquelineatus*	100%	GBGCA6212-13	BOLD:AAC0628
	3. *Lutjanus quinquelineatus*	100%	ABFJ026-06	BOLD:AAC0628
F08	1. *Lutjanus johnii*	100%	FOAC011-05	BOLD:AAC7492
	2. *Lutjanus johnii*	100%	FOAC013-05	BOLD:AAC7492
	3. *Lutjanus johnii*	100%	FOAC012-05	BOLD:AAC7492
K01	1. *Lutjanus malabaricus*	100%	FOAH623-08	BOLD:AAA7595
	2. *Lutjanus malabaricus*	100%	FOAC620-05	BOLD:AAA7595
	3. *Lutjanus malabaricus*	100%	FOAC616-05	BOLD:AAA7595
K02	1. *Lutjanus gibbus*	100%	MBFA937-07	BOLD:AAB3276
	2. *Lutjanus gibbus*	100%	MINDA173-23	BOLD:AAB3276
	3. *Lutjanus gibbus*	100%	SBF457-11	BOLD:AAB3276
K03	1. *Lutjanus gibbus*	100%	MBFA937-07	BOLD:AAB3276
	2. *Lutjanus gibbus*	100%	MINDA173-23	BOLD:AAB3276
	3. *Lutjanus gibbus*	100%	SBF457-11	BOLD:AAB3276
K04	1. *Lutjanus gibbus*	100%	BIFZC141-17	BOLD:AAB3276
	2. *Lutjanus gibbus*	100%	MINDA173-23	BOLD:AAB3276
	3. *Lutjanus gibbus*	100%	SBF457-11	BOLD:AAB3276
K05	1. *Lutjanus decussatus*	100%	ZOSKT706-16	BOLD:AAF0336
	2. *Lutjanus decussatus*	100%	BIFZC119-17	BOLD:AAF0336
	3. *Lutjanus decussatus*	100%	FADLI117-17	BOLD:AAF0336
K06	1. *Lutjanus quinquelineatus*	100%	GBMNB7467-20	BOLD:AAC0628
	2. *Lutjanus quinquelineatus*	100%	ANGBF38839-19	BOLD:AAC0628
	3. *Lutjanus quinquelineatus*	100%	GBGCA6212-13	BOLD:AAC0628
K07	1. *Lutjanus quinquelineatus*	100%	GBMNB7468-20	BOLD:AAC0628
	2. *Lutjanus quinquelineatus*	100%	BIFZC159-17	BOLD:AAC0628
	3. *Lutjanus quinquelineatus*	100%	FOAC036-05	BOLD:AAC0628
K08	1. *Lutjanus johnii*	99.69%	GBMNA14814-19	BOLD:AAC7492
	2. *Lutjanus johnii*	99.08%	FOAM228-10	BOLD:AAC7492
	3. *Lutjanus johnii*	99.08%	DBMR132-19	BOLD:AAC7492

due to migration from one water body to another. Larval dispersal caused by due to ocean current transport can also cause gene flow (Saleky and Dailami, 2021; Limmon *et al*., 2024).

The existence of the Indonesian Throughflow and the monsoon season create the ability for snapper larvae to disperse far with the ocean current system. These ocean currents move from the Pacific Ocean to

