

# Molecular Identification of Checkered Snapper (*Lutjanidae*) from Fakfak and Kaimana Waters, Papua

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## Abstract

Accurate and rapid species identification of snappers (*Lutjanus* spp.) is essential for biodiversity assessment and fisheries resource management, particularly in regions with high biodiversity such as the waters of Kaimana and Fakfak, West Papua. This study aimed to identify the economically important checkered snapper (*Lutjanus decussatus*) using a molecular approach based on DNA barcoding of the cytochrome c oxidase subunit I (COI) gene marker. Six fish tissue samples were collected from the waters of Kaimana and Fakfak, followed by molecular analyses including DNA extraction, PCR amplification, and sequencing. The obtained sequences had a length of 644 base pairs. Sequence data were analyzed by comparison with reference databases using BLAST and the Barcode of Life Data System (BOLD). The results showed that all sample sequences exhibited 100% similarity to reference sequences of *L. decussatus* in the databases and clustered into a single clade consistently supported by high bootstrap values and low intraspecific genetic distances. The interspecific and outgroup sequence was clearly separated from the main clade, indicating distinct interspecific divergence. These findings demonstrate the effectiveness of COI-based DNA barcoding for species identification of *L. decussatus*, and the obtained genetic information can support further studies on genetic diversity and resource management in West Papua.

**Keywords:** DNA barcoding, *Lutjanus decussatus*, phylogenetics

## INTRODUCTION

The Fakfak and Kaimana waters, located in the Bird's Head Seascape region of Indonesia, constitute one of the most biodiverse marine ecosystems in the world. These coastal and reef environments host approximately 995 species of shallow reef fish, contributing substantially to the broader regional ichthyofauna of 1,150 reef fish species documented in the Bird's Head Seascape area (Allen & Erdmann, 2009). This exceptional biodiversity reflects favorable ecological conditions that support high species diversity, particularly through the presence of extensive coral reef habitats functioning as critical nurseries, spawning grounds, and feeding areas for numerous fish species (Rogers *et al.*, 2018). The coral reef ecosystems of Fakfak and Kaimana represent a globally significant conservation priority, making sustainable management of reef fish populations essential for long-term ecosystem stability and regional food security (Chatterjee, 2017).

Among the diverse fish species inhabiting these waters, the checkered snapper, *Lutjanus decussatus*, represents a critical component of Fakfak and Kaimana reef fish communities both ecologically and economically. Ecologically, this species functions as an important apex predator within coral reef ecosystems, feeding on smaller fish and crustaceans, thereby contributing to population control and maintaining ecosystem trophic balance (Palufi *et al.*, 2024). As a keystone predator, *L. decussatus* plays a disproportionate role in regulating the structure and stability of reef fish communities, making population conservation directly relevant to broader ecosystem health (Putra, 2023). Economically, *L. decussatus* is a highly valued commercial species, commanding premium market prices across Indonesian coastal markets and supporting livelihoods for local fishing communities (Nanami & Yamada, 2008).

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Despite its ecological and economic importance, *L. decussatus* populations face unprecedented threats from multiple anthropogenic pressures. Intensive fishing pressure targeting economically important snappers has increased substantially in Fakfak and Kaimana waters in recent decades, driven by rising coastal demand and expansion of commercial fishing operations (Eddy *et al.*, 2021). In addition to overfishing, populations are threatened by destructive fishing practices including bottom trawling, coastal habitat degradation, pollution, and the synergistic effects of global climate change on coral reef ecosystems (Martinez *et al.*, 2018). The combination of these stressors has led to substantial population declines of *L. decussatus* throughout Indonesian waters; this species is currently documented reductions in population size and fishing catch data over recent decades (IUCN, 2009). Given the ecological and economic significance of *L. decussatus* in the Fakfak-Kaimana region, conservation planning requires robust baseline data on population structure and species-level monitoring.

To address the critical need for accurate species identification and monitoring in conservation efforts, molecular identification through DNA barcoding has emerged as a powerful complementary tool to support rapid, accurate species confirmation across all ontogenetic stages and specimen conditions. Unlike morphological identification, DNA barcoding does not require specialized taxonomic expertise and provides species-level confirmation for specimens that are morphologically degraded, processed, or at developmental stages where traditional identification is unreliable (Ward *et al.*, 2005). DNA barcoding has been successfully applied to support fisheries monitoring, species verification in catch records, and biodiversity assessments across multiple tropical fish families, including the Lutjanidae (snappers) (Ward *et al.*, 2009; Jin *et al.*, 2021).

