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DNA barcoding of 19 *Lutjanus* species to support fisheries management in the Bird's Head Seascape, Papua, Indonesia

ABDUL HAMID A. TOHA[1], EMMANUEL MANANGKALANGI[1], BAYU PRANATA[1,♥], MUHAMMAD DAILAMI[2], ANTAJALA R. MAULANA[2], DESTIA F. ARIYANI[2], MARTHIN D. T. GRATIA[2], RAKHMAD N. PRATAMA[2], SHINTA E. NURCAHYANI[1], SINDI MILANSARI[1], JENI[3], RADWA F. H. PUTRI[4], ZETH PARINDING[5], ESIE M. WANGI[6], ANDI FAJERIANI WYRASTI[7], CORAZON E. M. P. MAYOR[8]

[1]Department of Fisheries, Faculty of Fisheries and Marine Science, Universitas Papua. Jl. Gunung Salju, Manokwari 98314, West Papua, Indonesia. Tel./fax.: +62-986-211430, ♥email: b.pranata@unipa.ac.id
[2]Department of Aquaculture, Faculty of Fisheries and Marine Science, Universitas Brawijaya. Jl. Veteran, Malang 65145, East Java, Indonesia
[3]Department of Biology Education, Faculty of Teacher Training and Education, Universitas Papua. Jl. Gunung Salju, Manokwari 98314, West Papua, Indonesia
[4]Department of Chemistry, Faculty of Mathematics and Natural Science, Universitas Brawijaya. Jl. Veteran, Malang 65145, East Java, Indonesia
[5]Wasur National Park Office. Jl. Garuda Leproseri No. 03, Merauke 99611, Papua, Indonesia
[6]National Park Office of Teluk Cenderawasih Bay. Jl. Drs Esau Sesa Jl. Sowi Gn., Manokwari 98315, West Papua, Indonesia
[7]Department of Mathematics Education, Teacher Training and Education Sciences, Universitas Papua. Jl. Gunung Salju, Manokwari 98314, West Papua, Indonesia
[8]Postgraduate Program, Universitas Papua. Jl. Gunung Salju, Manokwari 98314, West Papua, Indonesia

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Abstract. *Toha AHA, Manangkalangi E, Pranata B, Dailami M, Maulana AR, Ariyani DF, Gratia MDT, Pratama RN, Nurcahyani SE, Milansari S, Jeni, Putri RFH, Parinding Z, Wangi EM, Wyrasti AF, Mayor CEMP. 2025. DNA barcoding of 19* Lutjanus *species to support fisheries management in the Bird's Head Seascape, Papua, Indonesia. Biodiversitas 26: 5287-5302.* Red snapper species (family Lutjanidae) are economically important reef fishes traded at local, national, and international levels. However, species-level taxonomic resolution and conservation assessments remain limited in the Bird's Head Seascape (BHS) region of West Papua, Indonesia. This study applied DNA barcoding of the mitochondrial cytochrome C Oxidase subunit I (COI) gene to assess *Lutjanus* species diversity and distribution within the Kaimana and Fakfak Marine Protected Areas (MPAs). A total of 77 specimens were successfully sequenced, resulting in a barcoding success rate of 100%. Nineteen species were identified: *Lutjanus argentimaculatus*, *L. bengalensis*, *L. biguttatus*, *L. bohar*, *L. decussatus*, *L. ehrenbergii*, *L. erythropterus*, *L. fulviflamma*, *L. fulvus*, *L. gibbus*, *L. quinquelineatus*, *L. lemniscatus*, *L. malabaricus*, *L. monostigma*, *L. rufolineatus*, *L. russellii*, *L. sebae*, *L. timoriensis*, and *L. vitta*. Thirteen species were recorded in Fakfak and twelve in Kaimana, with several species unique to each MPA. No genetic differences were observed among individuals within the same species. In contrast, the genetic distance between different species ranged from 2.8% to 19.3%. A total of nineteen monophyletic groups were recovered in the NJ phylogenetic trees, which were supported by high bootstrap values (91-100%). *Lutjanus decussatus* formed two subgroups in the phylogenetic trees. All identified species are currently listed as Least Concern (LC) by the IUCN, though population trends for most remain unknown, and some are declining. These findings demonstrate the utility of DNA barcoding for accurate species identification in data-poor tropical regions and provide a genetic baseline for future monitoring efforts. The confirmed presence of commercially exploited and potentially declining species underscores the need for site-specific fishery assessments and adaptive management strategies. Integrating molecular tools into routine monitoring can enhance enforcement of species-based catch limits and support sustainable fisheries management within Indonesia's MPAs.

Keywords: Bird's Head Seascape, COI, DNA barcoding, *Lutjanus*, Papua

INTRODUCTION

The Bird's Head Seascape (BHS) in Papua is recognized as a priority conservation area at regional, national, and international levels (Ahmadia et al. 2016). This seascape encompasses 23 Marine Protected Areas (MPAs), covering a total area of 5,191,771.79 hectares (Purwanto et al. 2021). Among these, the waters of Kaimana and Fakfak are particularly rich in marine biodiversity and have been designated as conservation zones through an ecosystem-based approach. Despite their protected status, these areas continue to face various threats (Varkeya et al. 2010), including the impacts of climate change, illegal activities, and unregulated coastal development (Mangubhai et al. 2012). In addition, the majority of local communities in BHS live below the poverty line and primarily depend on fisheries for their livelihoods (Huffard et al. 2012). The Kaimana and Fakfak waters fall within Indonesian Fisheries Management Area (*Wilayah Pengelolaan Perikanan Negara Republik Indonesia/WPPNI*) 715. The integration of WPPNRI and MPA frameworks is considered one of the most effective strategies for conserving species diversity and maintaining ecosystem processes (Starger et al. 2015). Among policy makers there are efforts to reconcile overlapping objectives and better understand between MPA and fisheries management (Weigel et al. 2014). However, significant gaps remain in implementing effective fisheries management within MPAs (Bennett et al. 2014). These challenges complicate the management of economically important fish species in BHS conservation areas, including those within the Kaimana and Fakfak MPAs.

Fish provide direct economic value and are a vital source of animal protein for humans (Pradeepkiran 2019). In the BHS, economically important fish include over 1,750 species of reef fish (California Environmental Associates 2018). However, the status of many fish stocks remains uncertain due to limited and often inaccurate species classification (Purwanto et al. 2021). Taxonomic identification is a cornerstone for fisheries research, conservation planning, and the sustainable use of marine resources (Ardura et al. 2013; Rahayu et al. 2023; Fadli et al. 2024). Traditional morphological identification, although widely used, requires extensive ichthyological expertise and can be confounded by factors such as ontogenetic variation and phenotypic plasticity (Bakar et al. 2018). In regions like Wallacea and Papua, several studies have shown that closely related species exhibit overlapping morphological traits that hinder reliable identification (Hubert et al. 2015). For example, *Lutjanus* species are frequently misidentified in fish markets and monitoring datasets due to cryptic morphology and high intra-species variability (Fadli et al. 2020). Misidentification can lead to flawed assessments of biodiversity, misinformed catch reporting, and ineffective management interventions (Fadli et al. 2020; Tlusty et al. 2023). In such cases, molecular approaches such as DNA barcoding offer a standardized, accurate tool for species-level identification and have been increasingly adopted in the Indo-Pacific region to resolve taxonomic ambiguity (Sembiring et al. 2015; Antil et al. 2022).

DNA barcoding using the cytochrome C Oxidase subunit I (COI) gene marker has become a powerful tool for animal species identification across a wide range of applications (Heller et al. 2018; Elyasigorji et al. 2023). This molecular approach has been employed for species description (Bakar et al. 2018), facilitating the discovery of new species (Allen et al. 2013; Iwatsuki et al. 2015), detecting invasive species (Iwatsuki et al. 2015), identifying endangered species (Kress 2017). In addition to species identification, COI gene data are widely used to analyze genetic diversity and relationships among marine organisms (DeBoer et al. 2014a, 2014b; Toha et al. 2016, 2020, 2022, 2025). Despite its proven utility, DNA barcoding research focused on the identification, diversity assessment, and genetic relationship analysis of economically important fish species within marine conservation areas remains limited. Therefore, this study would apply DNA barcoding to (i) identify species, (ii) analyse diversity, and (iii) explore genetic relationships among species within the genus *Lutjanus* in the Kaimana and Fakfak MPAs.

