Morphological Characteristics and Genetic Relationship of Red Snappers (*Lutjanus timoriensis, Lutjanus malabaricus, Lutjanus erythropterus*) in Papuan Waters

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Abstract

Environmental and genetic variables can exert an influence on alterations in morphological traits. Within fish species inhabiting diverse aquatic settings, there can be observed variations in morphological traits. Genetically, variations in fish morphological characteristics can occur through mating and gene flow. To date, there has been a lack of research conducted on the variability in morphological traits and genetic relationships between Lutjanus timoriensis, L. malabaricus, and L. erythropterus. Thus, the current research aimed to identify variations in the morphological characteristics as well as in the intra- and inter-specific relationships between three red snapper species from the genus Lutjanus. Cytochrome oxidase I (COI) gene was used to study the molecular relationship among species of red snapper. The results showed that L. timoriensis had high intraspecific morphological variation in young individuals. Morphologically, L. timoriensis, L. malabaricus, and L. erythropterus are very similar. Unlike L. malabaricus and L. erythropterus, young and adult L. timoriensis have black patches in the pectoral fin axils. The adult L. erythropterus has a comparatively small mouth, no hump on its head, and no black saddle at the base of its tail. Meanwhile, L. malabaricus has a comparatively large mouth, a head with a hump, and a black saddle at the base of its tail. In terms of body size, L. erythropterus is larger than L. timoriensis and L. malabaricus. Based on NCBI and Bold System data, molecular analyses determined that the observed fish were L. timoriensis, L. malabaricus, L. erythropterus with a similarity of between 99.85 and 100%. The phylogenetic tree construction demonstrated that L. malabaricus, L. timoriensis and L. erythropterus were closely related.

Keywords: *genetic; morphology; Lutjanus; COI; mtDNA*

Introduction

Red snapper is a demersal fish of the family Lutjanidae that lives in coral reefs in the East Pacific, Indo-West Pacific, and East and West Atlantic (Souza *et al*., 2019; Andriyono *et al*., 2022). The genus *Lutjanus* Bloch 1790 contains 70 species, including 43 species from the Indo-West Pacific region and 30 species in Indonesia (Allen *et al.,* 2013; Halim *et al*., 2022). This genus exhibits morphological attributes that encompass a range of body sizes, varying from small to large and is characterized by an oval and fusiform body shape (Allen, 1985). Red snappers have a continuous dorsal fin with a slight incision between the hard and soft spines. There are 10 or 11 hard spines and 11 to 16 soft spines on the dorsal fin. The pectoral fins of this species have 15-18 soft spines. The dorsal and anal fins are covered in scales, whereas the caudal fin is emarginate, truncated, or, in uncommon cases, bifurcated. Snappers vary widely in color, often having a reddish, yellow, gray, or brown background with striped patterns. They are often characterized by large blackish spots on the upper sides and under the front of the soft dorsal fin.

In a recent study conducted by Sala *et al.* (2022; 2023), *L. malabaricus,* and *L. erythropterus* were identified within the waters of Papua. These species have high similarity in morphological characteristics, as evidenced by the presence of

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yellow stripes in the recently identified species (Bakar *et al.,* 2018). High morphological variation species may make identification more difficult and potentially lead to misidentification (Li *et al*., 2018; Tapilatu *et al*., 2021; Dwifajri *et al*., 2022). Variation in morphological features can be influenced by both environmental and genetic factors. The morphological characteristics of an organism can exhibit variation in response to distinct and diverse environmental conditions (Shuai *et al.*, 2018; Nishio *et al*., 2019). According to Dunn *et al*. (2020), and Endo and Watanabe (2020), fish commonly exhibit a greater degree of morphological diversity, which can be observed both within and between groups. Therefore, in this study, the three species were examined the morphological characteristics that distinguish them. In addition to morphological approaches, analysis of the genetic characteristics of a species is used to understand the evolutionary relationships between species.