Prior DNA barcoding studies on snappers in Indonesian and neighboring waters provide important methodological guidance for the present study. Bakar *et al.* (2018) successfully developed DNA barcodes for *L. decussatus* collected from Malaysian waters, establishing baseline molecular reference sequences for this species. Subsequently, Fadli *et al.* (2024) expanded this work to include Lutjanidae species from Aceh waters (northern Sumatra), generating additional reference data for multiple snapper species in Indonesian waters. However, similar molecular characterization of *L. decussatus* from the Fakfak-Kaimana region remains absent from the scientific literature, representing a significant geographic gap in the available molecular reference database for this species.

DNA barcoding utilizes standardized short genetic markers that enable rapid and accurate species identification through molecular comparison (Koblmüller *et al.*, 2024). The approach is based on analysis of sequence variation at homologous gene regions, detecting barcoding gaps (the threshold difference between intraspecific and interspecific sequence variation) that distinguish species reliably (Elsaied *et al.*, 2021). The cytochrome c oxidase subunit I (COI) gene has become the universal standard marker for animal DNA barcoding, with a standardized amplicon length of approximately 650 base pairs (Hebert *et al.*, 2003). In fish identification, the COI barcode has demonstrated accuracy exceeding 98% across diverse teleost taxa, including reef-associated families such as Labridae, Pomacanthidae, and Carangidae (Ward *et al.*, 2005). The high discriminatory power of COI barcoding stems from its intermediate evolutionary rate—sufficient to accumulate species-level diagnostic differences while remaining too slow to generate excessive within-species variation that would obscure interspecific boundaries (Hebert *et al.*, 2003).

This study aims to contribute baseline molecular data supporting *L. decussatus* conservation and sustainable fisheries management in Fakfak and Kaimana waters. Specifically, we propose to: (1) establish DNA barcoding reference sequences for *L. decussatus* populations in Fakfak-Kaimana waters using the standard COI gene marker; (2) conduct phylogenetic analysis to assess the genetic relationships and evolutionary position of Fakfak-Kaimana populations relative to previously sequenced *L. decussatus* individuals from Malaysian and other Indonesian waters.

## MATERIAL AND METHODS

Samples consisted of ventral fins from putative *Lutjanus decussatus* specimens collected from fish markets in Fakfak and Kaimana, West Papua (Figure 1). Species identification was conducted through morphological observation based on diagnostic characters provided by FishBase ([www.fishbase.org](http://www.fishbase.org), Froese & Pauly, 2026 ). A total of six fin tissue samples were randomly selected, comprising three samples from Fakfak and three samples from Kaimana, and preserved in 96% ethanol. Molecular analyses were carried out at the Brainy Bee Molecular Biology Laboratory, Malang.

DNA isolation was performed using a Geneaid extraction kit following manufacturer users protocol and methods that is used by Dailami *et al.*, (2025), including tissue cell separation, cell lysis, DNA binding, purification, and elution using an elution buffer. A DNA barcoding fragment of the COI gene was amplified using universal primers designated for fish, namely the forward primer Fish-F1: 5'-TCAACCAACCACAAAGACATGGCAC-3' and the reverse primer Fish-R1: 5'-TAGACTTCTGGGTGGCCAAAGAATCA-3' (Ward *et al.*, 2005). PCR amplification was conducted using 40  $\mu$ L of PCR master mix containing Taq Green Polymerase, with 2  $\mu$ L of DNA template. The PCR cycling conditions consisted of an initial denaturation at 94°C for 15 seconds, followed by 35 cycles of denaturation at 94°C for 30 seconds, annealing at 51°C for 30 seconds, and extension at 72°C for 45 seconds, with a final extension at 72°C for 10 minutes (Dailami *et al.*, 2025). Amplification products were visualized by electrophoresis, targeting a band size of approximately 655 base pairs. Positive samples were subsequently sent for sequencing to a service provider in Malaysia, Apical Scientific Sdn. Bhd.

