

### [biodiv] Submission Acknowledgement

1 message

Ahmad Dwi Setyawan <smujo.id@gmail.com> To: Bayu Pranata <br/>b.pranata@unipa.ac.id> Wed, Nov 2, 2022 at 8:47 AM

Bayu Pranata:

Thank you for submitting the manuscript, "Phylogenetic of red snapper (Lutjanidae) in Yapen Island Waters" to Biodiversitas Journal of Biological Diversity. With the online journal management system that we are using, you will be able to track its progress through the editorial process by logging in to the journal web site:

Submission URL: https://smujo.id/biodiv/authorDashboard/submission/12746 Username: 050693

Osemanie, 030033

If you have any questions, please contact me. Thank you for considering this journal as a venue for your work.

Ahmad Dwi Setyawan

Biodiversitas Journal of Biological Diversity



Bayu Pranata <b.pranata@unipa.ac.id>

#### [biodiv] Editor Decision

1 message

Ayu Astuti <smujo.id@gmail.com>

Fri. Nov 4. 2022 at 1:31 AM

To: Bayu Pranata ob.pranata@unipa.ac.id>, Ridwan Sala oridwansala@gmail.com>, Aradea Bujana Kusuma oradea.bujana@gmail.com>

Bayu Pranata, Ridwan Sala, Aradea Bujana Kusuma:

We have reached a decision regarding your submission to Biodiversitas Journal of Biological Diversity, "Phylogenetic of red snapper (Lutjanidae) in Yapen Island Waters".

Our decision is: Revisions Required

Biodiversitas Journal of Biological Diversity

A-12746-Article Text-1065495-1-4-20221101 (wecompress.com).doc 1097K



#### [biodiv] Editor Decision

1 message

Smuio Editors <support@mail.smuio.id>

Sun. Dec 4, 2022 at 12:01 AM

To: Bayu Pranata <b.pranata@unipa.ac.id>, Ridwan Sala <ridwansala@gmail.com>, Aradea Bujana Kusuma <aradea.bujana@gmail.com>

Bayu Pranata, Ridwan Sala, Aradea Bujana Kusuma:

We have reached a decision regarding your submission to Biodiversitas Journal of Biological Diversity, "Phylogenetic of red snapper (Lutjanidae) in Yapen Island Waters".

Our decision is: Revisions Required

Reviewer A:

Dear Authors.

Thank you for submitting this paper that explores red snapper diversity in Yapen island waters, and more generally using the NCBI database. This is an interesting and useful study that provides some clear information on evolutionary relationships.

There are some revisions required in order to consider this manuscript for publication. I have included specific feedback on the word document version of the manuscript, please find attached. Make sure that any changes to the manuscript are shown using highlighted text or tracked changes. Additionally, please address the following key areas when making revisions:

- 1. Methods. Make sure the methods are clearly provided with regards to the number of samples that were collected, how maturity of fish was assessed, and where the samples were taken from. This is important from a repeatability standpoint.
- 2. Wording. Check through for several word choice and grammar errors. I have highlgihted these where possible in the text.

With these revisions, the paper should be in a stronger position overall.

Recommendation: Revisions Required

Biodiversitas Journal of Biological Diversity

A-12746-Article Text-1068164-1-4-20221202.doc 1266K



## [biodiv] New notification from Biodiversitas Journal of Biological Diversity

2 messages

DEWI NUR PRATIWI <support@mail.smujo.id> Reply-To: Ahmad Dwi Setyawan <editors@smujo.id> To: Bayu Pranata <b.pranata@unipa.ac.id> Mon, Dec 12, 2022 at 12:32 PM

You have a new notification from Biodiversitas Journal of Biological Diversity:

You have been added to a discussion titled "Uncorrected Proof" regarding the submission "Phylogenetic of red snapper (Lutjanidae) in Yapen Island Waters".