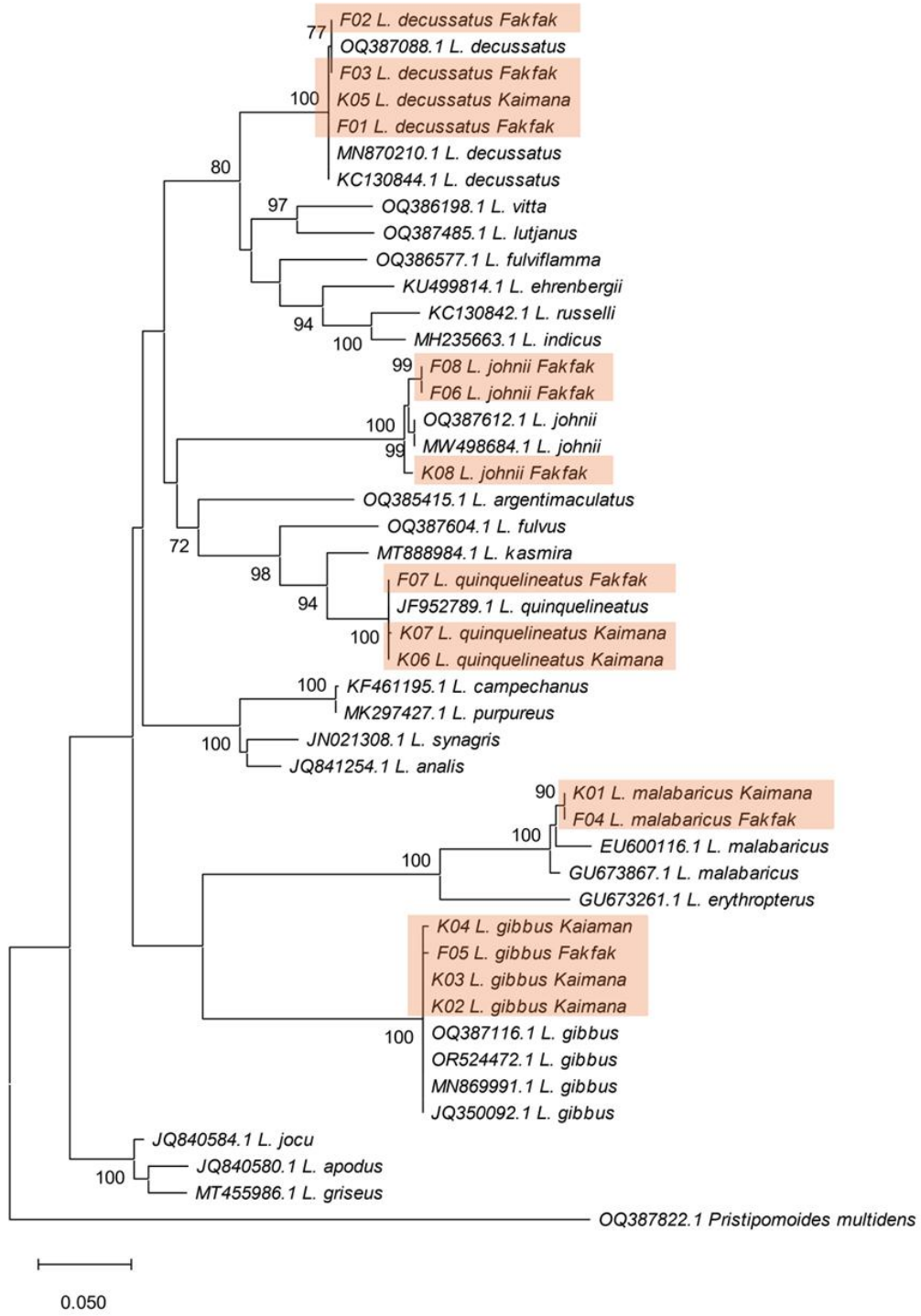

Figure 3. Phylogenetic tree of the genus *Lutjanus* spp. from Kaimana and Fakfak with comparison with the sequences from GenBank.

the Indian Ocean by passing through one of the following one of which is the waters of Eastern Indonesia. Snapper larvae originating from the western Pacific waters can be carried by the Through the Indonesian archipelago, including reaching the waters of West Papua. Most of Indonesia's seawater comes from the Pacific Ocean in two directions, namely water directly from the Pacific Ocean that fills the waters of the Eastern region and the China Sea that fills the waters of the Java Sea (Taufiqurrahman *et al*., 2020). This phenomenon can make the existence of genetic sharing in a sustainable manner.

Genetic distance

Genetic distance was calculated using the Maximum Composite Likelihood method. Genetic distance intraspecies *L. quinquelineatus* was 0.001, *L. johnii* was 0.007, *L. gibbus* was 0.002, *L. decussatus* was 0.001, and *L. malabaricus* was 0.016. The genetic distance intraspecies shows low results and each species has a close distance between individuals within the species. The genetic distances among the studied Lutjanidae species range from 0.00 to 0.31, with a mean distance of 0.165 and a median of 0.160. This range indicates considerable genetic diversity within the family, reflecting its evolutionary history and speciation processes in marine environments. The distribution of genetic distances follows a pattern consistent with the taxonomic classification of snappers, where closely related species exhibit lower genetic distances while more distantly related taxa show higher values.

The similar pattern was also found by researchers Limmon *et al*. (2024), in the *L. gibbus* species, which has a genetic distance of 0.0000-0.0165, which is said to be included in the genetic distance value classified as low and has a close relationship between individuals within the species. The genetic distance value is classified as low and has a close relationship between individuals within the species. The value of a very close relationship can be caused by the origin of the same parent. The results of the average genetic distance value between *Lutjanus* samples and GenBank sequences show results in the range of 0.001-0.016, which indicates that *Lutjanus* samples and GenBank sequences have a close genetic distance. This proves that the results of species identification using BLAST and BOLD system are accurate and appropriate. The genetic distance value of 0.000-0.009 is included in the low value (Nei, 1972). The relationship of a species is said to be closer if it has a smaller genetic distance value.

