Morphological and Genetic Characteristics of Red Snapper (*Lutjanidae*) in Nabire Waters

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Abstract

The Lutjanidae family has a wide range of varieties, posing challenges in their morphological identification. Molecular identification is crucial for augmenting the current morphological data as a comprehensive database for documenting the presence of economically significant fish species in Nabire water. This article provides a comprehensive analysis of the morphological and genetic characteristics of red snapper species, as well as assessment of the evolutionary connections among snapper fish found in the water. This study aims to analyze the morphological and genetic characteristics of red snapper species and assess the evolutionary relationships among red snapper found in the sea waters. The DNA extraction procedure was conducted according to the instructions provided by the Geneaid gSYNC DNA extraction kit. The molecular marker used is the DNA barcode of the cytochrome oxidase subunit I (CO1) gene. A total of 29 individuals were identified, representing 8 species, namely Lutjanus timoriensis, Lutjanus gibbus, Lutjanus bohar, Lutjanus papuensis, Pinjalo lewisi, Etelis coruscans, Pristipomoides multidens, and Aphareus rutilans. The molecular analysis indicated that there was a fragment length of 620 base pairs (bp). P. multidens and L. gibbus had the greatest genetic distance (0.22), whilst the species L. bohar and L. gibbus had the smallest genetic distance (0.11). The phylogenetic tree reconstruction yielded 8 monophyletic clades. Based on morphological and genetic analysis, eight species of the Lutjanidae family were identified in Nabire waters. Research is needed on biological parameters such as size when first caught, optimum length of capture and size when first gonad mature.

Keywords: *Papua, Lutjanus, Phylogenetic, CO1, mtDNA*

Introduction

Nabire is one of the cities located in the Cenderawasih Bay National Park Area (TNTC), Papua Island. This conservation area has more than 209 species of fish, one of which is snapper (*Lutjanidae*) (Suraji *et al*., 2015). Snappers are highly economical, making them a favorite fishing target, result in high fishing intensity targeting snapper. The level of utilization of coral fishery resources (Red Snapper and Grouper) in TNTC sea waters and surrounding waters is at the level of excessive utilization (Overfishing) (Decree of the Minister of Maritime Affairs of the Republic of Indonesia No. 19/2022).

The genus *Lutjanus* Bloch 1790 is the Lutjanidae family with the largest number of species. Snappers, belonging to the Lutjanidae family, are demersal fish that typically inhabit coral or coral reefs. They can be found in either large or small groups, and occasionally spend solitary lives (Allen, 1985). There are 44 species of the genus *Lutjanus* found in the Indo-West Pacific region (Allen *et al.,* 2013; Iwatsuki *et al.,* 2015). The Lutjanidae family lives in coral reef waters and is distributed in the East Pacific and West Indo-Pacific, East and West Atlantic (Souza *et al*., 2019).

Snappers possess distinct morphological features, including a sizable oral cavity, pointed canines, and a tail that is either blunt or divided into two prongs (Andriyono *et al*., 2020). Snappers are typically categorized into two distinct types: red snappers and white snappers. The primary attribute of red snappers is its predominant body color, which is a combination of red and white. Some snapper species such as *L. malabaricus, L. timoriensis* and *L. erypthropterus* have comparable physical traits that pose a challenge in direct differentiation (Pranata *et al*., 2024). Red snappers are difficult to differentiate using morphological analysis alone. Therefore, doing

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molecular analysis is essential to supplement the existing morphological data.

DNA sequence analysis has been employed to aid in the identification of species that provide challenges in terms of morphological traits recognition. Utilizing the genes for cytochrome c oxidase subunit 1 (CO1) in mitochondria as genetic markers to accurately identify and classify specific species (Powers *et al*., 2018). Bingpeng *et al*. (2018) state that molecular identification is a valuable tool for reliably differentiating species that share similar external morphological traits, which are challenging to separate based solely on their physical characteristics.

Sala *et al*. (2023) conducted research on the combined analysis of nine species of snappers (*L. vitta*, *L. decussatus*, *L. ehrenbergii*, *L. rufolineatus*, *L. fulvus*, *L. malabaricus*, *L. erytropterus*, *Pristipomoides multidens*, and *Aphareus rutilans*) by molecularly identifying using the Cytochrome Oxydase-1 (CO1) marker. In addition, Irmawati *et al*. (2020) investigated the morphometric properties of barramundi (*Lates calcarifer*) in the coastal waters of Bone, Wajo, Takalar, and North Kalimantan. A study on the morphometrics diversity and phenotypic relationship of red snappers has been carried out in the northern waters of Papua (Sala *et al.,* 2022). Pranata *et al.* (2024) used the CO1 gene to understand genetic connectivity between populations of the genus Lutjanus. No molecular identification of snappers in Nabire waters has been conducted. Therefore, it is crucial to examine the morphological and genetic characteristics of red snappers as well as investigate the kinship (*phylogenetic*) of snappers in Nabire sea waters.

Materials and Methods

The collection of snapper samples was conducted using a purposeful random sampling technique. Snapper specimens were gathered from various sites in traditional market of Nabire, including the Smoker Fish Market, Tapioca Afternoon Market, Oyehe Central Market, and Kalibobo Central Market (Figure 1). Subsequently, snapper morphometrics were measured at the aquatic resources laboratory, in the Faculty of Fisheries and Marine Sciences, University of Papua. In addition, molecular analysis was conducted at the BRIN Biosystematics and Evolution Research Center.