Genetic variation is the primary material for natural selection, which permits species to adapt to environmental changes. The loss of genetic variation will diminish the adaptive capacity of species (Des Roches *et al.,* 2021; Hoban *et al*., 2021; Torda and Quigley, 2022). Excessive exploitation of fish populations, natural selection, loss of genetic variation (Gandra *et al.,* 2021), and mutations can result in genetic changes.

In this study, the Cytochrome C Oxidase Subunit I (COI) mitochondrial gene was used to identify species and understand genetic relationships between species. Several researchers have used this gene for identification and understanding the level of diversity and genetic relationships of species (Pranata *et al.,* 2018; 2020; Dwifajri *et al.,* 2022; Sala *et al.,* 2023; Pranata *et al*., 2024). The aim of this study was to identify variation in morphological characteristics and genetic relationships of *L. timoriensis, L. malabaricus,* and *L. erythropterus* in Papuan waters. Information about the morphological variations of the three species is expected to facilitate the process of identification and genetic relationships of them.

Materials and Methods

This research was conducted in the waters of Papua, Indonesia. Genetic analysis was carried out on 34 samples of Red Snapper fish originating from 6 research locations (Jayapura, Yapen, Nabire, Manokwari, South Manokwari and Raja Ampat) (Figure 1.).

Tissue samples of fish were obtained from fish landing ports and markets. Initial morphological identification refers to the identification book of Moore and Cocas (2016). One centimeter of red snapper dorsal fin tissue was taken and put into a tube containing 80% ethanol for genetic analysis.

The morphological, morphometric and meristic characteristics of Red Snapper were documented and measured for weight, total length, standard length, body depth, head length, eye diameter, number of pectoral fin spines, number of dorsal fin spines, and number of anal fin spines for each specimen.

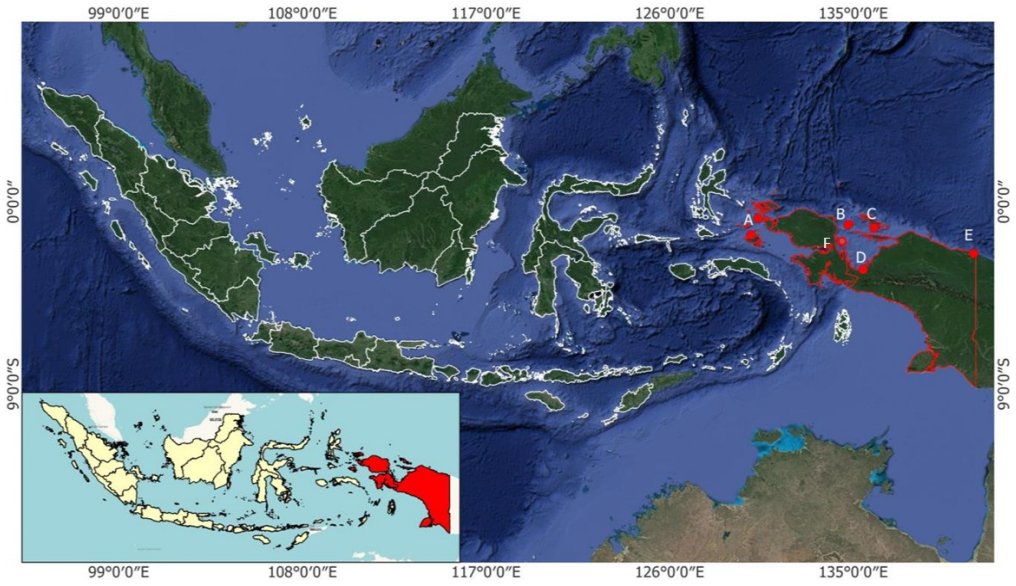


Figure 1. Red Snapper sampling site in the northern part of of Papua, including Raja Ampat (A), Manokwari (B), Yapen (C), Nabire (D), Jayapura (E) and South Manokwari (F).