The genetic distance of *L. apodus, L. jocu,* and *L. griseus* form a closely related group ranging from 0.03 to 0.04. This suggests recent speciation events or ongoing gene flow among these species, which also share similar ecological niches in Western Atlantic reef ecosystems. In addition, *L. russelli* and *L. indicus* exhibit a genetic distance of only 0.04, indicating their close evolutionary relationship. This aligns with their morphological similarities and overlapping distribution in the Indo-Pacific region. Moreover, *L. campechanus* and *L. purpureus* display a genetic distance of 0.001, suggesting they may represent the same biological species despite being described as separate taxa. This finding has significant implications for fisheries management and taxonomy of commercially important snappers. Lastly, *L. synagris* and *L. analis* show a genetic distance of 0.04, reflecting their close phylogenetic relationship despite some morphological differences.

Conclusion

This study successfully employed DNA barcoding technology to identify five species of snapper fish (*Lutjanus* spp.) from Kaimana and Fakfak, West Papua. A total of 16 samples were analyzed, yielding 654 base pair sequences of the COI gene that were identified as *Lutjanus decussatus, L. gibbus, L. quinquelineatus, L. malabaricus, and L. johnii*. Homology analysis using BLAST NCBI and BOLD System revealed high similarity rates (99.08-100%) with query cover of 93-100%, confirming the accuracy of species identification. Relationship analysis through phylogenetic tree reconstruction and genetic distance calculations demonstrated close intraspecific relationships (0.001-0.016) and distant interspecific relationships (>0.1000). The phylogenetic tree illustrated that all Lutjanidae species were separated into monophyletic clades, indicating the reliability of the phylogenetic reconstruction. The branching phenomenon observed in some samples with sequences from different locations suggests gene flow, possibly due to larval dispersal through the Indonesian Throughflow current system. This study demonstrates that DNA barcoding technology is an effective tool for accurate identification of snapper species from Kaimana and Fakfak waters, providing valuable data for aquaculture development and conservation efforts for these economically important fish species in West Papua.

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References

Akbar, N., Zamani, N.P. & Madduppa, H.H. 2014. Genetic diversity of yellowfin tuna (*Thunnus albacares*) from two populations in the Molucca Sea, Indonesia. *Depik*, 3(1): 65–73. https://doi.org/10.13170/depik.3.1.1304

Andriyono, S., Pradana, N.A., Sulmartiwi, L., Hidayani, A.A., Alam, M.J., Damora, A. & Habib, A. 2022. Molecular identification of snapper (Perciformes: Lutjanidae) landed at Pondokdadap Fishing Port of Sendang Biru,

Malang, Indonesia. *Ilmu Kelautan: Indonesian Journal of Marine Sciences*, 27(4): 307-314. https://doi.org/10.14710/ik.ijms.27.4.307-31 4

Dailami, M., Widyawati, Y. & Toha, A.H.A. 2021. Genetic identification of anchovy from Cenderawasih Bay using DNA barcoding approach. *Musamus Fish. Mar. J.*, 3: 154–166. https://doi.org/10.35724/mfmj.v3i2.3521

Dailami, M., Yuniarti, A., Widodo, M.S., Pratama, R.N., Ariyani, D.F., Siahaan, M.D.T.G. & Mayor, C.E.M.P. 2025. Genetic identification of Nilem fish using the DNA barcoding approach. *J. Trop. Divers.*, 1(1): 33–42. https://doi.org/10.64283 /jot diverse.2025.1(1):3

Darmawan, R.F., Mahrus, H., Bahri, S. & Zulkifli, L. 2024. Molecular and phylogenetic character analysis of tuna (*Auxis rochei*) based on the CO1 gene in the waters of West Lombok. *J. Biol. Trop.*, 24(2): 89–94. https://doi.org/10.29303/jbt. v24i2.6768

Fadli, N., Rahayu, S.R., El Rahimi, S.A., Damora, A., Muchlisin, Z.A., Ramadhaniaty, M., Razi, N.M., Siregar, A.R., Ummamah, A.R., Samad, A.P.A., Habib, A. & Siti-Azizah, M.N. 2024. DNA barcoding of commercially important snappers (Genus *Lutjanus* (Pisces: Lutjanidae)) from Aceh Waters, Indonesia. *Int. J. Des. Nat. Ecodyn.*, 19(3): 787-794.