Data collection of snapper morphology and morphological data tabulation

The snapper samples collected at the research site were subsequently identified based on their morphology. The initial morphological identification referred to the identification book authored by Moore and Colas (2016). The red snapper samples were distinguished by analyzing their morphometric and meristic traits. Photographs were taken of each red snapper sample, and their morphometric and meristic traits were then measured. The morphological analysis involved the description of snapper fish characteristics, including body color, fin count, and tail shape (Sala *et al*., 2023). The morphometric variables assessed in this study included the overall length, standard length, and weight of the fish.

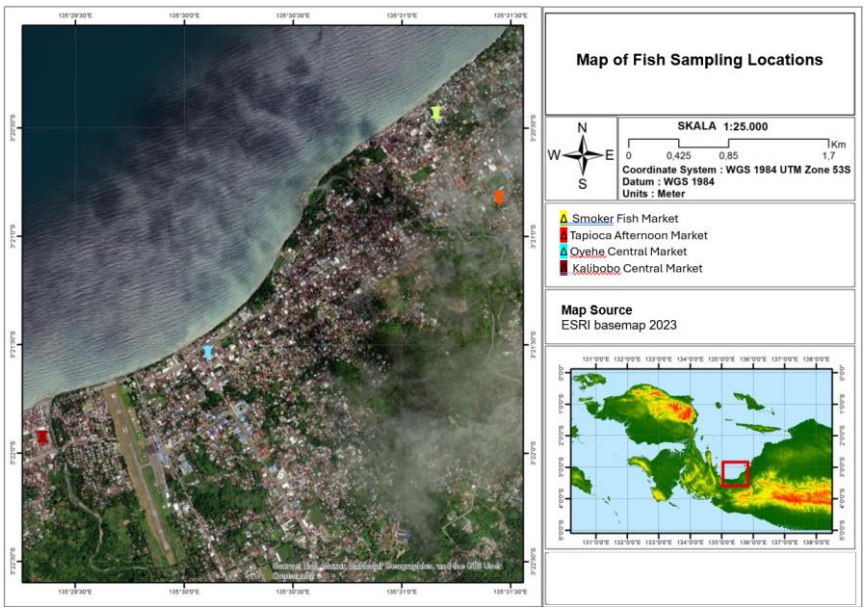

Figure 1. Red Snapper sampling sites

Table 1. Morphometric Characteristics of Red Snapper (*Lutjanidae*) found in Nabire waters

Species	Number of Individuals	Dorsal Fin	Dorsal Rays	Anal Spins	Anal Rays	Caudal Fin
Lutjanus gibbus	7	X	15	III	9	Truncate with rounded lobes
Lutjanus papuensis	3	X	14-15	III	9	Truncate
Lutjanus bohar	3	X	14-15	III	9	Truncate
Lutjanus timoriensis	10	XI	14-17	III-IV	9	Truncate
Aphareus rutilans	1	X	11	III	8	Forked
Etelis coruscans	1	IX	12	III	8	Forked
Pinjalo lewisi	3	XI-XII	12-14	III	8-9	Truncate
Pristipomoides multidens	1	X	14	III	8	Forked

Data collection of snapper genetic characteristics

Before conducting genetic analysis, a 2 cm section from the dorsal fins of a snapper fish that had previously undergone morphometric measurements was removed. The fish fin sample was purified using mineral water. Prior to being immersed in a tube holding a solution consisting of 70% alcohol, the tube containing the specimen was thereafter assigned a unique identifier in the format of the researcher's initials, the fish species, the location of the sample, and the sample number. The DNA extraction procedure was conducted according to the instructions provided by the Geneaid gSYNC DNA extraction kit. The amplification of the cytochrome oxidase subunit I (CO1) gene was performed using a specific set of CO1 primers designed by Ward *et al*. (2005): F1 5′-TCA ACC AAC CAC AAA GAC ATT GGC AC-3′ and 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 1.5 µL each primer. Thermal cycle settings were set at 95C for 4 min initial denaturation, followed by 35 cycles of denaturation at 95C for 30 sec, annealing at 54C for 45 sec, elongation at 72C for 1 min, post PCR at 72C for 7 min. The PCR results were then observed using a 1% agarose gel. This electrophoresis process was carried out using a voltage of 100 volts for 30 min and visualized with a UV transilluminator. Samples that had been declared successful underwent screening at 1st Base in Malaysia.

Data analysis

MEGA X software was used to perform sequence editing and alignment. The data sequence was then matched to the database available on the NCBI (National Center for Biotechnology Information) (Kumar *et al*., 2018). BLAST (Basic Local Alignment Search Tool) method was used for finding the similarity of the species found in the present study with the species available in the GenBank (*available online at www.ncbi.nml.nih.gov*). Kimura 2-parameter was used to analyze the genetic distance between species with 1,000x bootstrap replication. The phylogenetic tree was obtained using the neighbor – joining method with Bootstrap 1000x, using Mega XI software (Tamura *et al.,* 2021).