MATERIALS AND METHODS

Study area

This study commenced in 2024. Red snapper (*Lutjanus* spp.) samples were collected from various conservation areas within the waters of Kaimana (3°54'55.6"S, 133°58'21.3"E) and Fakfak (3°22'5.32"S, 132°26'11.52"E) of the West Papua, Indonesia (Figure 1). DNA extraction, amplification, and electrophoresis were conducted at the Brain Bee Laboratory, located in Malang, East Java, Indonesia.

Tissue sampling

Snapper fish tissue samples number per species (Table 2) were collected from fish landing sites and local markets in Fakfak and Kaimana from November 2023-December 2024 (Figure 1). Each specimen was photographed by camera or mobile cell. No ethical permit was required for sampling since samples were obtained from commercial fishers and fish markets. Morphological identification of the specimens was conducted using the identification guide by Moore and Colas (2016). Approximately one centimeter of caudal fin tissue was excised from each red snapper specimen and preserved in a tube containing 95% ethanol.

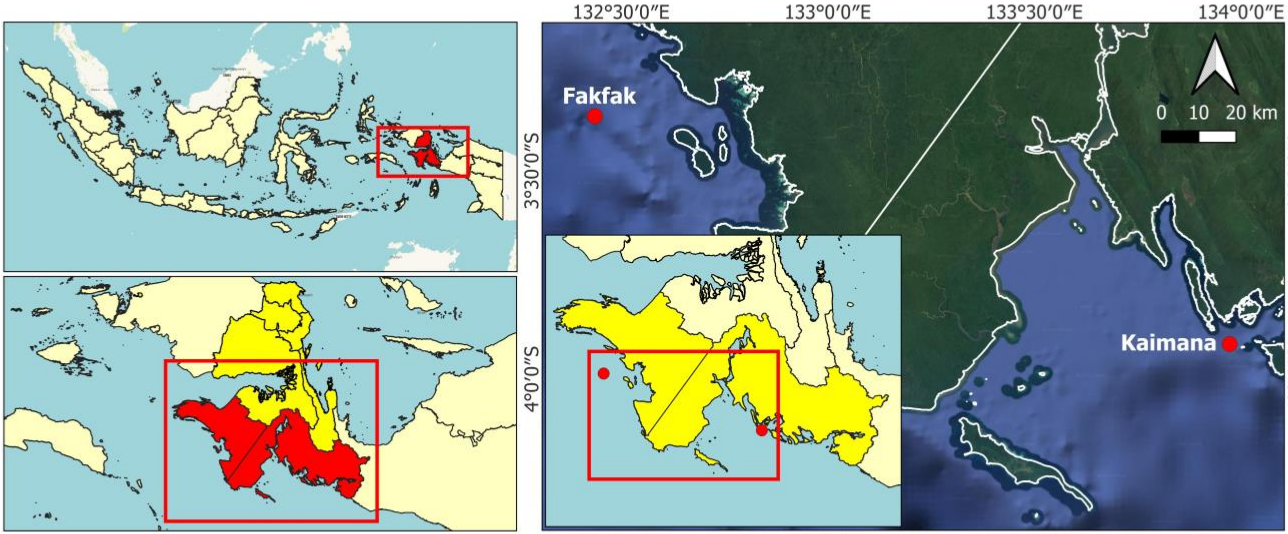


Figure 1. Study area in Kaimana and Fakfak waters, West Papua, Indonesia

Table 1. Accession numbers of COI gene sequences for *Lutjanus* species retrieved from NCBI

Species name	Accession number	Sampling location	Reference
Lutjanus argentimaculatus (Forsskål, 1775)	OR524461.1	Davao Gulf, Philippines	Nanola et al. (2023)
Lutjanus bohar (Forsskål, 1775)	OQ385529.1	Albay, Philippines	Bemis et al. (2023)
Lutjanus bengalensis (Bloch, 1790)	OL512911.1	Zhongsha Atoll, South China Sea	Huang et al. (2021)
Lutjanus biguttatus (Valenciennes, 1830)	OQ386009.1	Negros Oriental, Philippines	Bemis et al. (2023)
Lutjanus decussatus (Cuvier, 1828)	OQ386044.1	Aurora, Philippines	Bemis et al. (2023)
Lutjanus ehrenbergii (Peters, 1869)	KP194151.1	Lizard Island, Australia	Steinke et al. (2014)
Lutjanus erythropterus (Bloch, 1790)	GU673202.1	Australia	iBOL (2010)
Lutjanus fulviflamma (Forsskål, 1775)	MN870212.1	Ambon Island, Indonesia	Limmon et al. (2020)
Lutjanus fulvus (Forster, 1801)	PV170549.1	-	Bemis et al. (2025)
Lutjanus gibbus (Forsskål, 1775)	KF009614.1	Aurora, Philippines	Yambot et al. (2013)
Lutjanus lemniscatus (Valenciennes, 1828)	EF609397.1	Queensland, Australia	Ward and Holmes (2007)
Lutjanus malabaricus (Bloch & Schneider, 1801)	OR758620.1	Kupang, Indonesia	Wijayanti et al. (2023)
Lutjanus monostigma (Cuvier, 1828)	MK657561.1	Austral Islands, French Polynesia	Delrieu-Trotter et al. (2019)
Lutjanus quinquelineatus (Bloch, 1790)	EF609399.1	Western Australia, Australia	Ward and Holmes (2007)
Lutjanus rufolineatus (Valenciennes, 1830)	OR524478.1	Davao Gulf, Philippines	Nanola et al. (2023)
Lutjanus russellii (Bleeker, 1849)	OQ385435.1	Quezon, Philippines	Bemis et al. (2023)
Lutjanus sebae (Cuvier, 1816)	OQ386404.1	Metro Manila, Philippines	Bemis et al. (2023)
Lutjanus timoriensis (Quoy & Gaimard, 1824)	OQ385512.1	Metro Manila, Philippines	Bemis et al. (2023)
Lutjanus vitta (Quoy & Gaimard, 1824)	NC042930.1	Indonesia	Andriyono et al. (2018)

Table 2. Accession numbers of the COI gene sequences in NCBI for the *Lutjanus* species from this study

Species name	Accession number	Sampling location	Reference
Lutjanus bohar	PQ812433.1 PQ812432.1	Kaimana, Indonesia	This study
Lutjanus bohar	PQ804604.1 PQ804605.1	Fakfak, Indonesia	This study
Lutjanus decussatus	PQ804618.1 PQ804619.1 PQ804622.1 PQ804602.1	Fakfak, Indonesia	This study
Lutjanus decussatus	PQ812470.1	Kaimana, Indonesia	This study
Lutjanus ehrenbergii	PQ812610.1 PQ812612.1	Fakfak, Indonesia	This study
Lutjanus ehrenbergii	PQ812596.1	Kaimana, Indonesia	This study
Lutjanus erythropterus	PQ812437.1 PQ812438.1 PQ812442.1	Kaimana, Indonesia	This study
Lutjanus fulviflamma	PQ812597.1	Kaimana, Indonesia	This study
Lutjanus fulvus	PQ812603.1	Fakfak, Indonesia	This study
Lutjanus gibbus	PQ812450.1	Kaimana, Indonesia	This study
Lutjanus malabaricus	PQ804621.1	Fakfak, Indonesia	This study
Lutjanus monostigma	PQ812590.1	Kaimana, Indonesia	This study
Lutjanus lemniscatus	PQ804603.1	Fakfak, Indonesia	This study
Lutjanus malabaricus	PQ804621.1	Fakfak, Indonesia	This study
Lutjanus quinquelineatus	PQ812455.1 PQ812460.1	Kaimana, Indonesia	This study
Lutjanus timoriensis	PQ804625.1	Fakfak, Indonesia	This study
Lutjanus vitta	PQ812609.1	Fakfak, Indonesia	This study

Extraction, amplification and sequencing

DNA extraction was performed using the Genomic DNA Mini Kit (Tissue). Amplification of the COI gene was carried out using forward primer FishF1: (5'- `TCA ACC AAC CAC AAA GAC ATTGGC` -3') and reverse primer FishR1: (5'- `TAG ACT TCT GGG TGG CCA AAGAAT CA` -3') (Ward et al. 2005).