Fahmi, M.R., Kusrini, E., Hayuningtiyas, E.P., Sinansari, S. & Gustiano, R. 2020. DNA barcoding using COI gene sequences of wild betta fighting fish from Indonesia: phylogeny, status and diversity. Indones. *Fish. Res. J.*, 26(2): 97-105. https://doi.org/10.15578/ifrj. 26.2.2020.97-105

Galal-Khallaf, A., Elberri, A.I., Hasieb, H.E., Said, R.E.M., Mohammed-AbdAllah, E., Osman, A.G.M. & Mohammed-Geba, K. 2024. DNA barcoding for different egyptian and qatari common snappers reveals unexpected cryptic lineages in the blackspot snapper *Lutjanus ehrenbergii*. *Mar. Biodivers.*, 54: 85. https://doi.org/10. 1007/s12526-024-01477-x

Giamurti, A.S.R., Bambang, A.N. & Fitri, A.D.P. 2015. Marketing analysis catch product of red snapper (*Lutjanus* sp.) in Brondong Archipelagic Fishing Port, Lamongan, East Java. *Fish. Resour. Util. Manag. Technol.*, 4(4): 179–187.

Gold, J.R., Voelker, G. & Renshaw, M.A. 2011. Phylogenetic relationships of tropical western Atlantic snappers in subfamily Lutjaninae (Lutjanidae: Perciformes) inferred from mitochondrial DNA sequences. *Biol. J. Linn. Soc.*, 102(4): 915–929. https://doi.org/10.1111/ j.1095-8312.2011.01621.x

Halim, L.J., Rahim, I., Mahboob, S., Al-Ghanim, K.A., Amat, A. & Naim, D.M. 2022. Phylogenetic relationships of the commercial red snapper (*Lutjanidae* sp.) from three marine regions. *J. King Saud Univ. Sci.*, 34(2): 101756.

Holmes, S. 2003. Bootstrapping phylogenetic trees: theory and methods. *Stat. Sci.*, 18(2): 241-255. https://doi.org/10.1214/ss/1063994979

Kumar, S., Stecher, G., Suleski, M., Sanderford, M., Sharma, S. & Tamura, K. 2024. MEGA12: Molecular evolutionary genetic analysis version 12 for adaptive and green computing. *Mol. Biol. Evol.*, 41(12): msae263. https://doi.org/10. 1093/molbev/msae263

Limmon, G.V., Tetelepta, J.M.S., Pattikawa, J.A., Natan, J., Laimeheriwa, B.M. & Leatemia, B.M. 2024. Connectivity study of *Lutjanus gibbus* in conservation areas and fishing areas in the Fisheries Management Area of the Republic of Indonesia (FMA-RI 714) using DNA barcoding. *TRITON:* J. *Manajemen Sumberdaya Perairan*, 20(1): 16–27.

Lutz, I., Miranda, J., Santana, P., Martins, T., Ferreira, C., Sampaio, I., Vallinoto, M. & Gomes, G.E. 2023. Quality analysis of genomic DNA and authentication of fisheries products based on distinct methods of DNA extraction. *PLoS ONE,* 18(2):e0282369. https://doi.org/10.137 1/journal.pone.0282369

Macphee, A., Oriowo, T.O., Sternberg, N., Khatkar, M.S., Zenger, K.R. & Guppy, J.L. 2025. Comparison of whole genome sequencing performance from fish swabs and fin clips. *BMC Res. Notes*, 18: 15. https://doi.org/10.1186/ s13104-024-07075-1

Maharani, A.N.M.S., Sala, R., Toha, A.H.A., Purbani, D.C., Mokodongan, D.F., Kusuma, A.B. & Pranata, B. 2025. Morphological and genetic characteristics of red snapper (Lutjanidae) in Nabire Waters. *Ilmu Kelautan: Indonesian Journal of Marine Sciences*, 30(1): 83-91. https://doi.org/10.14710/ik.ijms.30.1.83-91

Martínez-Juárez, L.F., Schmitter-Soto, J.J., Cabanillas-Terán, N. & Mercado-Silva, N. 2024. Diet variability of snappers (Teleostei: Lutjanidae) in a bay-to-reef ecosystem of the Mexican Caribbean. *Water Biol. Secur.*, 3: 100211. https ://doi.org/10.1016/j.watbs.2023.100211

Meilana, L., Wardiatno, Y., Butet, N.A. & Krisanti, M. 2016. Morphological character and molecular identification with COI gene marker of horseshoe crabs (*Tachypleus gigas*) at coastal waters of Northern Java Island. *J. Ilmu Teknol. Kelautan Trop.*, 8(1): 145–158.