Result and Discussion

Morphological characteristics

Based on the results of morphological identification, 29 red snapper individuals were found belonging to eight species from five genera and two subfamilies of snapper in Nabire waters. The snapper species that were successfully identified included *Lutjanus papuensis*, *Lutjanus timoriensis*, *L. gibbus*, *L.bohar*, *P. lewisi*, *E. coruscans*, *A. rutilans*, and *P. multidens*. An analysis of the snapper's morphometric parameters was conducted (Table 1).

These results add to the number of species and genera of red snapper (Lutjanidae) found in Papuan waters from previous studies (Sala *et al*., 2022; Sala *et al*., 2023). Therefore, the total number of species in the genus *Lutjanus* in the Indo-Pacific is 44 (Iwatsuku *et al*., 2015) and 15 species in which (34%) of them, are found in Papuan waters, including those from this study. In this study, a species of *L. gibbus* with the following morphometric characteristics: dorsal rays X, 15; anal rays III, 9; caudal fin truncate with rounded lobes. These results, however, differ from the description of (Thi *et al*., 2015).

Morphometric characters of *L. papuensis* that include dorsal rays X, 14–15; anal rays III, 9; and caudal fin truncate in this study differ from the findings of Allen *et al*. (2013): dorsal rays (X. 13), anal rays (III, 8), and caudal fin slightly emarginate. L. bohar has the following morphometric characters: dorsal rays X, 14–15; anal rays III, 9; caudal fin truncate. This species is quite similar to the morphometric characters described by Allen (1985):

dorsal spines (total) 10; dorsal soft rays (total) 13–14; anal spines 3; and anal soft rays 8.

Lutjanus timoriensis has the following morphometric characters: dorsal rays XI, 14–17; anal rays III–IV, 9; caudal fin truncate. This morphometric character was similar to the short description of Allen (1985). *A. rutilans* possessing the subsequent morphometric characters: dorsal rays X, 11; anal rays III, 8; caudal fin forked. The dorsal fin and dorsal rays of the *A. rutilans* species in this research are different from the findings in Nabire Waters (Sala *et al*., 2023).

Etelis coruscans has the following morphometric features: dorsal rays XI, 12; anal rays III, 8; and a forked caudal fin. These morphometric characters differed from those found by Allen (1985), especially in dorsal spines (total) 10 and dorsal soft rays (total) 11. The morphometric characteristics of *P. lewisi* are as follows: dorsal rays X, 14; anal rays III, 8–9; caudal fin truncate. These characteristics differ from those found by Randall *et al*. (1987), especially in dorsal spines (XII) and dorsal soft rays (13). *P. multidens* has the following morphometric characteristics: dorsal rays X, 14; anal rays III, 8; forked caudal fin. Sala *et al*. (2023) identified *P. multidens* as having dorsal rays IX-X, 11, which are different from this study.

Genetic characteristics

A genetic analysis was conducted on eight species of red snapper discovered in the sea waters of Nabire. These species included *L. papuensis, L. timoriensis, L. gibbus, L. bohar, P. lewisi, E. coruscans, A. rutilans* and *P. multidens*. The mitochondrial DNA markers—specifically, the CO1 gene—have proven to be a useful tool for identifying aquatic species of the Lutjanidae family (Fadli *et al*., 2024). Furthermore, the CO1 gene is widely used in DNA barcoding and is useful in species differentiation (Bakar *et al*., 2018; Nur *et al*., 2022).

The length of each sample fragment of CO1 gene was 620 base pairs (bp). The red snapper samples exhibited a similarity ranging from approximately 92.47% to 100% according to the GenBank data from the National Center for Biotechnology Information (NCBI) (Table 2). Bhattacharjee *et al*. (2012) classified species similarity in BLAST results into three categories. Results are considered significant if they reach 97% - 100% similarity with the database sequence, sufficient if they reach 92% - 96% similarity, and have no similarity if they are less than 91%.