Extraction, amplification, and sequencing

DNA extraction was carried out based on the instructions from the Geneaid gSYNCTM DNA extraction kit. COI gene amplification was done using primers developed 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 contained Go Taq Green 25 µL, 5 µL primer, 1.5 µL DNA template and 19.5 µL nuclease free water. The thermal cycle was set as follows: initial denaturation at 95˚C for 4 min and followed by 35 cycles of denaturation at 95˚C for 30 sec, annealing at 54˚C for 45 sec, elongation at 72˚C for one min, post PCR at 72˚C for 7 min. The amplification results underwent electrophoresis for visualizing the DNA fragments in the PCR product. The result of the PCR was sent to the 1st BASE Sequencing Service Sdn. Bhd. (Malaysia).

Data analysis

DNA sequencing results were edited and aligned using MEGA XI software. DNA sequence data was matched to databases available at the National Center for Biotechnological Information (NCBI) online (www.ncbi.nml.nih.gov) and the Barcode of Life Data System (BOLD System). Phylogenetic analysis was done based on the Neighbor Joining (NJ) method (Saitou and Masatoshi, 1987) and the Kimura 2-parameter model (K2P) (Kimura, 1980) using Mega XI software (Tamura *et al.,* 2021).

Result and Discussion

Morphological and morphometric characteristics

Observations revealed that the morphological characteristics of *L. timoriensis* varied among individuals. Red snapper morphology includes a reddish dorsal side, a red or pink to silvery white abdomen, and black pectoral fin axils. Juvenile fish have a distinctive characteristic wherein a dark saddle, accompanied by a border of pearly white, is present on the top edge of the tail's base. The cranial region exhibits a pronounced inclination, while the posterior dorsal and anal fins possess a pointed morphology, resembling a triangle form. The head is steeply sloping, the posterior dorsal and anal fins are pointed or triangular, the caudal fin is truncate or slightly emarginated. The fish has 17 soft pectoral fin spines. On the dorsal fin, this fish has XI hard spines and 14-15 soft spines. The number of hard spines in the anal fin is III and the number of soft spines is 8. *L. timoriensis* displays intraspecific diversity (Figure 2.). These results are the same as those described by Allen (1985).

L. malabaricus has a reddish body color. Juveniles have brown or black bands (see lines in Figure 3A). Juveniles have a conspicuous black saddle along the base of the tail with a white leading edge (see oval in Figure 3A.), while adults only have a black saddle at the base of the tail (see oval in Figure 3B). Occasionally, juveniles have a series of narrow horizontal reddish markings (see Figure 3A.). The cranium of *L. malabaricus* is humped. The caudal fin is either truncate or slightly emarginated. The pectoral fin has 16 soft spines, the dorsal fin has XI hard spines and 14-15 soft spines, the anal fin has III hard spines and 8-9 soft spines, and the pectoral fin has 16 soft spines.

L. erythropterus individuals possess diverse morphological characteristics. This species morphology is characterized by a silvery pink body color, a comparatively small mouth, a slanted head shape, and a truncated tail shape. Juvenile individuals have a horizontal stripe on the body and a prominent black saddle with a white leading margin along the base of the tail (Figure 4B.). In contrast, adult fish lack a black prominence at the base of their tail (Figure 4A.). Nonetheless, some specimens (TL 56 cm) possess a black saddle at the base of the tail (Latumeten *et al.,* 2018.). There are 16 spines on the pectoral fin, XI hard spines and 15-16 soft spines on the dorsal fin, and III hard spines and 9-10 soft spines on the anal fin.

Morphologically, *L. timoriensis, L. malabaricus* and *L. erythropterus* look very similar. Juvenile individuals of all three species have horizontal stripes on their bodies and prominent black saddles with a white leading edge along the base of the tail (Figure 5.). However, there are several distinctions in morphological characteristics between the three species among adults. *L. malabaricus*, for instance, has a black saddle at the base of its tail (see oval Figure 6B), whereas mature *L. timorensis* does not (see oval Figure 6A). *L. timorensis* has black saddles on the pectoral fin axils (see circle Figure 6A.), while *L. malabaricus* does not (see circle Figure 6B). Compared to *L. malabaricus, L. timorensis* has a gentler dorsal fin that is more pointed or appears triangular (see triangle Figure 6A and B.). The mouth of *L. erythropterus* is comparatively smaller compared to that of *L. timoriensis* and *L. malabaricus*. The dorsal and anal fins of *L. erythropterus* and *L. malabaricus* have similar morphologies. The pectoral fin length of *L. erythropterus* is shorter than that of *L. timoriensis* and *L. malabaricus.*