Link: https://smujo.id/biodiv/authorDashboard/submission/12746

Ahmad Dwi Setyawan

Biodiversitas Journal of Biological Diversity

**DEWI NUR PRATIWI** <support@mail.smujo.id> Reply-To: Ahmad Dwi Setyawan <editors@smujo.id> To: Bayu Pranata <b.pranata@unipa.ac.id> Mon. Dec 12, 2022 at 12:35 PM

You have a new notification from Biodiversitas Journal of Biological Diversity:

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Bayu Pranata <b.pranata@unipa.ac.id>

# [biodiv] New notification from Biodiversitas Journal of Biological Diversity

1 message

**DEWI NUR PRATIWI** <support@mail.smujo.id> Reply-To: Ahmad Dwi Setyawan <editors@smujo.id> To: Bayu Pranata <b.pranata@unipa.ac.id> Tue, Dec 20, 2022 at 8:34 AM

You have a new notification from Biodiversitas Journal of Biological Diversity:

There is new activity in the discussion titled "Proof Reading" regarding the submission "Phylogenetic of red snapper (Lutjanidae) in Yapen Island Waters".

Link: https://smujo.id/biodiv/authorDashboard/submission/12746

Ahmad Dwi Setyawan

Biodiversitas Journal of Biological Diversity



# [biodiv] Editor Decision 1 message

Wed, Dec 21, 2022 at 10:35 PM

Ayu Astuti <support@mail.smujo.id>
To: RIDWAN SALA <ridwansala@gmail.com>, ARADEA BUJANA KUSUMA <aradea.bujana@gmail.com>, BAYU PRANATA <b.pranata@unipa.ac.id>

RIDWAN SALA, ARADEA BUJANA KUSUMA, BAYU PRANATA:

We have reached a decision regarding your submission to Biodiversitas Journal of Biological Diversity, "Phylogenetic of red snapper (Lutjanidae) in Yapen Island Waters, Papua, Indonesia".

Our decision is to: Accept Submission

Biodiversitas Journal of Biological Diversity

# Phylogenetic of red snapper (Lutjanidae) in Yapen Island Waters, Papua, Indonesia

Abstract. Red Snapper are an economically valuable fishery resource. Most of these snapper species are inhabitants of coral reef ecosystems. The variety of red snapper species can be found in the northern waters of Papua, particularly in the Yapen Regency. However, information regarding the biological and ecological aspects of these fish is virtually unavailable. On the other hand, the utilization intensity of red snapper as a source of community income continues to increase. By using DNA barcode sequences, this study attempted to examine species diversity and relationship among Lutjanidae family species. The study was carried out from June to August 2022 in the waters of Yapen Regency, by taking fish samples from the fish caught by local hand lining fishers. Red snapper samples were identified morphologically and molecularly. According to molecular analyses, it was identified nine red snapper species from the genera of Lutjanus, Aphareus and Pristipomoides. The morphological characteristics of the species from the genus of Aphareus are similar to those from the genus of Pristipomoides, yet different from those of the species from the genus of Lutjanus. The phylogenetic tree consisted of four clades with significant bootstrap values ranging from 98 to 99%. Clades 1, 2 and 3 comprise the species from the genus of Lutjanus, while clade 4 contained species from the genera of Aphareus and Pristipomoides. The greatest genetic distance was found between Lutjanus fulvus and Pristipomoides multidens, while the smallest genetic distance was found between Lutjanus vitta and Lutjanus ehrenbergii. Based on the study results, some management implications are discussed.

**Keywords:** Molecular, Papua waters, Phylogenetic, Biodiversity (9 pt)

**Abbreviations** (if any): All important abbreviations must be defined at their first mention there. Ensure consistency of abbreviations throughout the article.

27 Running title: Diversity of Red Snapper Species

INTRODUCTION

Red Snappers are demersal fish belonging to the Lutjanidae family and the Lutjanus genus. Red Snappers inhabit coral reef environments and are dispersed over the East and Indo-West Pacific, East and West Atlantic (Souza et al. 2019), the home to forty-three species of snappers from the Lutjanidae family (Allen et al. 2013).

Snappers belonging to the genus Lutjanus have small to large body sizes, oval bodies, and triangular-shaped heads. Snappers vary in coloring, typically having a reddish, yellow, gray, or brown background with a darker pattern of stripes or stems and frequently having a huge black spot on the upper side under the dorsal soft front fin (Allen, 1985). The species from the genus Lutjanus have remarkably similar morphologies, including one with a recently altered yellow stripe (Iwatsuki et al. 2015). High morphological similarity across species and genera might make identification more difficult. The morphological similarity has the potential to lead to identification mistakes.