Amplification was done using PCR Master mix materials including ddH$_2$O (21 μL), Taq-Green (25 μL), Primer F1 (1 μL) - R1 (1 μL), and DNA template (2 μL). PCR amplification was run under the following conditions: initial denaturation at 94.0°C for 180 seconds, followed by denaturation at 94.0°C for 30 seconds, annealing at 50.0°C for 30 seconds, and extension at 72.0°C for 45 seconds, with a final extension at 72.0°C for 300 seconds. The PCR amplification stage was repeated for 35 cycles. DNA sequencing was performed using the Sanger dideoxy termination method. Sequencing was conducted by 1st BASE Malaysia through the sequencing services provided by PT. Genetika Sains Indonesia.

Data analysis

The DNA sequencing results were edited and aligned using MEGA XI software (Tamura et al. 2021). Species identification was conducted using the BLAST (Basic Local Alignment Search Tool) algorithm via the NCBI database (www.ncbi.nml.nih.gov). Analyses of genetic diversity parameters—including the number of haplotypes, polymorphic sites, and average number of nucleotide differences—were performed using DnaSP software (Librado and Rozas 2009). Further analyses of nucleotide composition, transition and transversion mutations and genetic distance were also conducted using MEGA XI (Tamura et al. 2021). Phylogenetic relationships were inferred using the Neighbor-Joining (NJ) method based on the Kimura 2-Parameter (K2P) model, implemented in MEGA XI (Tamura et al. 2021). The mtDNA COI gene sequences of *Lutjanus* species obtained from NCBI were used for phylogenetic analysis (Table 1). The sequence data generated in this study have been entered into the NCBI website and can be accessed through the access codes in Table 2.

Although *Lutjanus* species had been identified previously, this study was necessary to (i) fill geographic gaps in genetic data, (ii) verify species sold in Indonesian markets, (iii) detect cryptic diversity, (iv) establish a baseline for fisheries management in the BHS, and (v) strengthen the global COI reference database with high-quality, locally sourced sequences.

RESULTS AND DISCUSSION

We have identified commercially important fish species of the genus *Lutjanus* using both morphological and molecular approaches. Representative color photographs of voucher specimens collected from the waters of Kaimana and Fakfak are presented in Figure 2.

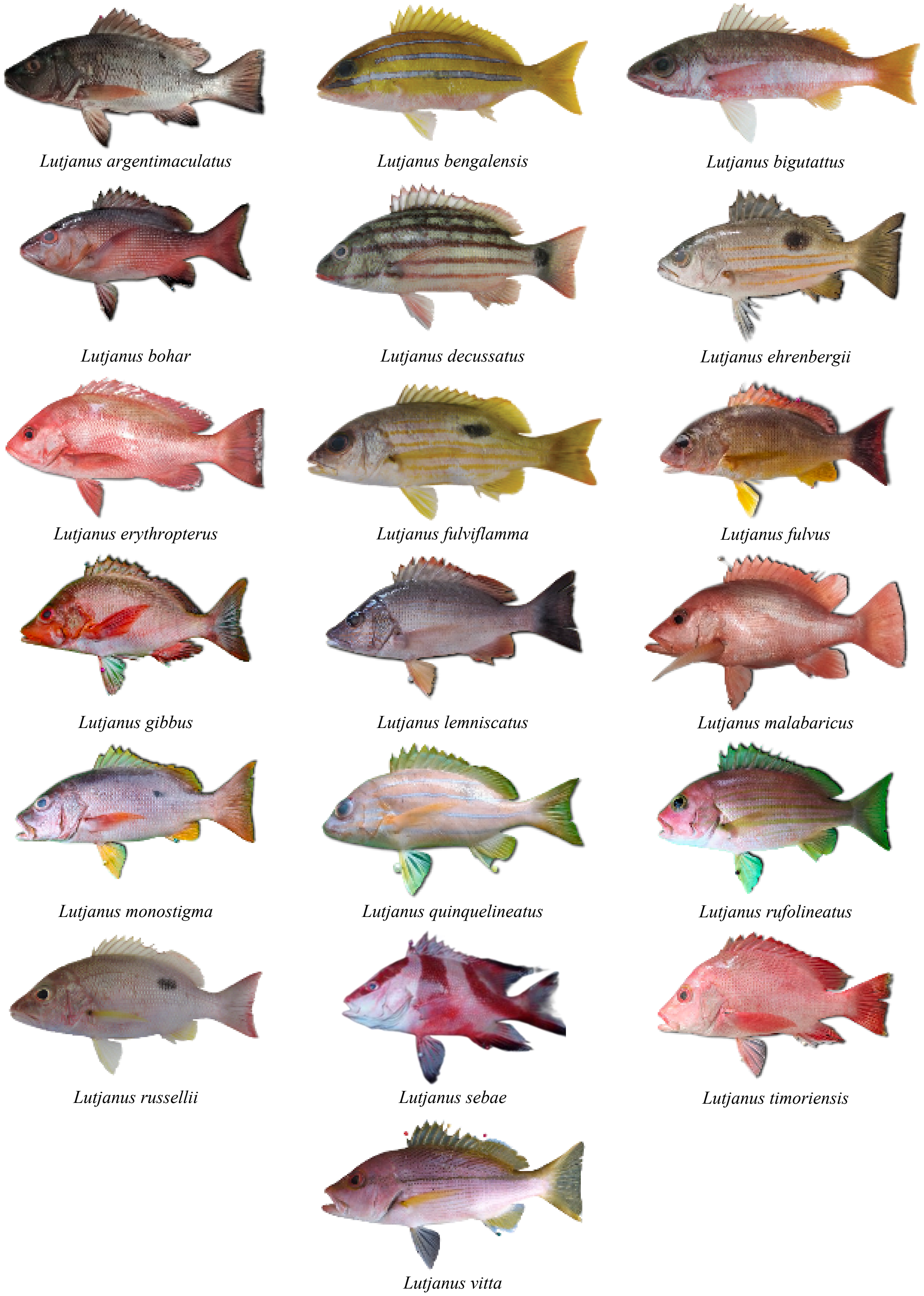
Molecular sequence data provided robust verification of species identification for specimens collected from fish markets in Fakfak and Kaimana. BLAST analysis of COI gene fragment sequences (654 base pairs) from 77 individual samples of *Lutjanus* revealed the presence of 19 species, with sequence similarity levels ranging from 99.85% to 100% (Table 3).

This study identified 12 species of *Lutjanus* in the waters of Kaimana and 13 species in the waters of Fakfak. The species commonly found in both locations included *L. argentimaculatus, L. bohar*, *L. decussatus*, *L. ehrenbergii*, *L. lemniscatus*, *L. malabaricus*. Species found exclusively in Kaimana were *L. quinquelineatus*, *L. monostigma*, *L. gibbus*, *L. fulviflamma*, and *L. erythropterus*. In contrast, species identified only in Fakfak waters included *L. gengalensis*, *L. fulvus*, *L. rufolineatus*, *L. russellii*, *L. sebae*, *L. timoriensis*, and *L. vitta*. A summary of the nucleotide composition and mutational characteristics of the COI gene sequences for *Lutjanus* species is presented in Table 4.

In general, the nucleotide composition of the *Lutjanus* genus shows the highest percentage of Thymine (T) and Cytosine (C), followed by Adenine (A) and Guanine (G). Analysis of the COI gene sequences revealed polymorphism within the genus, with a total of 65 polymorphic sites identified. The population of the genus *Lutjanus* species showed varying levels of haplotype diversity. Diversity analysis was performed only for species represented by more than one individual. A summary of haplotype diversity and related genetic diversity indices is presented in Table 5.