Several morphometric characteristics were observed from the third species. The results of these observations indicate that *L. erythropterus* has the largest body depth (average 10.6 cm) among the three species. Allen and Erdmann (2012), showed

that the body depth of *L. erythropterus* was greater than that of *L. timoriensis* and *L. malabaricus. L. erythropterus* has an average head length of 8.2 cm. These findings are identical to those of Andriyono *et al.* (2022). *L. erythropterus* and *L. malabaricus* have an eye diameter of 1.9 cm, while *L. timoriensis* has an eye diameter of 1.8 cm. Individual red snapper size structure caught is important information for its management in Papuan waters. The average total length of *L. timoriensis, L. malabaricus*, and *L. erythropterus* is 23–23.7 cm (Table 1). The average size of *L. timoriensis, L. malabaricus*, and *L. erythropterus* fish caught in Papuan waters was not yet reaching the optimum harvest size (Mous *et al.*, 2021) (Table 2.). Several research results in Indonesia showed that the maximum length of *L. malabaricus* is 69.5 cm (Rapi *et al*., 2020) and L. erythropterus is 26.4 cm (Andriyono *et al*., 2022).

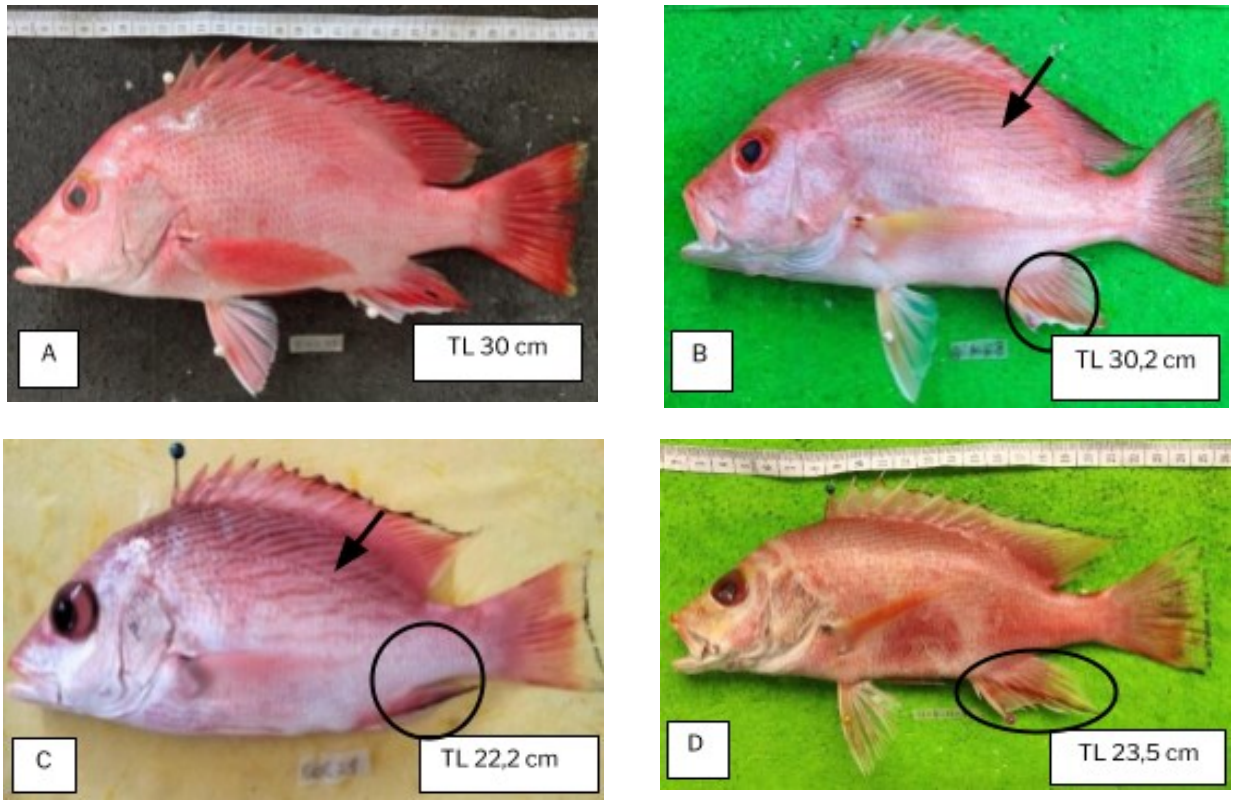


Figure 2. The Intraspecific Morphological Variation of *L. timoriensis*
Note: *L. timoriensis* displays intraspecific diversity. The pectoral fins of *L. timorensis* were reddish (Figure 2A.) and yellowish (Figure 2B.). On occasion, in juvenile fish a red horizontal line (see arrow Figure 2B, C) on the body and a white horizontal line (see circle Figure 2B, C.) on the lower tip of the anal fin preceded by black. However, Figure 2D shows that the anal fin tip consists of a combination of black, white, and yellowish (see oval Figure 2D.). The caudal and dorsal fin tips are black and preceded by yellow (Figures 2A, C, and D.).

Table 1. Morphometric Characteristics of the genus *Lutjanus*

Morphometric Characters	*L. timoriensis*		*L. malabaricus*		*L. erythropterus*	
	Min-Max (cm)	Mean	Min-Max (cm)	Mean	Min-Max (cm)	Mean
Total length (TL)	17.1-42	23.7	20-52	28.3	21.5-49.5	23
Standard length (SL)	13.6-35.6	19.2	16-44.5	23.1	17.4-42.5	26.8
Body depth (BD)	6-13.4	7.9	6.5-18.2	9	7.9-15.4	10.6
Head length (HL)	5.3-14.2	7.7	4-18	7.9	4.7-12.8	8.2
Eye Diameter (ED)	1.3-2.8	1.8	1.8-1.9	1.9	1.2-2.5	1.9