Reef fish, including groupers and snappers, are economically significant. This condition can lead to the intense exploitation of reef fish resources in diverse Indonesian sea regions. Through Decree No. 19/2022 issued by the Indonesian Minister of Marine Affairs, the Indonesian government has concluded that reef fish in fisheries management areas (FMA), such as FMA 717, which encompasses the northern portion of West Papua, are overexploited with utilization rates exceeding one.

The waters of the northern part of Papua, including the waters of the *Yapen* Regency, are the State Fisheries Management Area of the Republic of Indonesia (WPPNRI). According to Decree Number 50/KEPMEN-KP/2017 of the Minister of Maritime Affairs and Fisheries of the Republic of Indonesia, the red coral fish resources in these and nearby waters have been fully exploited. The lack of proper catch identification hinders the management of red snapper fisheries.

**Comment [A1]:** Discuss these implications in a bit more detail.

**Comment [A2]:** Remove this statement

Comment [A3]: Italicise the genus name

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In general, red snappers include *L. malabaricus*, *L. timoriensis*, and *L. erythropterus*, but the Indo-West Pacific contains forty-three species of red snappers. Sadly, there is currently no information regarding the quantity of Red Snapper species in the area. Red snapper species may have varying susceptibilities to fishing activities. Therefore, distinct management methods are needed to maintain sustainable fishing. Developing conservation plans and long-term management methods requires knowledge of the species and subpopulations involved (Bakar et al. 2018).

The search for species identity based on morphology does not provide full confidence compared to using a molecular approach due to the "evolution convergent" (Zou and Zhang, 2016). The morphological traits of organisms inhabiting distinct environments can vary (Heino, 2014; Shuai et al. 2018). Using genetic markers, species identity can be accurately determined. Molecular studies have relied significantly on genetic markers (Chan et al. 2021). Genetic markers are genomic DNA segments that provide information about taxonomic differentiation (Patwardhan et al. 2014; Grover et al. 2016). DNA sequences have been employed as genetic markers for species identification and the discovery of novel species (Allen et al. 2013; Iwatsuki et al. 2015). Mitochondrial DNA (mtDNA) is one of the genomic DNA segments frequently employed in molecular studies.

Mitochondrial DNA has been utilized extensively to answer questions concerning genetic diversity, population evolution, and cellular structure (Gupta et al. 2015). The cytochrome oxidase c subunit I (COI) gene was employed in this study for molecular identification and phylogenetic construction. The COI gene is a "DNA barcode" (Pentinsaari et al., 2016). It is a dependable tool for monitoring biodiversity and reconstructing phylogenetic data (Pei et al. 2017). The purpose of phylogenetic studies is to recreate the evolutionary history of a species or taxon (Jarvis et al., 2017), which is represented by a tree-like diagram (Ramos et al. 2021).

Earlier research on the family Lutjanidae employed the COI gene for genetic analysis (Gold et al. 2015; Bakar et al. 2018; Fadli et al. 2020; Shan et al. 2021; Halim et al. 2022). Researchers also used the COI gene to identify a new species of red snapper (*L. papuensis*) in the Cendrawasih Bay of Papua New Guinea (Allen et al. 2013). In this study, we gathered Lutjanidae specimens from fish landing places in Yapen. We identified the species based on their morphological and molecular characteristics. The current study aimed to ascertain the number of species within the family Lutjanidae and to reconstruct a phylogenetic tree to establish the evolutionary link between species.

#### MATERIALS AND METHODS

#### Study sites

 We conducted this study in Yapen Regency, Papua, between June and August of 2022 (Figure 1). Extraction, electrophoresis, and amplification were conducted at the Genetic Laboratory of *Universitas Bengkulu*. Additionally, we collected data for 16 Lutjanidae nucleotide sequences from the National Center for Biotechnology Information (NCBI (https://www.ncbi.nlm.nih.gov) (Table 1).