Table 3. BLAST results of *Lutjanus* species collected from the waters of Kaimana and Fakfak in BHS

Sampling localities	Species name	Sample size (N)	BLAST (Percent identity)	GenBank accessions
Kaimana Waters	*Lutjanus argentimaculatus*	4	99.85-100	OR524461.1
	Lutjanus bohar	7	99.85-100	OQ385529.1
	Lutjanus bengalensis	6	99.69-100	OL512911.1
	Lutjanus decussatus	5	96.94-100	OQ386044.1
	Lutjanus ehrenbergii	1	99.69	KP194151.1
	Lutjanus erythropterus	12	99.85-99.69	GU673202.1
	Lutjanus fulviflamma	2	100	MN870212.1
	Lutjanus gibbus	3	99.69	KF009614.1
	Lutjanus lemniscatus	1	100	EF609397.1
	Lutjanus malabaricus	1	99.85	OR758620.1
	Lutjanus monostigma	2	100	MK657561.1
	Lutjanus quinquelineatus	7	99.85-100	EF609399.1
Fakfak Waters	*Lutjanus argentimaculatus*	1	99,85	OR524461.1
	Lutjanus bohar	5	99.85-100	OQ385529.1
	Lutjanus biguttatus	1	100	OQ386009.1
	Lutjanus decussatus	5	96.94-100	OQ386044.1
	Lutjanus ehrenbergii	4	99.85-100	KP194151.1
	Lutjanus fulvus	2	100	PV170549.1
	Lutjanus lemniscatus	1	100	EF609397.1
	Lutjanus malabaricus	1	99.85	OR758620.1
	Lutjanus rufolineatus	1	100	OR524478.1
	Lutjanus russellii	1	100	OQ385435.1
	Lutjanus sebae	1	100	OQ386404.1
	Lutjanus timoriensis	1	100	OQ385512.1
	Lutjanus vitta	2	100	NC042930.1
	Total number of Individuals	77		



Lutjanus argentimaculatus

Lutjanus bengalensis

Lutjanus biguttatus

Lutjanus bohar

Lutjanus decussatus

Lutjanus ehrenbergii

Lutjanus erythropterus

Lutjanus fulviflamma

Lutjanus fulvus

Lutjanus gibbus

Lutjanus lemniscatus

Lutjanus malabaricus

Lutjanus monostigma

Lutjanus quinquelineatus

Lutjanus rufolineatus

Lutjanus russellii

Lutjanus sebae

Lutjanus timoriensis

Lutjanus vitta

Figure 2. Color photographs of commercially important snapper species (*Lutjanus* spp.); voucher specimens from Kaimana and Fakfak waters

Table 4. Characteristics of the COI gene sequences in *Lutjanus* species

Species	Sample number	Nucleotide composition (%)				G+C (%)	A+T (%)	Tr	Tv	Ps	Average codons
		T	C	A	G						
Lutjanus argentimaculatus	5	26.3	30.2	23.8	19.6	49.8	50.1	7	1	8	218
Lutjanus bengalensis	6	28.2	28.5	25.2	18.1	46.6	53.4	7	1	8	
Lutjanus biguttatus	1	29.1	27.7	24.5	18.8	46.5	53.6	-	-	-	
Lutjanus bohar	12	30.3	25.8	25.3	18.6	44.4	55.6	6	-	6	
Lutjanus decussatus	10	27.9	28.9	24.5	18.5	47.4	52.4	26	2	28	
Lutjanus ehrenbergii	5	28.8	28.1	23.8	19.3	47.4	52.6	2	2	4	
Lutjanus erythropterus	12	28.9	28.7	23.3	19.1	47.8	52.2	3	-	3	
Lutjanus fulviflamma	2	27.7	29.2	24.5	18.6	47.8	52.2	1	-	1	
Lutjanus fulvus	2	29.1	28.4	24.6	17.9	46.3	53.7	2	-	2	
Lutjanus gibbus	3	30.1	26.5	25.1	18.4	44.9	55.2	2	-	2	
Lutjanus lemniscatus	2	28.2	28.6	23.5	19.7	48.3	51.7	-	-	-	
Lutjanus malabaricus	2	28.3	28.3	23.8	19.6	47.9	52.1	-	-	-	
Lutjanus monostigma	2	28.6	28.6	23.8	18.9	47.5	52.4	1	-	1	
Lutjanus quinquelineatus	7	28.8	27.9	24.6	18.7	46.6	53.4	2	-	2	
Lutjanus rufolineatus	1	28.2	28.1	24.5	18.8	46.9	52.7	-	-	-	
Lutjanus russellii	1	28.9	28.1	24.5	18.4	46.5	53.4	-	-	-	
Lutjanus sebae	1	28.3	28.9	25.1	17.7	46.6	53.4	-	-	-	
Lutjanus timoriensis	1	29.1	29.1	23.1	18.8	47.9	52.2	-	-	-	
Lutjanus vitta	2	29.1	27.9	24.0	18.9	46.8	53.1	0	-	0	

Note: Tr: Transition, Tv: Transversion, Ps: Polymorphic site

Table 5. Genetic diversity of species from the genus *Lutjanus*

Species	Sample size (N)	Number of haplotypes, (h)	Haplotype (gene) diversity, (Hd)	Nucleotide diversity (per site), (Pi)
Lutjanus argentimaculatus	5	5	1.000	0.00398
Lutjanus bohar	12	6	0.682	0.00204
Lutjanus bengalensis	6	5	0.933	0.00438
Lutjanus biguttatus	1	-	-	-
Lutjanus decussatus	10	6	0.889	0.01356
Lutjanus ehrenbergii	5	3	0.800	0.00306
Lutjanus erythropterus	12	3	0.530	0.00151
Lutjanus gibbus	3	2	0.667	0.00204
Lutjanus fulviflamma	2	2	1.000	0.00153
Lutjanus fulvus	2	2	1.000	0.00306
Lutjanus lemniscatus	2	1	0	0
Lutjanus malabaricus	2	1	0	0
Lutjanus monostigma	2	2	1.000	0.00153
Lutjanus quinquelineatus	7	3	0.524	0.00116
Lutjanus rufolineatus	1	-	-	-
Lutjanus russellii	1	-	-	-
Lutjanus sebae	1	-	-	-
Lutjanus timoriensis	1	-	-	-
Lutjanus vitta	2	1	0	0

Note: The species *Lutjanus rufolineatus*, *Lutjanus russellii*, *Lutjanus sebae*, *Lutjanus timoriensis*, and *Lutjanus biguttatus* each contain one individual within the species, so they cannot reflect haplotype diversity

The haplotype and nucleotide diversity results in Table 4 provide important insights into the genetic structure of *Lutjanus* species in the BHS. The analysis of haplotypes across multiple species was conducted to assess both intraspecific genetic variation (within species) and interspecific divergence (between species), which together form the basis for understanding evolutionary processes and biodiversity patterns. By comparing haplotypes across different species, this study aimed to evaluate the extent of hidden genetic diversity, detect potential cryptic lineages, and establish whether COI sequences can reliably discriminate closely related taxa.

No significant differences were observed in the genetic distance values among individuals within genus *Lutjanus* (Table 6). Representative morphological photographs of *Lutjanus* species with the lowest and highest genetic distance values are shown in Figures 3 and 4, respectively.