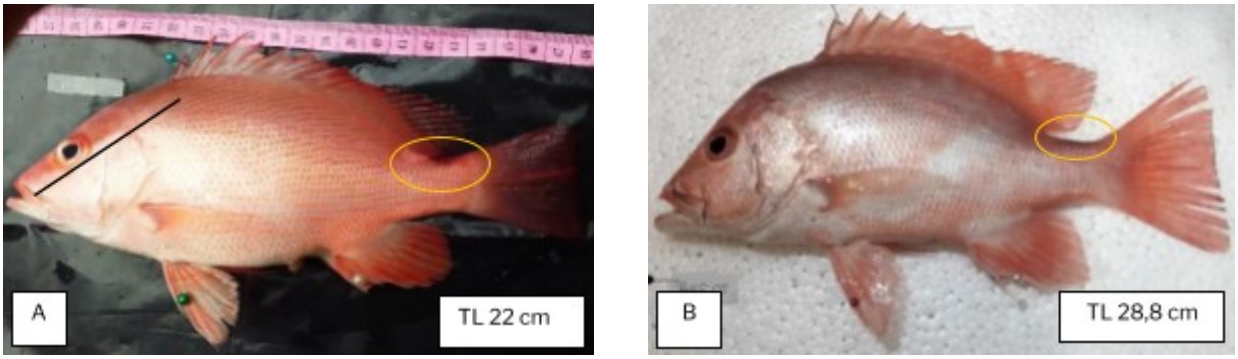


Figure 3. The Intraspecific Morphological Variation of *L. malabaricus*

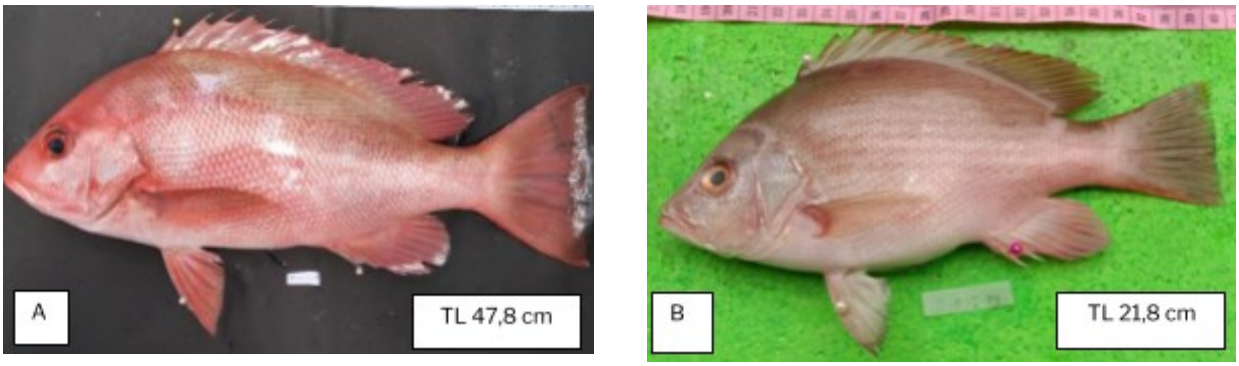
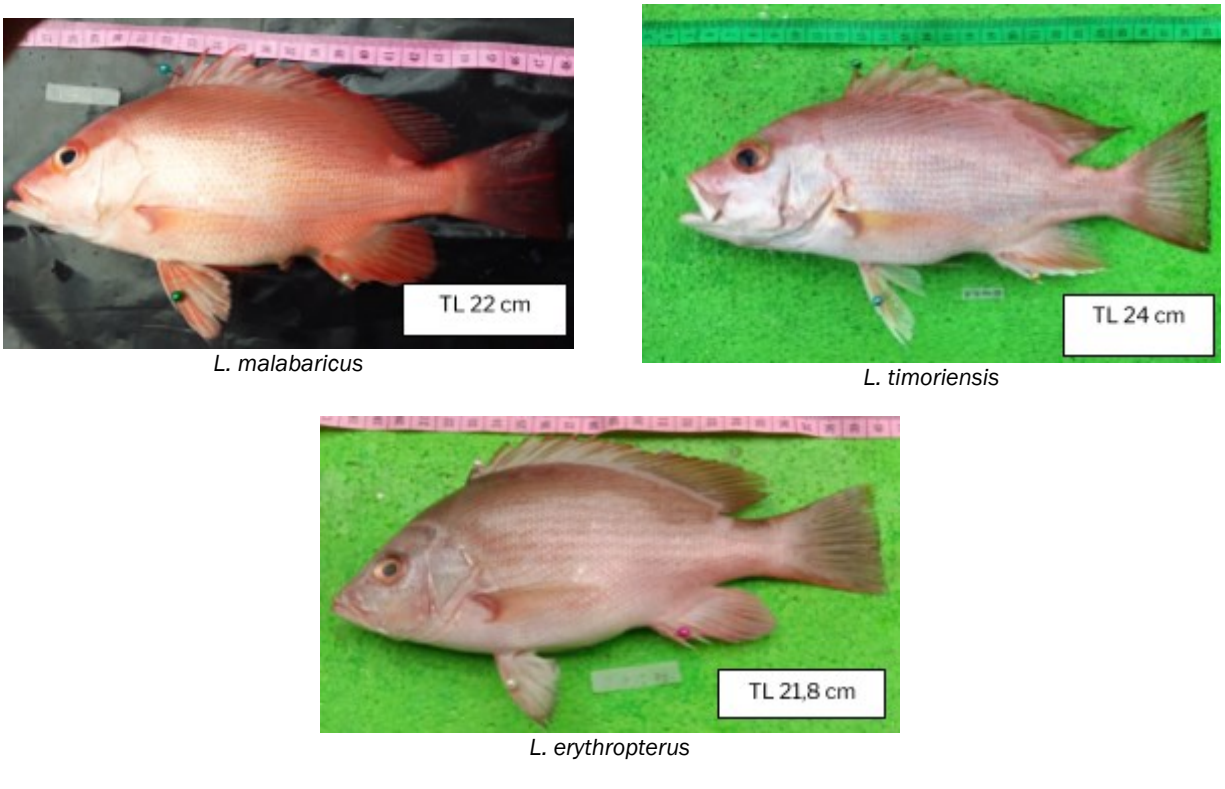