Figure 1. Sampling locations in the Yapen Regency

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Table 1. Lutjanidae sequence from NCBI

Species	Location	Access code		
Lutjanus vitta	Indonesia: Java	MH085866		
Lutjanus vitta	Malaysia	MG002627		
Lutjanus decussatus	Indonesia: Maluku, Ambon Island, Ambon	MN870144		
Lutjanus ehrenbergii	Indonesia: Maluku, Ambon Island, Ambon	MN870091		
Lutjanus ehrenbergii	Indonesia: Maluku, Ambon Island, Ambon	MN870134		
Lutjanus rufolineatus	Indonesia	GU673676		
Lutjanus rufolineatus	Indonesia: Maluku, Ambon Island, Ambon	MN870325		
Lutjanus fulvus	Philippines: Aurora, Region 3	KF009613		
Lutjanus fulvus	Indonesia	MK256673		
Lutjanus malabaricus	Philippines: Aurora, Region 3	KF009618		
Lutjanus erythropterus	Indonesia	GU673841		
Lutjanus erythropterus	Australia	GU673202		
Lutjanus erythropterus	Malaysia	MG002616		
Pristipomoides multidens	Australia: West Coast	MK092068		
Aphareus rutilans	China	NC063973		
Aphareus rutilans	China	ON152703		

# Sampling method

Samples were selected using purposive random sampling. Snappers were obtained from Fish Landing Places (TPI), fish markets, and fishing spots. Early morphological identification referred to the identification book of Moore & Colas (2016); White et al. (2013). One centimeter of red snapper dorsal fin tissue was removed and placed in a tube containing ethanol 80%.

#### Morphological identification

The identification of red snapper samples was based on their morphometric and meristic characteristics. We measured and photographed the weight, total length, standard length, number of pectoral fin spines, number of dorsal fin spines, and number of anal fin spines of each specimen.

# Extraction, amplification, and sequencing

DNA extraction followed the instructions from the Geneaid gSYNCTM DNA extraction kit. Amplification of the cytochrome oxidase subunit I (COI) gene used a set of COI primers developed by A. Ward et al. (2005): F1 5'-TCA ACC AAC CAC AAA GAC ATT GGC AC-3' dan R1 5'-TAG ACT TCT GGG TGG CCA AAG AAT CA-3'. PCR mix Go Taq Green Master Mix consisted of Go Taq Green 25 µL, 1.5 µL DNA template, 19.5 µL nuclease free water, and 5 µL primer. The thermal cycle setting was 95°C for 4 minutes during initial denaturation, followed by 35 cycles of denaturation at 95°C for 30 seconds, annealing at 54°C for 45 seconds, elongation at 72°C for 1 minute, post PCR at 72°C for 7 minutes. The amplification results were electrophoresed to visualize the presence of DNA in the PCR product. The purified PCR results were then sent to 1st BASE Sequencing Service Sdn. Bhd. (Malaysia) for sorting purposes.

#### Data analysis

The DNA sequencing results were aligned and edited using MEGA X software. The sequence data were then matched with the database available online at the NCBI (National Center for Biotechnological Information) genbank (www.ncbi.nml.nih.gov). The BLAST (Basic Local Alignment Searc Tool) method was used to assess the homology between the sequences from the study sample and the genetic data sequences stored in the genbank (Toha et al. 2020). BLAST results were tabulated to show the identity of the study sample. Genetic distance analysis and phylogenetic tree reconstruction were carried out using the Neighbor Joining Method, Kimura-2 parameter model and bootstrap value of  $1000 \times$  with the help of MEGA X software.

#### RESULTS AND DISCUSSION

# 111 Result

Morphological character analysis

The morphometric and meristic characteristics of the Lutjanidae red snappers found in this study are described in Table 2. The morphological identification results showed that nine species belonged to the genera *Lutjanus*, *Aphareus* and *Pristipomoides*. *L. vitta*, *L. decussatus*, *L. rufolineatus*, *L. malabaricus*, *L. erythropterus*, *L. ehrenbergii* and *L. fulvus* 

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Comment [A9]: 80% ethanol

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Comment [A12]: Results

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belong to the genus *Lutjanus*; *A. rutilans* comes from the genus *Aphareus*; and *P. multidens* belongs to the genus *Pristipomoides*. The three species with maximum and minimum length and weight are *L. erythropterus* (Max. 49.5 cm – Min. 45 cm), *P. multidens* (Max. 48.8 cm – Min. 42 cm) and *L. malabaricus* (Max. 29 cm – Min. 52 cm). Other species include *L. erythropterus* (Max. 1986 gr – Min. 1560 gr), *P. multidens* (Max. 1380 gr – Min. 804 gr) and *L. malabaricus* (Max. 2611 gr – Min. 378 gr).