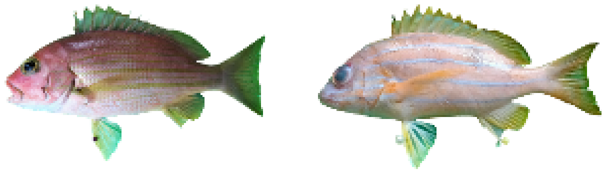
Table 6. Estimates of net evolutionary differences between species of the genus *Lutjanus*

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
Lutjanus argentimaculatus	**0.00**																		
Lutjanus bohar	0.174	**0.00**																	
Lutjanus decussatus	0.130	0.141	**0.01**																
Lutjanus ehrenbergii	0.139	0.152	0.105	**0.00**															
Lutjanus erythropterus	0.169	0.166	0.164	0.184	**0.00**														
Lutjanus fulviflamma	0.140	0.153	0.086	0.092	0.169	**0.00**													
Lutjanus fulvus	0.115	0.148	0.131	0.139	0.160	0.138	**0.00**												
Lutjanus gibbus	0.158	0.115	0.157	0.152	0.170	0.156	0.153	**0.00**											
Lutjanus lemniscatus	0.127	0.156	0.054	0.096	0.178	0.097	0.137	0.174	**0.00**										
Lutjanus malabaricus	0.159	0.175	0.172	0.181	0.099	0.154	0.160	0.174	0.179	**0.00**									
Lutjanus monostigma	0.127	0.148	0.102	0.080	0.166	0.098	0.141	0.168	0.093	0.163	**0.00**								
Lutjanus bengalensis	0.135	0.148	0.124	0.119	0.173	0.127	0.100	0.162	0.113	0.179	0.117	**0.00**							
Lutjanus quinquelineatus	0.121	0.138	0.144	0.130	0.162	0.137	0.089	0.147	0.145	0.179	0.131	0.063	**0.00**						
Lutjanus rufolineatus	0.118	0.152	0.132	0.123	0.153	0.130	0.091	0.152	0.128	0.167	0.124	0.061	0.028	**n/c**					
Lutjanus russellii	0.146	0.152	0.093	0.081	0.162	0.086	0.145	0.166	0.094	0.161	0.079	0.135	0.137	0.125	**n/c**				
Lutjanus sebae	0.177	0.188	0.185	0.178	0.155	0.161	0.189	0.193	0.189	0.136	0.163	0.173	0.169	0.168	0.161	**n/c**			
Lutjanus timoriensis	0.161	0.169	0.181	0.165	0.115	0.147	0.159	0.149	0.189	0.102	0.149	0.171	0.151	0.150	0.153	0.153	**n/c**		
Lutjanus biguttatus	0.139	0.148	0.092	0.102	0.171	0.100	0.133	0.175	0.083	0.182	0.102	0.132	0.138	0.128	0.101	0.186	0.179	**n/c**	
Lutjanus vitta	0.146	0.139	0.088	0.096	0.191	0.104	0.128	0.170	0.069	0.191	0.091	0.120	0.128	0.115	0.099	0.186	0.187	0.073	**0.00**

Note: Analysis included 77 nucleotide sequences. Yellow shading denotes the highest and lowest interspecific genetic distance values, while boldface indicates intraspecific genetic distances. “n/c” is used for species represented by a single individual, for which intraspecific genetic distance could not be determined. Standard error estimate(s) are were obtained by a bootstrap procedure (1,000 replicates). Analyses were conducted using the Kimura 2-parameter model. The analytical procedure encompassed 77 coding nucleotide sequences using 1st, 2nd, 3rd, and non-coding positions. The pairwise deletion option was applied to all ambiguous positions for each sequence pair resulting in a final data set comprising 654 positions. Evolutionary analyses were conducted in MEGA12

Lutjanus sebae *Lutjanus gibbus*

Figure 3. Morphology of species with the highest genetic distance (0.193)



Lutjanus rufolineatus *Lutjanus quinquelineatus*

Figure 4. Morphology of species with the lowest genetic distance (0.028)

Nineteen monophyletic groups corresponding to nineteen species, as deduced by BLAST, were generated from NJ phylogenetic trees. Both trees show a high percentage of bootstrap values that supported all the clustered species. Phylogenetic tree revealed that all samples exhibit close genetic relationships and no clear pattern in terms of species division based on the sample's localities. A comprehensive phylogenetic tree encompassing all *Lutjanus* species identified in this study is presented in Figure 5. Additionally, a comparative phylogenetic analysis was conducted by integrating COI gene sequences from NCBI with those generated in this study (Figure 6).

Discussion

Application of DNA barcoding

This study contributes novel insights by documenting 19 *Lutjanus* species—representing 59% of Indonesia's snapper diversity—within a relatively small area of the Bird's Head Seascape, establishing one of the most comprehensive molecular baselines for the genus in the region. This fine-scale genetic resolution is unprecedented in Papuan fisheries, where catch monitoring has largely depended on morphology-based identifications prone to errors. Beyond verifying species identities, DNA barcoding uncovers hidden biodiversity that traditional methods overlook, enhancing fisheries governance through more accurate stock assessments and targeted management of cryptic or vulnerable lineages. These findings also support more effective enforcement against illegal, unreported, and unregulated fishing in globally significant marine biodiversity hotspot.

Additionally, this study reveals potential cryptic speciation within *L. decussatus*, demonstrated by its subdivision into two well-supported phylogenetic subgroups with significant genetic divergence. This suggests that what is currently treated as a single species may actually consist of genetically distinct lineages that could be reproductively isolated or geographically structured. Such cryptic diversity has important implications for biodiversity assessments and fisheries management, as these hidden lineages may differ in their ecological roles, susceptibility to fishing pressure, and conservation priorities. The integration of DNA barcoding with phylogenetic analysis not only facilitates accurate species identification but also uncovers evolutionary patterns that traditional morphology-based methods often miss. This molecular approach provides a valuable framework for refining *Lutjanus* taxonomy and informs targeted conservation strategies in the Bird's Head Seascape, highlighting the essential role of genetic data in sustainable marine resource management.

Homology

Accurate species identification is fundamental for understanding fish biology and ensuring food safety for consumers. DNA barcoding has become a vital tool in this context, particularly for verifying the identity of both whole and processed fish products in which key morphological characteristics may no longer be present (Bemis et al. 2023). In this study, identification of 19 *Lutjanus* species using COI gene sequences yielded high similarity to reference sequences, ranging from 99.85% to 100%. According to Bemis et al. (2023), sequence identity of ≥97.5% is considered reliable for species-level identification, whereas sequences with ≤97.4% similarity require alignment with additional sequences and re-examination of voucher specimens for confirmation or revision.

The genus *Lutjanus* (Bloch, 1790) comprises 73 species globally (Froese and Pauly 2025a), with 43 species reported from the Indo-West Pacific region (Allen et al. 2013) and 32 species recorded in Indonesian waters (Froese and Pauly 2025b). Therefore, the identification of 19 species in the Fakfak and Kaimana waters of the Bird's Head Seascape (BHS) represents approximately 26% of the global *Lutjanus* diversity, 44% of species in the Indo-West Pacific, and 59% of those found in Indonesian waters.

The number of species found in Fakfak and Kaimana also exceeds those recorded in Yapen waters (Sala et al. 2023) and Nabire, Papua (Maharani et al. 2025). The composition of the species caught varies greatly at the two research locations. However, in general, the number of species found does not show a high difference. These differences may be influenced by the results and fishing operations, observation time, different habitats and species communities.

The application of DNA barcoding has proven to be highly effective for identifying *Lutjanus* species from mixed fishery catches. Morphological variation between juvenile and adult individuals within this genus—particularly in body coloration—often complicates field identification. For instance, juvenile and adult specimens of *Lutjanus timoriensis*, *L. malabaricus*, and *L. erythropterus* display highly similar and overlapping morphological features, leading to frequent misidentification (Sala et al. 2023; Milansari et al. 2025). Pranata et al. (2024b) documented these variations in detail and emphasized the complexity of distinguishing species based solely on coloration and external morphology. In such cases, DNA barcoding offers a reliable and objective method for accurate species identification.