Nei, M. 1972. Chapter 9: Genetic distance between populations. *Am. Nat.*, 106(949): 283–292.

Nurilmala, M., Indarwati, A.R. & Nugraha, R. 2022. Detection of fish gelatin with DNA biomarkers. *IOP Conf. Ser.: Earth Environ. Sci.*, 1033(1). https://doi.org/10.1088/1755-1315/1033/1/012046

Pranata, B., Sala, R., Kusuma, A.B., Toha, A.H.A., Purbani, D.C., Mokodongan, D.F. & Sipriyadi, S. 2024. Genetic diversity and connectivity of Red Snapper *Lutjanus gibbus* in the Papua Waters, Indonesia. *Biodiversitas*, 25(1): 276-286. https://doi.org/10.13057/biodiv/d250132

Randa, R.A.W.R.A., Boli, P., Purba, G.Y., Bawole, C.A.C.A. & Tebay, S. 2024. Effectiveness of the Fakfak Marine Protected Areas Management, Papua Barat, based on the Evika Instrument. *Igya Ser Hanjop: J. Pembangunan Berkelanjutan*, 6(2): 123–133. https://doi.org/10.47039/ish.6.2024.123-133

Rapi, N.L., Djumanto. & Murwantoko. 2022. Dynamic population of red snapper (*Lutjanus malabaricus*) in Pinrang Water. *J. Penelit. Perikan. Indones.*, 28(4): 177–185.

Rapi, N.L., Hidayani, M.T., Djumanto, D. & Murwantoko, M. 2019. Size distribution and length-weight relationship of red snapper (*Lutjanus malabaricus*) in Pinrang Waters Regency of Pinrang. *Agrikan: J. Agribisnis Perikanan*, 12(2): 317–321. https://doi.org/10.29239/j.agrikan.12.2.317-321

Sala, R., Kusuma, A.B. & Pranata, B. 2023. Phylogenetic of red snapper (Lutjanidae) in Yapen Island Waters, Papua, Indonesia. *Biodiversitas*, 24(2): 716-723.

Saleky, D. & Dailami, M. 2021. Konservasi genetik ikan kakap putih (*Lates calcarifer*) melalui pendekatan DNA barcoding dan analisis filogenetik di Sungai Kumbe Merauke Papua. *J. Kelaut. Trop.*, 24(2): 141–150. https://doi.org/10.14710/jkt.v24i2.10760

Sari, A.P., Wambrauw, L.T. & Maspaitella, M.R. 2019. Analysis of fishermen's income levels and capture fisheries marketing institutions in Fakfak Regency, West Papua Province. *JFRES: J. Fiscal and Regional Economy Studies*, 2(1): 78–86. https://doi.org/10.36883/jfres.v2i1.22

Shao, K-T., Ko, H-L., Chiu, Y-C., Huang, H-K., Chen, Y-F. & Chang, Y-W. 2025. Establishing a COI DNA barcoding reference database for Taiwanese coastal fish species from egg and larvae specimens. *ZooKeys*, 1247: 207-216. https://doi.org/10.3897/zookeys.1247.151795

Sjafrie, N.D.M. & Setyastuty, A. 2020. The Utilization of Sea Cucumber in Kaimana Regency, West Papua Province. Oseanologi Dan Limnologi Di Indonesia, 5(2), 121. https://doi.org/10.14203/oldi.2020.v5i2.309

Taufiqurrahman, E., Wahyudi, A.J. & Masumoto, Y. 2020. The Indonesian throughflow and its impact on biogeochemistry in the Indonesian Seas. *ASEAN J. Sci. Technol. Dev.*, 37(1): 29–35. https://doi.org/10.29037/AJSTD.596

Thahir, M.T. & Lagoa, Y. 2018. Analysis of marine water pollution index with Cu and Pb metal parameters in Raja Ampat Tourism Area of West Papua. *J. Sumberdaya Akuat. Indopasifik*, 2(2): 113–118.

Ward, R.D., Zemlak, T.S., Innes, B.H., Last, P.R. & Hebert, P.D.N. 2005. DNA barcoding Australia's fish species. *Philos. Trans. R. Soc. B Biol. Sci.*, 360(1462): 1847–1857. https://doi.org/10.1098/rstb.2005.1716

Yusuf, M., Riana, A.D., Rusneni, Ibrahim, M., Emakarim, L.R., Umar, N.A., Tajuddin, M. & Hajriani, S. 2024. Bio-economic analysis of red snapper (*Lutjanus* spp.) in the waters of Makassar City, South Sulawesi, Indonesia. *ECSOFiM: Economic and Social of Fisheries and Mar. J.*, 12(01): 1-15.