Table 2. The measurement of the morphometric and meristic characteristics of the Lutjanidae family

Species	Min. Length (cm)	Max. Length (cm)	Min. Wight (gr)	Max. Wight (gr)	Pectoral Fin	Dorsal fin	Dorsal rays	Anal spins	Anal rays	Caudal fin
L. vitta	22.6	27.3	137	300	14-16	IX-X	13-14	III	8-9	Emarginate
L. rufolineatus	22.7	25.9	252	312	16-17	XI	13	III	9	Emarginate
L. malabaricus	29	52	378	2611	16-17	XI	14-15	III	9-10	Truncate
L. erythropterus	45	49.5	1560	1986	16	XI	15-16	III	10	Truncate
L. ehrenbergii	17.7	22.3	89	200	14-15	IX-X	14	III	9	Truncate
L. fulvus	19	21.1	133	174	16	X	15	III	9	Emarginate
L. decussatus	17.8	17.9	96	102	16	X	14	III	9	Emarginate
A. rutilans	31	32.7	276	283	15-16	XII-XI	9-10	III	8	Forked
P. multidens	42	49.8	804	1380	16	IX-X	11	III	8	Forked

#### Molecular identification

 Molecular identification was conducted using the Basic Local Alignment Search Tool at the National Center for Biotechnology Information. Table 3 displays the results of the molecular identification. The identification results indicate that the DNA sequences of the nine species share a high degree of similarity (99.68% to 100%) with the NCBI database.

Table 3. The BLAST species data at GenBank National Center for Biotechnology Information (NCBI)

Specimen code	Species	Query Cover	Similarity	Accession	
BKS06	Lutjanus Malabaricus	99%	99.53%	KF009618	
BKS36	Lutjanus ehrenbergii	97%	99.54%	KP194151	
BKS47	Lutjanus fulvus	99%	99.68%	KF009613	
BKS29	Lutjanus vitta	99%	99.84%	NC042930	
BKS07	Lutjanus erythropterus	97%	100%	GU673202	
BKS12	Lutjanus rufolineatus	97%	100%	MN870075	
BKS17	Lutjanus decussatus	96%	99.84%	MN870144	
BKS18	Aphareus rutilans	100%	99.53%	KF009564	
BKS21	Pristipomoides multidens	99%	99.84%	KF430626	

#### Genetic distance analysis

Genetic distance analysis was conducted using the Kimura-2 parameter method and resulted in variations in genetic distance between nine species of red snappers. The largest genetic distance was found between *L. fulvus* and *P. multidens*. Meanwhile, *L. vitta* and *L. ehrenbergii* were discovered to have the smallest genetic distance. A small genetic distance number indicates a high level of morphological similarity of the observed species (the smaller the distance, the higher the morphological similarity of the observed species and vice versa).

Table 4. Genetic distance among collected red snappers of the Lutjanidae family

		1	2	3	4	5	6	7	8
1	BKS29_L. vitta								
2	BKS23_L. rufolineatus	0.115							
3	BKS47_L. fulvus	0.122	0.090						
4	BKS07_L. erythropterus	0.192	0.161	0.167					
5	BKS36_L. ehrenbergii	0.086	0.121	0.129	0.179				
6	BKS06_L. malabaricus	0.178	0.166	0.172	0.113	0.149			
7	BKS17_L. decussatus	0.082	0.125	0.125	0.165	0.097	0.176		
8	BKS18_A. rutilans	0.174	0.172	0.173	0.160	0.171	0.170	0.175	
9	BKS21_P. multidens	0.182	0.193	0.205	0.187	0.193	0.201	0.180	0.129

#### Phylogenetic analysis

Reconstruction of the phylogenetic tree was conducted using the Neighbor Joining Method, Kimura-2 parameter model and bootstrap value of  $1000 \times$  with the help of MEGA X software. The phylogenetic tree shows the genetic relationships and evolutionary history between species or taxa based on current molecular data (Jarvis et al. 2017). The phylogeny tree was constructed from the nine individual sequences obtained from this study and sixteen individual sequences from the GenBank (Table 1). We added sixteen individual DNA sequences from various countries to strengthen the position of the sequences obtained in this study.