Table 2. NCBI BLAST Results of Red Snapper (Lutjanidae) Analysis

No.	Samples	Species	*Query Cover (%)*	Similarity (%)	*Accession Number*
1	10_B2_F1	*Lutjanus gibbus*	98%	97.83%	MZ606201.1
2	11_C2_F1	*Lutjanus gibbus*	99%	100%	MF409615.1
3	12_D2_F1	*Lutjanus gibbus*	100%	100%	MF409615.1
4	13_E2_F1	*Lutjanus gibbus*	100%	100%	MF409615.1
5	14_F2_F1	*Lutjanus gibbus*	96%	99.84%	MZ606210.1
6	15_G2_F1	*Lutjanus gibbus*	97%	100%	MN870401.1
7	28_D4_F1	*Lutjanus gibbus*	85%	98.91%	KU943895.1
8	16_H2_F1	*Lutjanus papuensis*	97%	99.01%	HM422401.1
9	17_A3_F1	*Lutjanus papuensis*	97%	99.01%	HM422401.1
10	29_E4_F1	*Lutjanus papuensis*	97%	99.04%	HM422401.1
11	18_B3_F1	*Lutjanus bohar*	100%	100%	MZ606255.1
12	19_C3_F1	*Lutjanus bohar*	100%	99.84%	MZ606255.1
13	27_C4_F1	*Lutjanus bohar*	75%	99.58%	KY802100.1
14	20_D3_F1	*Lutjanus timoriensis*	99%	99.84%	KJ202176.1
15	21_E3_F1	*Lutjanus timoriensis*	99%	99.84%	KJ202176.1
16	22_F3_F1	*Lutjanus timoriensis*	98%	99.84%	OQ386744.1
17	25_A4_F1	*Lutjanus timoriensis*	98%	92.47%	OQ386866.1
18	34_B5_F1	*Lutjanus timoriensis*	98%	99.69%	KJ202176.1
19	35_C5_F1	*Lutjanus timoriensis*	98%	99.84%	KJ202176.1
20	36_D5_F1	*Lutjanus timoriensis*	97%	99.84%	OQ386744.1
21	23_G3_F1	*Pinjalo lewisi*	98%	99.68%	OQ387275.1
22	24_H3_F1	*Pinjalo lewisi*	82%	100%	LC484205.1
23	30_F4_F1	*Pinjalo lewisi*	97%	99.84%	OQ387275.1
24	26_B4_F1	*Aphareus rutilans*	97%	99.84%	EF609285.1
25	31_G4_F1	*Pristipomoides multidens*	96%	99.84%	MZ310705.1

Table 3. Average Nucleotide Composition of Red Snapper (Lutjanidae) in Nabire Waters

Spesies	T(U) %	C %	A %	G %	A+T Content %
Pristipomoides multidens	30,2	28,5	23,4	17,9	53,6
Pinjalo lewisi	28,6	30	24,8	16,6	53,4
Lutjanus timoriensis	28,9	29,8	23	18,3	51,9
Lutjanus papuensis	28,2	28,9	24,8	18,1	53
Lutjanus gibbus	30,3	27,2	25,2	17,3	55,5
Lutjanus bohar	30,5	26,4	25,5	17,6	56
Etelis coruscans	28,9	28,5	23,2	19,4	52,1
Aphareus rutilans	29,8	29,2	23,1	17,9	52,9

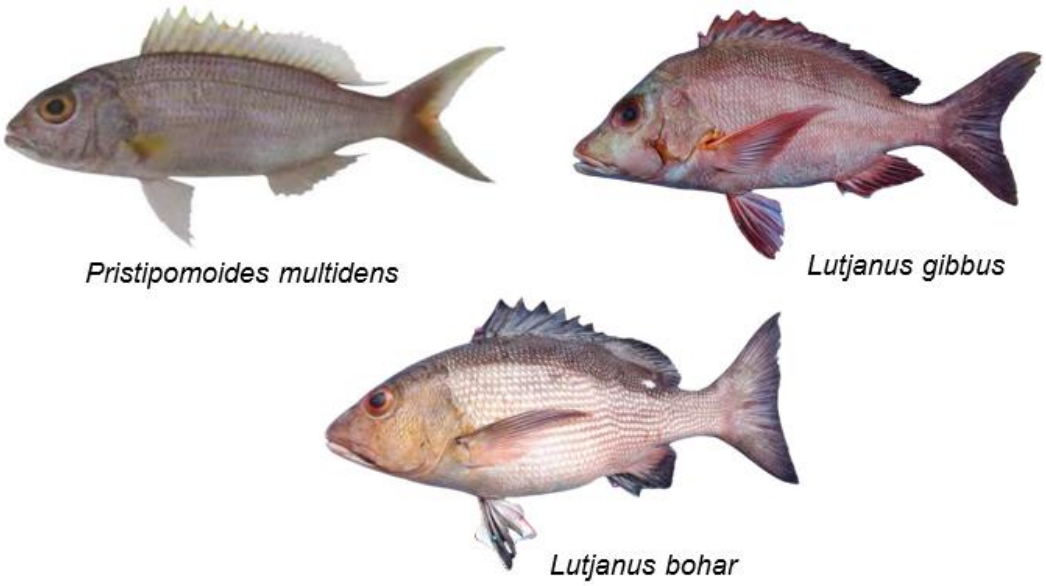

Figure 2. Morphological Forms of *P. multidens* (top left*), L. gibbus* (top right), *L. bohar* (bottom)

The nucleotide composition obtained exhibited varying contents. The greatest average found by examining the base composition of samples to categorize CO1 was T (Thymine), whereas the lowest average was G (Guanine) (Table 3).

The A+T composition of all snapper samples obtained exceeded the C+G composition by more than 50%. These findings align with the studies undertaken by Ramadan *et al*. (2023), which revealed an A+T composition above 50%. The nucleotide composition of *Lutjanus bohar* yielded a combined A+T content of 56%, whereas the study conducted by Ramadan *et al*. (2023) reported a combined A+T content of 51.24%. The variation in habitat allows for differences in composition. Ali *et al*. (2021) suggest that variations in nucleotide composition may arise as a result of a species' adaptations to its surroundings. Morphological differences can occur if fish species live in unique and different environmental conditions (Shuai *et al*., 2018).