Figure 4. The Intraspecific Morphological Variation of *L. erythropterus*



L. malabaricus

L. timoriensis

L. erythropterus

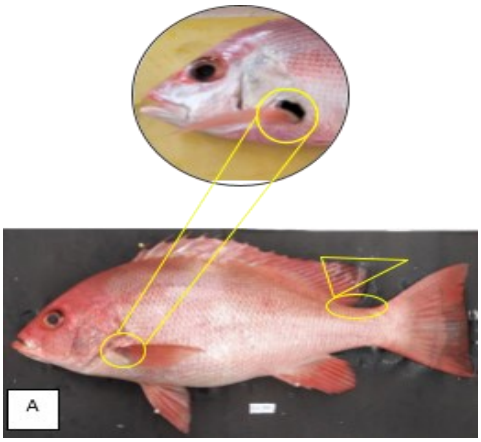
Figure 5. Interspecific Morphological Variation of Juvenile Individuals

Genetic relationship

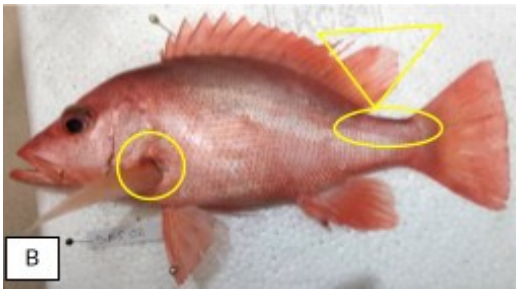
The Basic Local Alignment Search Tool (BLAST) method at the National Center for Biotechnology Information (NCBI) was used to identified the three *Lutjanus* species. BLAST results showed that 12 individuals were identified as *L. erythropterus*, 8 individuals were identified as *L. malabaricus* and 14 individuals were identified as *L. timoriensis*. The sample DNA sequence has a similarity level between 99.85-100% with the DNA sequence from the NCBI database.

Genetic distance is a measure of genetic differences between species or between populations within a species (Nei, 1987). The intraspecific genetic range of the three species ranges between 0% and 0.5% (Table 3.). According to the findings of Halim *et al.* (2022), five species belonging to the genus *Lutjanus* had an intraspecific genetic distance of between 0.1% and 0.7%. The interspecific genetic distance ranges between 10.2 and 11.4%. Past studies showed that the genetic distance between species of the genus *Lutjanus* was 18.3% (Afriyie *et al.,* 2020), 8.2% (Halim *et al.,* 2022) and 11.3% (Sala *et al.,* 2023). These results show that intraspecific genetic differences are smaller compared to interspecific genetic differences. The results of Xing Bingpeng *et al.* (2018), and Ude *et al.* (2020), showed that the average interspecific genetic distance was 31 times higher than the average intraspecific distance. Our results show similar morphology of the three species. Sala *et al.* (2023), explained that the greater the morphological similarity between observed species, the smaller the genetic distance value and vice versa.

Phylogenetic tree reconstruction has yielded two main clades. Clade 1 consists of 12 individuals of *L. erythropterus* and 8 individuals of *L. malabaricus*, while clade 2 consists of 14 individuals of *L. timoriensis*. The bootstrap value for the clade is approximately 100 (Figure 7). A high bootstrap value indicates that the branches in the phylogenetic tree formed are reliable. According to the reconstructed phylogenetic tree, L. *malabaricus* and *L. erythropterus* belong to the same clade. Several other research results also show that L. *malabaricus* and *L. erythropterus* come from the same clade (Bakar *et al.,* 2018; Velamala *et al.,* 2019; Halim *et al.,* 2022; Sala *et al.,* 2023). This indicates that the two species are closely related genetically.