Comment [A14]: Wording isn't clear here. What do you mean? Surely this would be based on the age of the specimenns

**Comment [A15]:** Explain clearly what numbers 1-8 are

#### Discussion

 Morphological characteristics

This study discovered members of the genus *Lutjanus* with small to large bodies. Species in the genus *Lutjanus* have an oval and deep body. They are also slender and fusiform in shape (Allen, 1985). Species belonging to the genus *Lutjanus* have a continuous dorsal fin with a tiny incision between the thorny and soft parts. The red snappers vary in appearance, typically having a reddish, yellow, gray, or brown background with a darker pattern of stripes or stems and are frequently distinguished by a huge blackish spot on the upper side beneath the dorsal fin. Some species, including *L. vitta*, *L. decussatus*, *L. ehrenbergii*, and *L. rufolineatus*, have black spots and stripes on their bodies. *L. vitta* is defined morphologically by diagonal lines above and horizontal lines below the lateral line. From the eye to the tail fin, a brownish-yellow stripe is present on this species. Moreover, *L. decussatus* is defined morphologically by the presence of eight bands and a big black patch at the base of the caudal fin. *L. ehrenbergii* is distinguished morphologically by the presence of three to four horizontal lines of variable width on the underside of the scales and a prominent black spot under the dorsal fin. *L. rufolineatus* is distinguished by the presence of 7-8 yellow horizontal stripes. In this study, we did not see any black spots and horizontal and vertical stripes in *L. fulvus*, *L. malabaricus* or *L. erythropterus*. The number of pectoral fin soft spines in the *Lutjanus* genus varies between 14-17, while the number of dorsal fin spines ranges between IX-XI and 13-14 soft spines. All species had the same number of hard spines on the anal fin, namely III, and 8-10 soft spines. Each member of the genus *Lutjanus* has a truncated and emarginate tail.

Those of the genus *Lutjanus* have a distinct body form than members of the genus *Aphareus*. Red snappers belonging to the genus *Aphareus* have an elongated and fusiform body (Allen, 1985). *A. rutilans* is one of the *Aphareus* species identified in this research. In addition to its body shape, *A. rutilans* lacks canines and vomerine teeth, unlike members of the genus *Lutjanus*. *A. rutilans* has little teeth in its jaws. The *Aphareus* genus has a forked tail, in contrast to the *Lutjanus* genus, which has a tail that is between emarginate and truncate. *A. rutilans* has a continuous dorsal fin with no grooves between the hard and soft spiny sections. The final dorsal and anal fins are stretched out.

The body of *Pristipomoides* species is comparable to that of *Aphareus* species. This study recognized *P. multidens* as one of the species from the genus *Pristipomoides*. *P. multidens* and *A. rutilans* have continuous dorsal fins, with no grooves between the hard and soft spines. Both species possess prolonged dorsal and anal fin rays in addition to a tail fork. *P. multidens* possesses canines and vomerine teeth on the front of its jaw.

The above description demonstrates that distinct morphological traits identify species from the genera *Lutjanus*, *Aphareus*, and *Protipomoides*, as well as species within the same genus. Nevertheless, the species of the three genera share similar meristic traits. Molecular analysis can clarify the species-level differentiation of these fish groups.

Morphology molecular agreement in genetic distance

Our current findings show that the two species of red snappers, *L. ehrenbergii* and *L. vitta*, have the smallest genetic distance value (0.086). A small genetic distance value suggests that two species share a close genetic kinship. Our findings indicate that the two species share a similar ancestor. There is a horizontal line on the body of both species, with a huge black spot on the body of *L. ehrenbergii* and a large yellow spot on the underside of the soft dorsal fin of *L. vitta*. Indentations exist between the hard and soft spiny portions of each species. The shape and number of spines on the anal fins of both species are likewise similar (Figure 2).