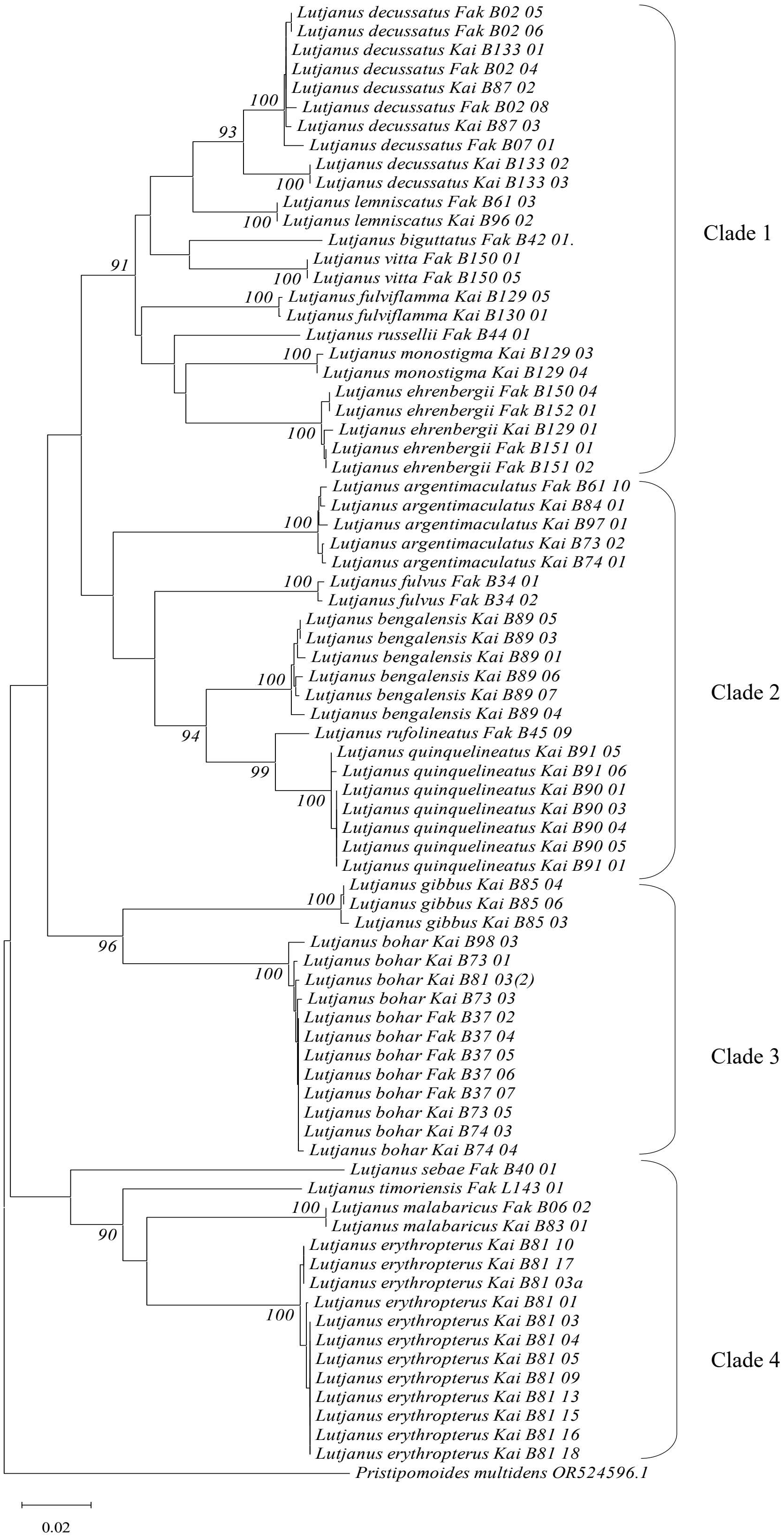


Figure 5. Molecular phylogeny of *Lutjanus* species. Bootstrap values (1000 replicates) greater than 90% are shown. The evolutionary history was inferred using the Neighbor-Joining method. The optimal tree with the sum of branch length = 1.163 is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1,000 replicates) is shown next to the branches. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were calculated using the Kimura 2-parameter method and are in units of the number of base substitutions per site. The analytical procedure encompassed 78 nucleotide sequences. The pairwise deletion option was applied to all ambiguous positions for each sequence pair resulting in a final data set consisting of 675 positions

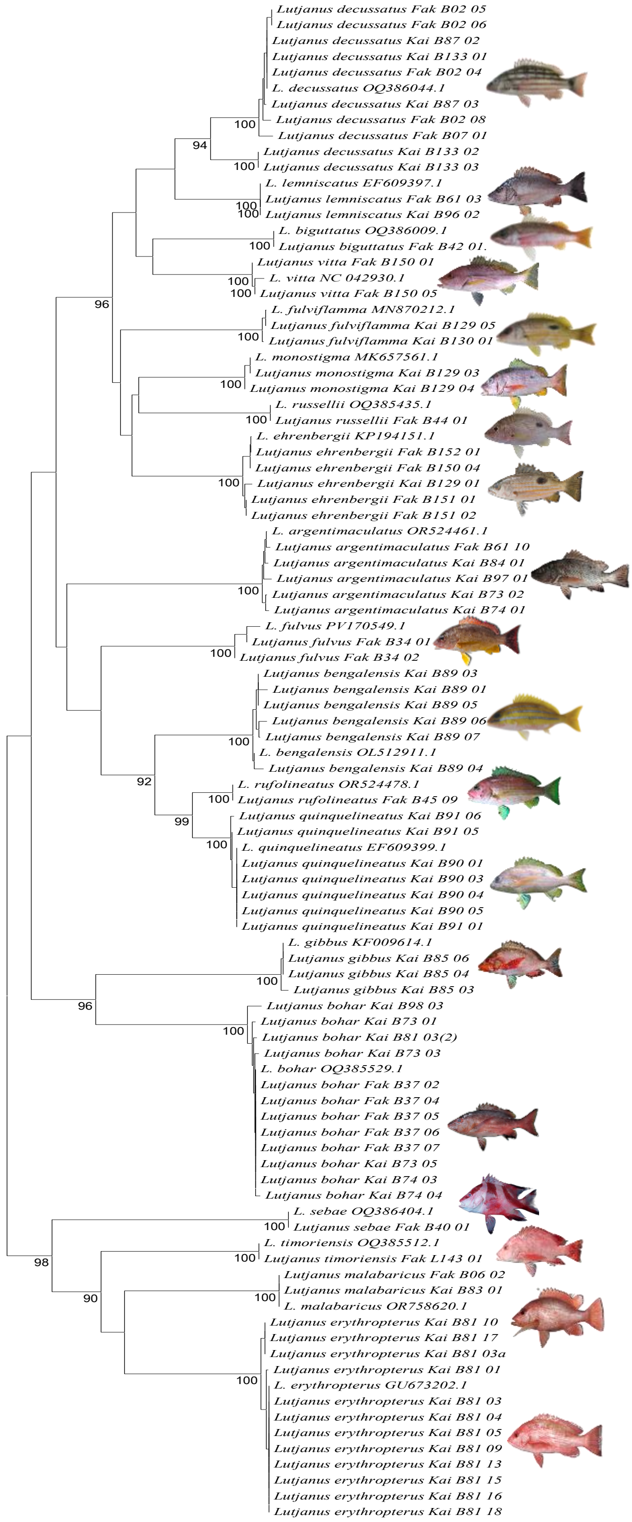


Figure 6. Neighbor-Joining (NJ) phylogenetic tree based on Kimura 2-Parameter (K2P) genetic distances, illustrating the evolutionary relationships among the Lutjanus species analyzed in this study alongside reference sequences retrieved from GenBank. The tree groups species according to their genetic similarity, with branch support assessed through bootstrap analysis using 1,000 replicates. Only bootstrap values above 80% are displayed on the tree, indicating strong confidence in the reliability of those branching points and overall clade formations. This figure confirms clear species delineations and supports the accuracy of species identifications derived from DNA barcoding

DNA barcoding is particularly valuable in overcoming the challenges associated with morphological identification in the field. DNA barcoding is a fast and accurate method for identifying species that relies on genetic data rather than external morphological traits, eliminating the need for expert taxonomic knowledge and offering a practical identification tool for non-specialists (Jiang et al. 2025). The DNA barcoding approach is very helpful in fisheries monitoring, and routine application of DNA barcode will produce high-quality information on the composition and exploitation of species of the genus *Lutjanus* in Papuan waters. Misidentification should be avoided, especially for commercially exploited species including commercially important Lutjanidae fish (Kausar et al. 2025).

Furthermore, this study has highlighted the potential presence of *L. timoriensis, L. malabaricus*, *L. bengalensis,* and *L. quinquelineatus,* suggesting the need for more comprehensive taxonomic investigations to verify their species status. Without the use of DNA barcoding, the recognition of these closely related groups might not have been possible, underscoring the power of DNA barcoding in uncovering hidden biodiversity.