L. timoriensis



L. malabaricus

L. erythropterus

Figure 6. Interspecific Morphological Variation of Adult Individuals
Note: *L. malabaricus* has a black saddle at the base of its tail (see oval Figure 6B.), whereas mature *L. timorensis* does not (see oval Figure 6A.). *L. timorensis* has black saddles on the pectoral fin axils (see circle Figure 6A.), while *L. malabaricus* does not (see circle Figure 6B.). *L. timorensis* has a gentler dorsal fin that is more pointed or appears triangular (see triangle Figure 6A and B.).

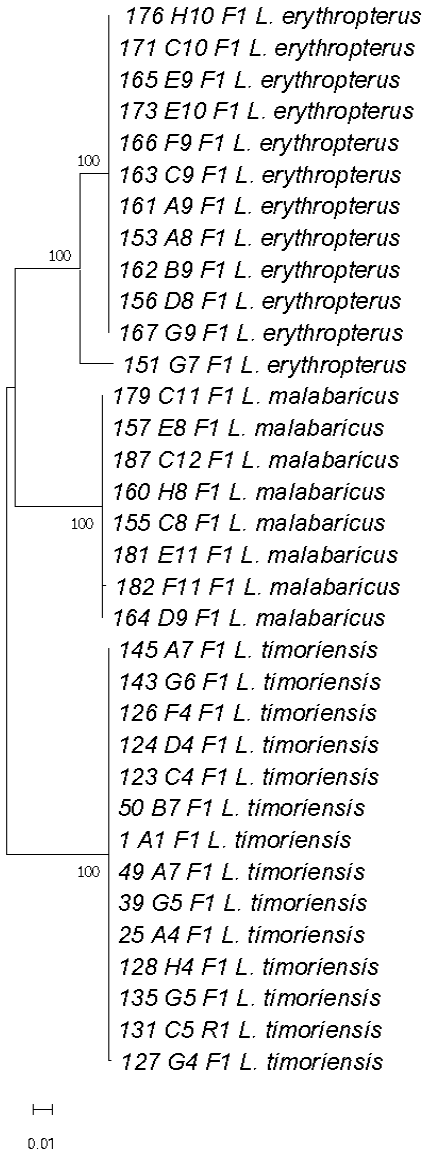


Figure 7. Evolutionary relationships of taxa

Table 2. Biological Aspects of the genus *Lutjanus* (Mous *et al*., 2022)

No.	Species	L_{max}	W_{mat}	L_{opt}
1	*L. malabaricus*	94	1822	67
2	*L. erythropterus*	70	773	50
3	*L. timorensis*	65	532	46

Notes: Lmax= Maximum attainable total length at Indonesian lattitudes (cm); Wmat= Weight at 50% maturity in gram; Lopt = Optimum Harvest Size (cm);

Table 3. The intraspecific and interspecific genetic distances of the genus *Lutjanus*

No.	Species	1	2	3
1	*L. erythropterus*	0.005		
2	*L. timoriensis*	0.114	0.000	
3	*L. malabaricus*	0.102	0.111	0.000

Notes: *Bold text indicates intra-specific genetic distance values*

Conclusion

There is intraspecific variation in morphological characteristics of the species *L. timorensis, L. malabaricus* and *L. erythropterus.* The three species can be identified based on their differences in morphological characters. When young and mature, *L. timoriensis* can be distinguished from *L. malabaricus* and *L. erythropterus* because it has black saddles in the axils of its pectoral fins. Young *L. malabaricus* can be distinguished from L. *erythropterus* as it has a wide slanting brown or black band from the upper jaw to the beginning of the dorsal fin. Juvenile Young *L. malabaricus* also has a relatively large mouth shape compared to L. *erythropterus*. While adult *L. malabaricus* has a humped head, relatively large mouth with a black saddle at the base of the tail, *L. erythropterus* has a non-humped head, relatively small mouth, and no black saddle at the base of the tail. Genetically, *L. malabaricus* and *L. erythropterus* demonstrate smaller genetic differences than *L. timoriensis*. The results of the kinship analysis show that *L. malabaricus* is closely related to *L. erythropterus.*

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