The farthest genetic distance (0.205) was found between *L. fulvus* and *P. multidens*. In this study, a high genetic distance score suggests a significant difference between physical traits. This is demonstrated by the morphological differences between *L. fulvus* and *P. multidens*. *P. multidens* has a moderately extended body shape, whereas *L. fulvus* has an oval body that ranges from relatively deep to thin and fusiform in shape. *P. multidens* possesses a continuous dorsal fin with no grooves separating the hard and soft spines. Both the dorsal and anal fins are stretched out. In contrast, *L. fulvus* has a small incision between the thorny and soft parts of the dorsal fin. In addition, *L. fulvus* has an emarginate tail while *P. multidens* has a forket tail. *L. fulvus* has a reddish yellow body color and yellow spots on the tops of its eyes, but *P. multidens* has a yellowish to pale pink body color. There are five or six golden dashed lines found on the body of *P. multidens*. The species also has a pair of gold and blue stripes on its snout and cheeks.

Comment [A16]: Include citation

Comment [A17]: Cite here





Lutjanus ehrenbergii

Lutjanus vitta





Lutjanus fulvus

Pristipomoides multidens

Figure 2. Comparison of morphological characters of some Lutjanidae species

Phylogenetic tree

 $\frac{187}{188}$ 

The phylogenetic analysis in this study resulted in four main clades, these clades have bootstrap values ranging from 96 – 99% (Figure 3). The first, second, and third clades are members of the genus *Lutjanus*, whilst the fourth clade is composed of members from the genera *Pristipomoides* and *Aphareus*. Each clade consists of descendants of a common ancestor. In our investigation, *L. vitta*, *L. decussatus*, and *L. ehrenbergii* formed the first clade. All three species are descended from a common ancestor. The three species share the same physical characteristics, which include an oval body shape and a slim build. These species have similar canines and body spots. The second clade is composed of the species *L. fulvus* and *L. rufolineatus*. Both share a common ancestor. The two species possess an oval body shape; nevertheless, their body depth is greater than that of species in the first group. *L. malabaricus* and *L. erythropterus* constitute the third clade. Both species have the same lineage and have comparable morphologies. The discovered individuals can be differentiated by the shape of their snouts.

The Neighbor Joining method for constructing the phylogenetic tree can boost the results of the genetic distance analysis. *L. ehrenbergii* and *L. vitta* have the smallest genetic distance value, forming a paraphyletic clade with a bootstrap value of 97%. *L. fulvus* and *P. multidens*, which generated polyphyletic tree branches, were discovered to have the highest genetic distance value. We did not find any new variation after several sequences from GenBank were combined in the phylogenetic analysis.

Implications of molecular analysis for fisheries resource management

The paradigm shifts in fisheries resource management, from traditional species-based management to the Approach to Fisheries Management Ecosystem (AFME) (Staples et al. 2014; Hutubessy and Mosse, 2015), do not eliminate the importance of understanding the characteristics of the managed resource species. It is believed that disregard for species-level resource characteristics has slowed the application of AFME in fisheries resource management (Howell et al. 2021). The significance of species-level characteristics in fish management lies in the fact that each fish species responds differently to the applied management intervention. For instance, the reaction to fishing effort influences catch size variations.

Reef fish, especially Red Snappers, are the dominant species of fish captured by fishers in Yapen waters. Red snappers, particularly *L. malabaricus*, *L. erythropterus*, and *P. multidens*, have a substantial economic value. To satisfy local market demand, intense fishing is conducted. The information on size structure of each individual fish is vital for fish management in Yapen seas, among other places. Table 5 indicates the optimal size for capturing *L. malabaricus* and *L. erythropterus*. In the meantime, the size of the captured *P. multidens* individuals did not meet the optimal harvest size, possibly due to their immature gonads.

L. rufolineatus individuals caught in this study had reached the second stage of gonadal maturity. Some L. ehrenbergii individuals had reached the first stage of gonadal maturity, whereas others had not. In the meantime, the size of captured L. vitta, L. fulvus, and A. rutilans had not yet reached the size of the fish to attain gonadal maturity. This finding cannot be inferred with certainty, however, because the size of the fish that initially experienced gonadal maturation is affected by changes in environmental factors. Excessive and indiscriminate fishing can reduce the size of fish in the wild.