Estimates of net evolutionary differences in genus Lutjanus

No genetic differences were observed among individuals within the same species, as shown in Table 5. These findings are consistent with those reported by Bakar et al. (2018) and Halim et al. (2022), who also found low intraspecific genetic distances within *Lutjanus* species. In contrast, the genetic distance between different species ranged from 2.8% to 19.3%, indicating clear interspecific divergence. Previous studies have reported varying levels of interspecific genetic distances within the genus *Lutjanus*, including 18.3% (Afriyie et al. 2020), 8.2% (Halim et al. 2022), 11.3% (Sala et al. 2023), and 13.3% (Kausar et al. 2025). As expected, the genetic divergence among individuals of the same species was consistently lower than that observed between species. This pattern supports the general principle that smaller genetic distances correlate with higher morphological similarity, while greater genetic distances often reflect more distinct morphological differentiation (Sala et al. 2023).

Lutjanus sebae and *L. gibbus* exhibited the highest interspecific genetic distance. This is consistent with their distinct morphological characteristics. *L. sebae* typically has a short and deep body shape, with juvenile and small adult individuals exhibiting vertical body bars. In contrast, *L. gibbus* has a more fusiform body with a distinctly forked caudal fin featuring rounded lobes, and juveniles are marked by a large round black spot at the base of the caudal fin. These pronounced morphological distinctions complement the observed high interspecific genetic distance between *L. sebae* and *L. gibbus*, measured up to approximately 19.3% in COI gene sequence divergence in this study as shown in both mitochondrial DNA barcoding and phylogenetic analyses (Halim et al. 2022; Sala et al. 2023). This correspondence between morphological and genetic data strengthens confidence in species delimitation and highlights the robustness of combining both approaches in fish taxonomy and fisheries management (Bakar et al. 2018; Jiang et al. 2025).

On the other hand, *L. quinquelineatus* and *L. rufolineatus* demonstrated the lowest genetic distance between species. Morphologically, both species share a fusiform body shape, a sharply sloped head profile, and similar fin structures—10-11 spiny dorsal fin rays, 13-15 soft dorsal rays, three spiny anal fins, and eight soft anal fins. Both species also exhibit horizontal stripes along the body and may have a round black spot below the anterior part of the dorsal fin. In *L. rufolineatus*, some specimens also display a black spot of similar size and location.

Genetic characteristics and genetic diversity

The COI gene is a mitochondrial DNA (mtDNA) marker widely used for rapid and cost-effective authentication and identification of various animal species (Hebert et al. 2003). It has been extensively applied in DNA barcoding studies, particularly for fish species identification (Toha et al. 2016, 2020; Sala et al. 2023; Pranata et al. 2024a, 2024b) and is a genetic sequence-based approach, based on standard gene regions (Toha et al. 2020).

In this study, the COI gene fragment for *Lutjanus* species was 654 base pairs (bp) in length. This is consistent with most DNA barcoding protocols, although slight variations were noted in other studies, such as 650 bp and 613 bp (Bakar et al. 2018; Halim et al. 2022). Differences in COI sequence length may result from variations in PCR primer sets or amplicon concentrations used during amplification (Toha et al. 2020). The percentage of nucleotide composition of T, C, and G from the genus *Lutjanus* species was quite similar. The G+C content was smaller when compared to the A+T content. These findings are in line with Chu et al. (2013) and Pranata et al. (2024), who also reported lower G+C composition in *L. gibbus*.

A total of 65 polymorphic sites were detected among the COI sequences, arising from both transition and transversion mutations. According to Sendell-Price et al. (2023), such mutations are fundamental to evolutionary processes, introducing variability into populations and enabling evolutionary adaptation. However, not all nucleotide mutations result in changes to amino acid sequences. Here are two primary types of nucleotide substitutions: transition and transversion mutations. Transition mutations involve substitutions between purines (A↔G) or between pyrimidines (C↔T), while transversions involve purine-to-pyrimidine changes or vice versa (A↔C, A↔T, G↔C, G↔T) (Aloqalaa et al. 2019). In this study, transition mutations were more frequent than transversions, likely due to the physicochemical similarities among nucleotides involved in transitions, which less frequently result in amino acid substitutions (Aloqalaa et al. 2019). Such mutations within the COI gene may reflect forms of genetic adaptation to specific aquatic environmental conditions (Fonseca et al. 2023).

The genetic diversity (haplotype) detected in this study ranged from 0.524-1 with a total of 42 haplotypes identified across all *Lutjanus* species sampled. Several species exhibited notably high levels of genetic diversity, including *L. argentimaculatus, L. bengalensis, L. decusatus, L. ehrenbergii, L. fulviflamma, L. monostigma* and *L. fulvus*. Although the number of individuals sampled varied between locations, this variation did not appear to influence the overall levels of haplotype or nucleotide diversity (Pereira et al. 2004). High levels of genetic diversity are often associated with increased adaptability and long-term population resilience (Toha et al. 2020).

Phylogenetic tree

The COI gene has proven to be useful for phylogenetic tree reconstruction (Kartavtsev et al. 2016). A total of nineteen monophyletic groups, each corresponding to a distinct species as identified through BLAST comparisons, were recovered in the NJ phylogenetic trees based on COI sequence data. In this study, phylogenetic analysis using COI sequences revealed well-resolved clades in which species were grouped according to their expected taxonomic relationships, with no evidence of interspecific clade mixing. The resulting phylogenetic tree delineated three primary clades based on morphological traits. Clade I comprises individuals that exhibit horizontal or vertical stripe patterns and/or prominent black spots on the body. Clade II includes two species lacking these stripe patterns, while Clade III encompasses four species without horizontal or vertical body stripes. Juvenile *L. sebae* show a stripe pattern. However, when they reach adult size, the stripe pattern will disappear. Clades have varying bootstrap values (Figure 5). A particularly interesting case was *L. decussatus*, which consistently formed two well-supported subgroups across both phylogenetic trees. This subdivision was also reflected in the genetic distance matrix, suggesting potential cryptic speciation or deep intraspecific divergence within this taxon. The existence of such substructure within *L. decussatus* warrants further investigation using additional markers (e.g., nuclear genes or genomic SNPs) and expanded sampling across its distribution range to assess whether these lineages represent reproductively isolated units or geographically structured populations. If confirmed, the recognition of cryptic taxa would have significant taxonomic and conservation implications.

These groupings were consistently supported by high bootstrap values, indicating strong confidence in the phylogenetic resolution of the sampled Lutjanus species. High bootstrap values indicate that the branches on the phylogenetic tree formed are reliable. Based on the phylogenetic tree reconstruction, *L. sebae, L. timoriensis, L. malabaricus* and *L. erythropterus* consistently form the same clade. This result aligns with findings from previous studies (Bakar et al. 2018; Halim et al. 2022; Sala et al. 2023). This confirms the phylogenetic proximity of these species within the genus *Lutjanus*. The phylogenetic structure did not reflect any geographic partitioning among samples, indicating that genetic variation within species is not strongly driven by locality (Pranata et al. 2024)—at least at the COI level. This may point to high gene flow across regions or limitations of the marker's resolution in capturing population-level structure. Barcode gap analysis further corroborated these findings by demonstrating distinct interspecific divergence across the majority of species, with minimal overlap between intra- and interspecific genetic distances. The absence of overlap in most cases confirmed the presence of a clear barcode gap, a critical criterion for reliable species delimitation using DNA barcoding (Fortaleza et al. 2025).

The NCBI database provides a platform for documenting the world's biodiversity and also provides reference sequences for comparison in the case of newly discovered species (Halim et al. 2022). In this study, no ambiguous sequences were detected (Figure 6), likely due to the high similarity of the reference sequences used. Grouping of misidentified sequences is one of the significant results of the phylogenetic tree. This is exemplified in the study of Halim et al. (2022), where several sequences did not group into the expected clade but instead into another species clade, indicating that the specimens were misidentified in the database. Lamendin et al. (2015) and Halim et al. (2022) also highlighted several misidentified sequences from the Lutjanidae family, especially *L. malabaricus* and *L. erythropterus*, submitted in the BOLD and NCBI databases. Another study (e.g., Cundy et al. 2023) has also documented cases of mislabeled *Lutjanus* sequences, particularly among species with overlapping morphologies. This is a common error because the study was conducted in several laboratories around the world with a large number of datasets.