Genetic distance

The genetic distance among red snapper individuals in Nabire sea waters varied from 0% to 0.08%, while the genetic distance between different species ranged from 0.11% to 0.22% (Table 4). This finding indicated that the genetic distance between the red snapper species was larger than the genetic distance between its individuals. The species *P. multidens* and *L. gibbus* exhibited the greatest genetic distance, with a score of 0.22. Conversely, the species *L. bohar* and *L. gibbus* displayed the lowest genetic distance of 0.11. The genetic distance between two species is directly proportional to the level of similarity between their sequence results. As the genetic distance increases between two species, the level of similarity between their sequence results also increases (Nei, 1972).

Among the two species that exhibited the greatest genetic distance, notable variations in morphological characteristics were evident,

particularly in terms of their distinct body structures. For instance, *P. multidens* shows a slender body morphology, but *L. gibbus* has a compact and broad body structure. In addition, *P. multidens* often has a tail with pointed ends, but *L. gibbus* generally possesses a tail tip that is more rounded in shape. *P. multidens* typically shows a silver body color, but *L. gibbus* tends to have a reddish body color (Figure 2). The two species that had a small genetic distance showed similar physical characteristics. Both species exhibited similar dorsal fin forms, anal fin shapes, pelvic fin shapes, and eye colors (Figure 2).

Phylogenetic tree

The phylogenetic analysis employed the *Neighbor Joining Tree* (NJT) approach, utilizing a bootstrap value of 1000x. The phylogenetic tree yielded two primary clades: the Lutjaninae clade and the Etelinae clade. The Lutjanidae family's sub-clade (Figure 3) has 8 distinct groups, with 7 of them having a bootstrap value of 100% and 1 group having a bootstrap value of 97%. These clades correspond to the number of species identified in this study. The clades are as follows: the first clade is *L. timoriensis*, the second clade is *P. lewisi*, the third clade is *L. bohar*, the fourth clade is *L. gibbus*, the fifth clade is *L. papuensis*, which belongs to the subfamily Lutjaninae, the sixth clade is *E. coruscans*, the seventh clade is *P. multidens*, and the eighth clade is *A. rutilans*, which belongs to the subfamily Etelinae.

Fadli *et al*. (2024) conducted a phylogenetic study of 15 species members of the *Lutjanus* genus using the CO1 gene. The CO1 gene was also using to phylogenetic analysis of some species belonging to the Lutjanidae family by Sala *et al*. (2023). Figure 3 demonstrates that the *P. multidens* clade and the *A. rutilans* clade are closely related based on the results of the phylogenetic tree reconstruction. This aligns with the findings derived from the research conducted by Sala *et al*. (2023). The phylogenetic tree analysis revealed that *P. multidens* and *L. bohar* were located in distinct subfamily clades, suggesting a substantial genetic distance (Table 4).