**Comment [A18]:** Why were these specific species selected? Remove the red underline from the images.

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Table 5. Maximum size, optimal harvest size and first gonadal maturity of the Lutjanidae family

Species	Lmax	Lopt	Lmat	Wmat	Reference
L. malabaricus	54	39	29	1822	Mous et al. (2021)
L. erythropterus	70	50	37	773	,
L. vitta	43	31	23	174	
A. rutilans	120	85	64	2129	
P. multidens	92	66	49	1356	
L. fulvus	40	-	21.45	-	Hassana et al. (2022)
3	-	_	22.5 (FL)	_	Shimose and Nanami (2014)
L. rufolineatus	-	_	14.6 – 18.1 (FL)	_	Taylor et al. (2018)
L. ehrenbergii	35	_	20.1	_	Allen (1985)
L. decussatus	35	-	-	-	•

Note: Lmax = maximum attainable total length at Indonesian lattitudes. Lmat = Length at maturation (cm). Lopt = Optimum Harvest Size (cm). Wmat = Weight at maturation in gram. FL = Fork Length

Another impact of overfishing is the loss of biodiversity, both at the species and molecular level. Our research provides basic information about the types of red snappers traded in the local market. This information can be used as a database for the presence of red snapper species in Yapen waters. We identified red snappers using morphological and molecular analysis approaches, so the results were perfectly accurate. Combining morphological and molecular approaches can reduce taxonomic ambiguity in observed species (Dwifajri et al. 2022). The combination of the two approaches is very much needed because of the high morphological similarity between red snapper species within one genus and between genera.

Data on taxonomic certainty can be used to create maps of species biodiversity, identify species with the potential for aquaculture development and high economic value, and formulate legislative protection strategies for endangered species. Currently, the central government has established a fully exploited status for Red Snapper fishing in the waters of Cenderawasih Bay, including Yapen Island and its surroundings. This status is solely granted to red snappers in general, but the Indo-West Pacific is home to forty-three species of red snappers. Alternatively, distinct species may have varying susceptibilities to capture. Consequently, conservation must occur not only at the family or genus level, but also at the species level. According to the above description, the success of a fishery resource management depends on information on the target species. To accurately determine the characteristics of a species, phylogenetic studies using a molecular method are necessary to trace species.

Comment [A19]: How was maturation identified? Discuss clearly

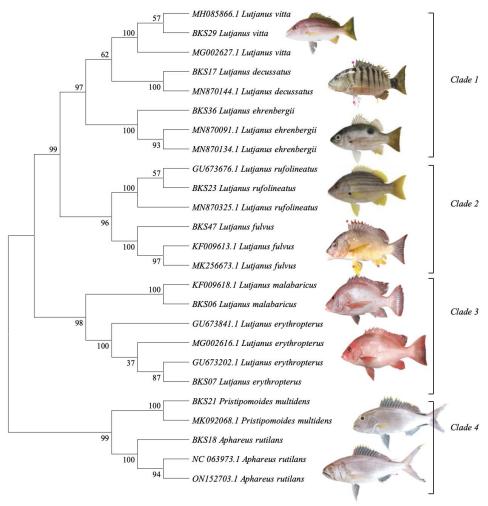


Figure 3. Evolutionary relationships of taxa Lutjanidae in Yapen Regency

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# ACKNOWLEDGEMENTS

We would like to thank the Ministry of Education, Culture, Research and Technology for funding this study through a research scheme with DIPA Directorate of Research, Technology and Community Service Directorate General of Higher Education, Research and Technology Ministry of Education, Culture, Research and Technology Number: 235/E5/PG.02.00.PT/2022. Research contract number No. 235/E5/PG/02.00.PT/2022 (dated May 30, 2022) and derivative contract No. 190.c/UN42.15/PG/2022 (June 7, 2022).

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**Comment [A20]:** Uuseful content here but provide a legend for evolutionary distance and highlight which of these are your own findings

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- Comment [A21]: Are the author intiials available?
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