From a conservation standpoint, these genetic findings are highly relevant. Phylogenetic attributes offered guidance to proactively manage multispecies fisheries and improve our understanding of ecological niches and ecosystem stability (Houk et al. 2021). Accurate species identification is critical for monitoring fisheries, enforcing trade regulations (e.g., CITES listings) (IUCN 2025), and implementing species-specific management plans. The detection of genetically distinct lineages, such as those seen within *L. decussatus*, could indicate the presence of previously unrecognized diversity that may be differentially impacted by fishing pressure or habitat degradation. Failing to distinguish such lineages could result in the oversight of at-risk populations and misinformed conservation priorities. Overall, the strong correspondence between genetic data and species-level taxonomy in this study highlights the value of molecular tools in supporting biodiversity conservation and sustainable fisheries management in marine ecosystems.

Lutjanus *fishery Management in BHS*

This study not only confirms the diversity of *Lutjanus* species in the Kaimana and Fakfak Marine Protected Areas, but also offers important insights into the genetic composition and structure of these populations. Comprehensive analyses revealed a high degree of taxonomic congruence between morphological and molecular identifications. Snapper and grouper are seafood products that are at high risk because the same species are marketed under different names in different countries, and extensive processing, which is common for imported products, removes diagnostic characters needed for morphological species identification (NOAA 2015).

The findings of this study also serve as a valuable reference for future biomonitoring efforts, including tracking trends in species diversity, detecting local extinctions, and uncovering cryptic or incipient speciation. Furthermore, they provide a scientific basis for marine biodiversity conservation and resource management**,** particularly in identifying areas of high biodiversity and implementing evidence-based conservation strategies (Huang et al. 2023). Lastly, as emphasized by Kovačić et al. (2020) and Limmon et al. (2020), species inventories based on well-curated collections and the integration of multiple data sources significantly enhance an understanding of fisheries.

The waters of Kaimana and Fakfak fall within Fisheries Management Area (WPPNRI) 715 (Decree of the Indonesian Minister of Marine and Fisheries Affairs No. 19/2022). This region possesses significant potential for both demersal and pelagic fishery resources. The estimated potential for demersal fish and reef fish, including the genus Lutjanus in Fisheries Management Area 715 is 185,562 tons. However, the exploitation level of reef fish resources—particularly red snapper—in WPPNRI 715, encompassing Kaimana, Fakfak, and surrounding waters, has reached an overexploited status.

A total of 67 species in the genus *Lutjanus* are registered with the IUCN. The IUCN has classified certain species as threatened because their populations have declined significantly, primarily as a result of overfishing driven by their economic, medicinal, or culinary importance (Dulvy et al. 2021). According to this number, only 9 are in the Data Deficient (DD) category and the rest are in the Least Concern (LC) status. All 19 species identified in the waters of Fakfak and Kaimana fall under the LC category. However, population trends for most of these species remain unknown, with the exception of *L. decussatus* and *L. sebae*, which are reported to show declining trends, and *L. monostigma*, whose population is considered stable (IUCN 2025). Notably, none of the *Lutjanus* species identified in this study are currently listed under CITES Appendix I, II, or III issued by the Convention on International Trade in Endangered Species of Wild Fauna and Flora valid from 7 February 2025 (CITES 2025). The conservation status of the *Lutjanus* species in the waters of Fakfak and Kaimana can be seen in Table 7.

All *Lutjanus* species identified in the waters of Fakfak and Kaimana are currently classified as Least Concern (LC) under the IUCN Red List criteria (IUCN 2025). This status indicates that, based on available data, these species do not meet the thresholds for categories such as Critically Endangered, Endangered, Vulnerable, or Near Threatened. The wildlife trade, including that of *Lutjanus* species, presents significant conservation and environmental security risks, especially in the absence of robust international regulatory framework to monitor trade in species not listed in the CITES appendix. Species listed in CITES are more likely to face reported conservation threats compared to species that are not listed. According to Fukushima et al. (2020), the risks of wildlife trade or commercialization to the survival of many species including lesser-known or less charismatic snappers, are often overlooked including all snappers in the genus *Lutjanus*, whose population trends are unknown via IUCN (IUCN 2025) and are not evaluated in CITES.

Table 7. Conservation status of the *Lutjanus* species in Fakfak and Kaimana waters according to IUCN* and CITES**

Species	IUCN/current population trend	CITES	Reference
Lutjanus argentimaculatus	LC/Unknown	-	Russell et al. (2016a)
Lutjanus bengalensis	LC/Unknown	-	Russell et al. (2019)
Lutjanus biguttatus	LC/Unknown	-	Russell et al. (2016n)
Lutjanus bohar	LC/Unknown	-	Russell et al. (2016b)
Lutjanus decussatus	LC/Decreasing	-	Curtis-Quick (2010)
Lutjanus ehrenbergii	LC/Unknown	-	Russell et al. (2016c)
Lutjanus erythropterus	LC/Unknown	-	Govender et al. (2019a)
Lutjanus fulviflamma	LC/Unknown	-	Russell et al. (2016f)
Lutjanus fulvus	LC/Unknown	-	Russell et al. (2016i)
Lutjanus gibbus	LC/Unknown	-	Russell et al. (2016e)
Lutjanus quinquelineatus	LC/Unknown	-	Russell et al. (2016d)
Lutjanus lemniscatus	LC/Unknown	-	Govender et al. (2019b)
Lutjanus malabaricus	LC/Unknown	-	Carpenter et al. (2019)
Lutjanus monostigma	LC/Stable	-	Russell et al. (2016g)
Lutjanus rufolineatus	LC/Unknown	-	Russell et al. (2016j)
Lutjanus russellii	LC/Unknown	-	Russell et al. (2016k)
Lutjanus sebae	LC/Decreasing	-	Russell et al. (2016l)
Lutjanus timoriensis	LC/Unknown	-	Russell et al. (2016m)
Lutjanus vitta	LC/Unknown	-	Russell et al. (2016h)

Note: *: International Union on Conservation of Nature, **: Convention on International Trade in Endangered Species of Wild Fauna and Flora, -: Not listed

The Government of Indonesia, through the Ministry of Marine Affairs and Fisheries, has issued Decree No. 123 of 2021 concerning the Snapper and Grouper Fisheries Management Plan. This policy initiative was driven in part by a 9.9% decline in the export value of snapper fishery products in 2020, as well as a shift in the status of resource utilization from fully exploited to overexploited. The decree prioritizes the management of three genera: *Lutjanus, Pristipomoides*, and *Aphareus*. These taxa were selected based on several practical considerations, including the dominance of their catch volumes, prevalence in trade, ease of data collection, and feasibility of monitoring efforts (Ministry of Marine and Fisheries Affairs No. 123/2021).

The management of marine resources in the waters of Fakfak and Kaimana is further supported by the designation of both regions as marine conservation areas**.** The Kaimana Marine Protected Area (MPA**)** encompasses a total area of 499,804.13 hectares (Decree of the Indonesian Minister of Marine and Fisheries Affairs No. 25/Kepmen-KP/2019). Similarly, the Fakfak MPA covers an area of 346,807.87 hectares, formalized under Decree No. 79/Kepmen-KP/2019. Taxonomic information of the genus *Lutjanus* species in Kaimana and Fakfak waters through DNA barcoding can help in providing accurate taxonomic data and information and species management.

This study confirmed the effectiveness of mitochondrial COI gene barcoding in accurately identifying nineteen *Lutjanus* species from the waters of Kaimana and Fakfak, including four morphologically similar species—*L. timoriensis*, *L. malabaricus*, *L. bengalensis*, and *L. quinquelineatus*—that are commonly misidentified in the field. The integration of BLAST results, phylogenetic analysis, and genetic distance metrics validated the reliability of COI for species delimitation in the Bird's Head Seascape (BHS) of Papua, Indonesia. These findings have direct implications for fisheries management by improving the accuracy of species identification in catch monitoring, enabling more reliable stock assessments, and helping to detect cryptic diversity, such as the genetic structuring observed in *L. decussatus*. The application of DNA barcoding can also support local enforcement efforts by providing a standardized tool to verify species identity in seafood trade and combat Illegal, Unreported, and Unregulated (IUU) fishing. As such, this molecular approach contributes to science-based fisheries governance and long-term conservation of marine biodiversity in one of the world's most ecologically valuable reef systems.

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