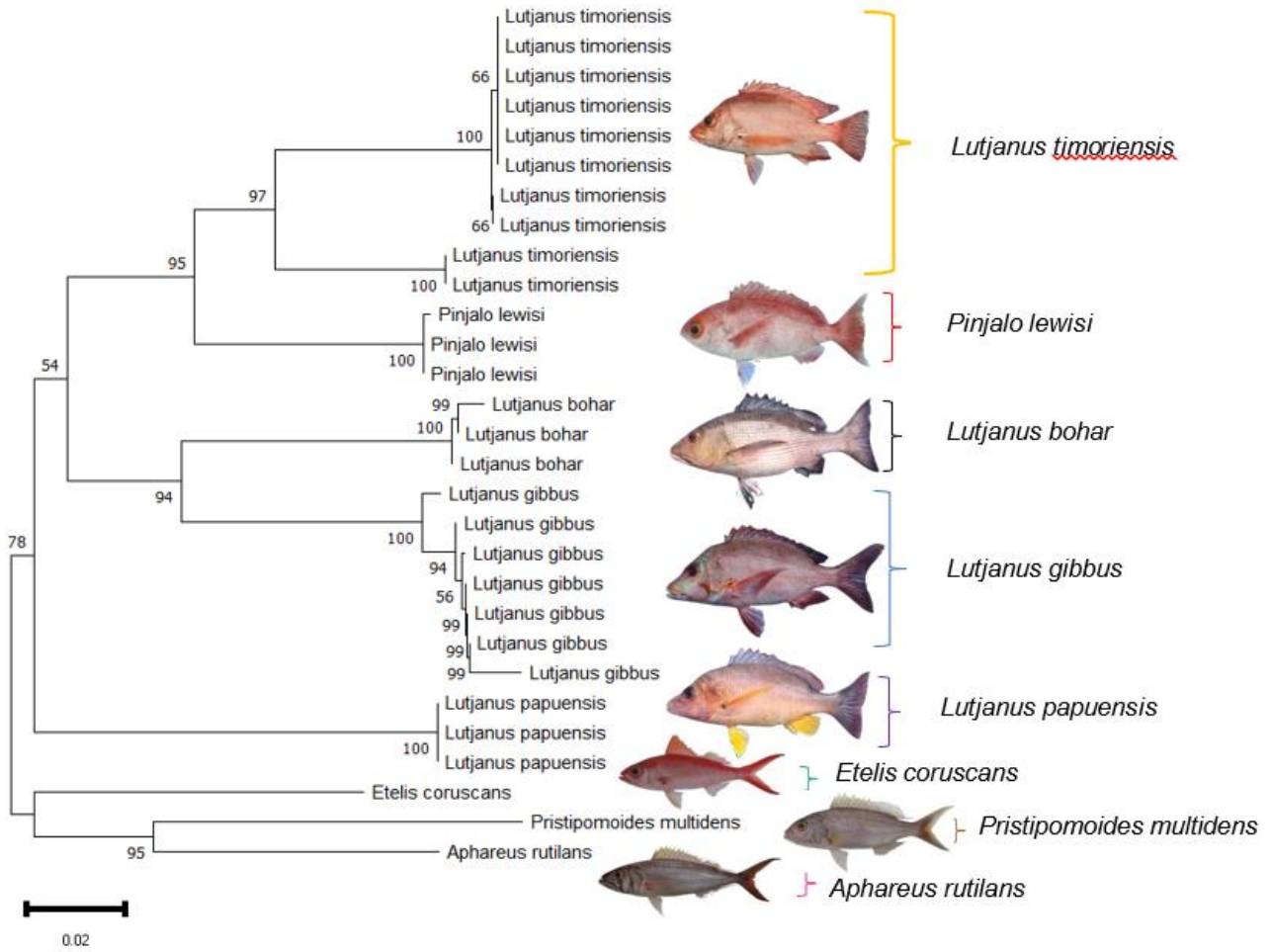

Figure 3. Phylogenetic tree reconstruction using the Neighbor Joining method with the Kimura-2 parameter model and Bootstrap 1000x

Table 4. Genetic Distance between *Lutjanus* Individuals and Species

No	Species	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
1	*P. multidens*																												
2	*P. lewisi*	0.18																											
3	*P. lewisi*	0.18	0.00																										
4	*P. lewisi*	0.18	0.00	0.00																									
5	*L. timoriensis*	0.20	0.09	0.09	0.09																								
6	*L. timoriensis*	0.20	0.11	0.11	0.11	0.08																							
7	*L. timoriensis*	0.20	0.11	0.11	0.11	0.08	0.00																						
8	*L. timoriensis*	0.20	0.11	0.11	0.11	0.08	0.00	0.00																					
9	*L. timoriensis*	0.20	0.11	0.11	0.11	0.08	0.00	0.00	0.00																				
10	*L. timoriensis*	0.20	0.11	0.11	0.11	0.08	0.00	0.00	0.00	0.00																			
11	*L. timoriensis*	0.20	0.09	0.09	0.09	0.00	0.08	0.08	0.08	0.08	0.08																		
12	*L. timoriensis*	0.20	0.11	0.11	0.11	0.08	0.00	0.00	0.00	0.00	0.00	0.08																	
13	*L. timoriensis*	0.20	0.11	0.11	0.11	0.08	0.00	0.00	0.00	0.00	0.00	0.08	0.00																
14	*L. timoriensis*	0.20	0.11	0.11	0.11	0.08	0.00	0.00	0.00	0.00	0.00	0.08	0.00	0.00															
15	*L. papuensis*	0.18	0.15	0.14	0.14	0.16	0.18	0.18	0.18	0.18	0.18	0.16	0.18	0.18	0.18														
16	*L. papuensis*	0.18	0.15	0.14	0.14	0.16	0.18	0.18	0.18	0.18	0.18	0.16	0.18	0.18	0.18	0.00													
17	*L. papuensis*	0.18	0.15	0.14	0.14	0.16	0.18	0.18	0.18	0.18	0.18	0.16	0.18	0.18	0.18	0.00	0.00												
18	*L. gibbus*	0.22	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.15	0.15	0.15											
19	*L. gibbus*	0.21	0.17	0.17	0.17	0.17	0.16	0.16	0.16	0.16	0.16	0.17	0.16	0.16	0.16	0.16	0.16	0.16	0.01										
20	*L. gibbus*	0.21	0.17	0.17	0.17	0.17	0.16	0.16	0.16	0.16	0.16	0.17	0.16	0.16	0.16	0.16	0.16	0.16	0.01	0.00									
21	*L. gibbus*	0.21	0.17	0.17	0.17	0.17	0.16	0.16	0.16	0.16	0.16	0.17	0.16	0.16	0.16	0.16	0.16	0.16	0.01	0.00	0.00								
22	*L. gibbus*	0.21	0.17	0.17	0.17	0.17	0.16	0.16	0.16	0.16	0.16	0.17	0.16	0.16	0.16	0.16	0.16	0.16	0.01	0.00	0.00	0.00							
23	*L. gibbus*	0.21	0.17	0.17	0.17	0.17	0.16	0.16	0.16	0.16	0.16	0.17	0.16	0.16	0.16	0.16	0.16	0.16	0.01	0.00	0.00	0.00	0.00						
24	*L. gibbus*	0.22	0.18	0.18	0.18	0.18	0.17	0.17	0.17	0.17	0.17	0.18	0.17	0.17	0.17	0.17	0.17	0.17	0.02	0.01	0.01	0.01	0.01	0.01					
25	*L. bohar*	0.20	0.17	0.17	0.17	0.16	0.17	0.17	0.17	0.17	0.17	0.16	0.17	0.17	0.17	0.16	0.16	0.16	0.12	0.11	0.12	0.12	0.12	0.12	0.12				
26	*L. bohar*	0.19	0.16	0.16	0.16	0.16	0.17	0.17	0.17	0.17	0.17	0.16	0.17	0.17	0.17	0.16	0.16	0.16	0.11	0.11	0.11	0.11	0.11	0.11	0.12	0.01			
27	*L. bohar*	0.19	0.16	0.16	0.16	0.16	0.17	0.17	0.17	0.17	0.17	0.16	0.17	0.17	0.17	0.16	0.16	0.16	0.11	0.11	0.11	0.11	0.11	0.11	0.12	0.00	0.00		
28	*E. coruscans*	0.16	0.15	0.15	0.15	0.17	0.17	0.17	0.17	0.17	0.17	0.17	0.17	0.17	0.17	0.16	0.16	0.16	0.18	0.19	0.18	0.18	0.18	0.18	0.20	0.18	0.17	0.17	
29	*A. rutilans*	0.13	0.15	0.15	0.15	0.17	0.19	0.19	0.19	0.19	0.19	0.17	0.19	0.19	0.19	0.17	0.17	0.17	0.18	0.19	0.19	0.19	0.19	0.19	0.20	0.18	0.17	0.17	0.16

Conclusion

The findings demonstrated that DNA barcoding to be a useful method for accurate fish identification. The results showed combining morphology and DNA barcoding analysis can effectively identify red snapper in Nabire, Papua. Twenty-nine red snapper individuals, taken from Nabire waters were morphologically and molecularly identified as *Lutjanus timoriensis, L. papuensis, L. bohar, L. gibbus, Pinjalo lewisi, Aphareus rutilans, Pristipomoides multidens,* and *Etelis coruscans. P. multidens* and *L. gibbus* exhibited the greatest genetic distance. Conversely, *L. bohar* and *L. gibbus* displayed the smallest genetic distance. The phylogenetic tree construction for *L. bohar* and *L. gibbus* revealed a monophyletic clade, indicating a close evolutionary relationship. It is supported by the high bootstrap values that indicate the robustness of the branches in each clade.

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References

Ali, F., Mamoon, A. & Abbas, E. 2021. Mitochondrial-based phylogenetic inference of worldwide species of Genus *Siganus*. *Egypt. J. Aquat. Biol. Fish.*, 25(1): 371–388. https://doi.org/10.21608/ejabf.2021.143930

Allen, G.R. 1985. FAO Species Catalogue. Vol. 6. Snappers of the world. An annotated and illustrated catalogue of lutjanid species known to date. *FAO Fish. Synop.*, 125(6): p.208.

Allen, G.R., White, W.T. & Erdmann, M.V. 2013. Two new species of snappers (Pisces: Lutjanidae: *Lutjanus*) from the Indo-West Pacific. *J. Ocean Sci. Foundation*, 6: 33-51. https://doi.org/10.5281/zenodo.103 6813

Andriyono, S., Novian, A.P., Laksmi, S., Andi, A.H., Jobaidul, A., Adrian, D. & Ahasan, H. 2020. 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.27.4.307-314

Bakar, A.A., Adamson, E.A.S., Juliana, L.H., Mohd, S.A.N., Wei-Jen, C., Man, A. & Md, D.N. 2018. DNA barcoding of Malaysian commercial snapper reveals an unrecognized species of the yellow-lined *Lutjanus* (Pisces: Lutjanidae). *Plos One,* 13: 1–12, https://doi.org/10.1371/journal.pone.0202945

Bhattacharjee, M.J., Laskar, B.A., Dhar, B. & Ghosh, S.K. 2012. Identification and evaluation of freshwater catfishes through DNA barcoding. *Plos One*, 7(11): e49950. https://doi.org/10.1371/journal.pone.0049950

Bingpeng, X., Heshan, L., Zhilan, Z., Chunguang, W., Yanguo, W. & Jianjun, W. 2018. DNA barcoding for identification of fish species in the Taiwan Strait. *Plos One,* 13(6): e0198109. https://doi.org/10.1371/journal.pone.0198109

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, A., 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. Ecodynamics*, 19(3): 787-794. https://doi.org/10.18280/ ijdne.190309

Irmawati, Tassakka, A.C.M.A.R., Nadiarti., Husain, A.A.A., Umar, M.T., Alimuddin, A. & Parawansa, B.S. 2020. Stock identification of Asian Seabass (*Lates Calcarifer* Bloch, 1790) using morphometric characteristics. *J. IPTEKS PSP*, 7(13): 42–52. https://doi.org/10.20956/jipsp.v7i13.8570

Iwatsuki, Y., Tanaka, F. & Allen, G.R. 2015. *Lutjanus xanthopinnis*, a new species of snapper (Pisces: Lutjanidae) from the Indo-west Pacific, with a redescription of *Lutjanus madras* (Valenciennes 1831), (Valenciennes). *J. Ocean Sci. Foundation*, 17: 23-42.

Kumar, S., Stecher, G., Li, M., Knyaz, C. & Tamura, K. 2018. MEGA X: molecular evolutionary genetics analysis across computing platforms. *Mol. Biol. Evol.*, 35(6): 1547-1549. https://doi.org/10.1093/molbev/msy096

Nur, N.F.M., Abdullah, S., Seah, Y.G., Mohd Nor, S.A.M., Tan, M.P., Habib, A., Jaafar, T.N.A.M. 2022. DNA barcoding of commercially important trevallies, *Carangoides* spp. (Carangiformes: Carangidae): a Baseline

report of species from Malaysia. *Thalassas: An Inter. J. Mar. Sci.*, 38: 227–253. https://doi.org/10.1007/s41208-022-00405-9

Moore, B. & Colas, B. 2016. Identification guide to the common coastal food fishes of the pacific Island region. Pacific Community. 140 pp

Nei, M. 1972. Genetic distance between populations. *The American Naturalist*, 106(949): 283-292. https://doi.org/10.10 8 6/282771

Pranata, B., Sala, R., Kusuma, A.B., Toha, A.H.A., Purbani, D.C., Mokodongan, D.F. & Sipriyadi. 2024. Genetic diversity and connectivity of Red Snapper *Lutjanus gibbus* in the Papua Waters, Indonesia. *Biodiversitas*, 25: 276-286. https://doi.org/10.13057/biodiv/d250 132

Pranata, B., Sala, R., Kusuma, A.B., Purbani, D.C., Mokodongan, D.F., Sipriyadi, S. & Azhar, M I. 2024. Morphological Characteristics and Genetic Relationship of Red Snappers (*Lutjanus timoriensis, Lutjanus malabaricus, Lutjanus erythropterus*) in Papuan Waters. *Ilmu Kelautan: Indonesian Journal of Marine Sciences*, 29(2): 191-200. https://doi.org/10.14710/ik.ijms.29.2.191-200

Powers, T., Harris, T., Higgins, R., Mullin, P. & Powers, K. 2018. Discovery and identification of species using CO1 DNA barcoding. *J. Nematol,* 50: 399-412. https://doi.org/10.21307/jofnem-2018-029

Ramadan, A., Almaaty, A.H.A., Al-Tahr, Z.M. & Allam, M. 2023. Phylogenetic diversity of some Snappers (Lutjanidae: Perciformes) inferred from mitochondrial 16S rRNA sequences. *Egypt. J. Aquat. Biol. Fish.*, 27(3): 307–317. https://doi.org/10.21608/ejabf.2023.301156

Randall, J.E., Allen, G.R. & Anderson Jr, W.D. 1987. Revision of the Indo-Pacific Lutjanid genus *Pinjalo*, with description of a new species. Bishop Museum Press. 17 pp.

Shuai, F., Shixiao, Y., Sovan, L. & Xinhui, L. 2018. Habitat effects on intra-species variation in functional morphology: Evidence from freshwater fish. *Ecol. Evol.,* 8(22): 10902-10913. https://doi.org/10.1002/ece3.4555

Souza, A.S., Eurico, A.D.J., Manolo, F.P., Marcelo, B.C., Luiz, A.C.B., Erik, G.M., Marcelo, N.S.V., Pedro, M.G.Jr. & Wagner, F.M. 2019. Phylogeography and historical demography of two sympatric atlantic snappers: *Lutjanus analis* and *L. jocu*. *Front. Mar. Sci.*, 6: p.545. https://doi.org/10.3389/fmars.2019.00545

Sala, R., Kusuma, A.B., Bataradewa, S. & Pranata, B. 2022. Morphometrics diversity and phenotypic relationship of the Red Snapper (*Lutjanus gibbus*) in Northern Papua Waters. *Egypt. J. Aquat. Biol. Fish.*, 26(5): 1211–1227.

Sala, R., Kusuma, A.B. & Pranata, B. 2023. Phylogenetic of red snapper (Lutjanidae) in Yapen Island Waters, Papua, Indonesia. *Biodiversitas*, 24(2): 716–723. https://doi.org/10.13057/biodiv/d240206

Suraji, Rasyid, N., Kenyo, A.S., Jannah, A.R., Wulandari, D.R., Saefudin, M., Ashari, M., Widiastutik, R., Kuhaja, T., Juliyanto, E., Afandi, Y.A, Wiyono, B., Syafrie, H., Handayani, S.C. & Soemodinoto, A. 2015. Profile of Conservation Area of Papua Province – West Papua. Directorate of Conservation of Areas and Fish Species, Directorate General of Marine Affairs, Coasts and Small Islands, Ministry of Marine Affairs and Fisheries. 74 pp.

Tamura, K., Glen, S. & Sudhir, K. 2021. MEGA 11: Molecular Evolutionary Genetics Analysis Version 11. *Mol. Biol. Evol.*, 38 (7): 3022-3027. https://doi.org/10.1093/molbev/msab120

Thi, O.T., Ha, Q.V.D. & Thuy, B.D. 2015. Phylogenetic relationships of emperors (Lethrinidae) and Snappers (Lutjanidae) in Vietnam based on mitochondrial DNA sequences. *Int. Conf. Biol. Environ. Food Eng*, Singapore. pp. 15-16

Ward, R.D., Zemlak, T.S., Bronwyn, H.I., Peter, R.L. & Hebert, P.D.N. 2005. DNA barcoding Australia's fish species. *Philos. Trans. Royal. Soc. Biol. Sci.*, 360(1462): 1847-57. https://doi.org/10.1098/rstb.2005.1716