Sequencing results were obtained as electropherograms in ABI format. These data were edited to generate consensus sequences and to remove low-quality regions using Molecular Evolutionary Genetics Analysis (MEGA) software version 12 (Kumar, *et al.*, 2024). The resulting sequences were compared with reference data in the GenBank database using Basic Local Alignment Search Tool (BLAST). Molecular identification analysis was further conducted using the Barcode of Life Data System (BOLD system) to identify the clustering of sample sequences into Barcode Index Numbers (BINs) based on sequence similarity (Ratnasingham *et al.*, 2024).

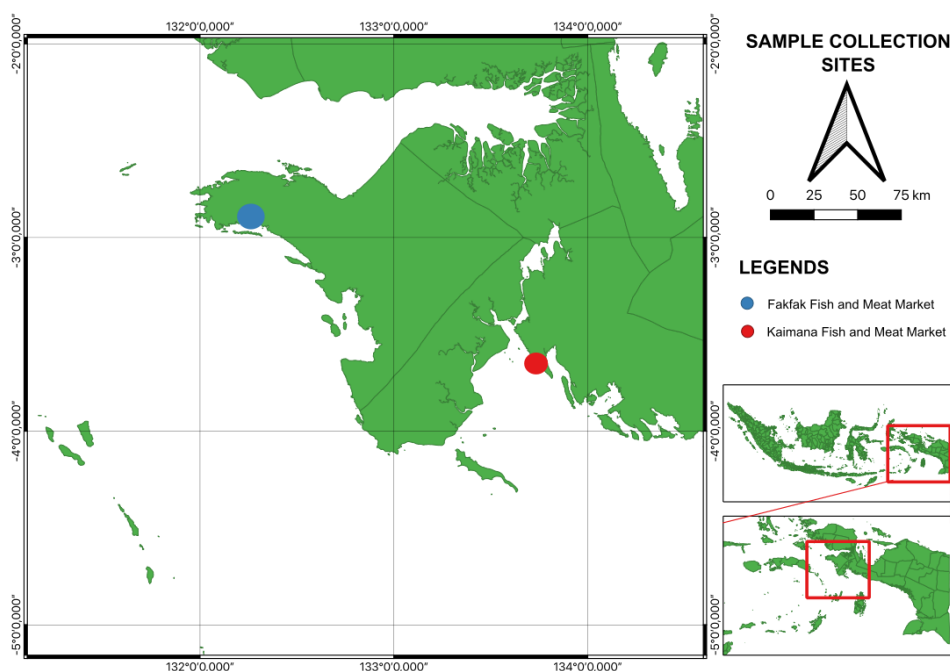


Figure 1. Map of the sampling locations

The sequence data obtained in this study consisted of three *L. decussatus* sequences from Fakfak and three sequences from Kaimana, which were classified as primary data, while the secondary data comprised sequences retrieved from the GenBank database. Secondary data, including intraspecific (within-species) were obtained from BLAST results with a total 12 sequences of *L. decussatus* in accordance with the intraspecific threshold proposed by Hebert, *et al.* (2003), with similarity differences less than 2%, and interspecific (between-species) sequences including *L. vitta*, *L. lutjanus*, *L. xanthopinnis*, and *L. lemniscatus*, selection of these species was based on previous phylogenetic studies by Bakar *et al.* (2018), which demonstrated that these taxa represent the closest relatives to *L. decussatus* within the genus *Lutjanus*, represented by two sequences that met the criteria of sequence lengths exceeding 650 base pairs and previously published records, *Pristipomoides multidentis* used as an outgroup. The data were downloaded from GenBank through the NCBI (National Center for Biotechnology Information).

Primary and secondary datasets were aligned using the ClustalW program in MEGA 12, with dataset details provided in Table 1. Data analyses, including nucleotide composition, genetic distance calculations, and phylogenetic reconstruction, were performed using MEGA 12. Phylogenetic reconstruction was conducted using the Maximum Likelihood (ML) method with the Kimura 2-parameter+Gamma distributed model and 1,000 bootstrap replications.

**Table 1.** Primary and secondary sequence datasets of *Lutjanus decussatus* used for genetic distance calculations and phylogenetic reconstruction, including sampling locations and GenBank NCBI accession numbers.

No	Species	Location	Acession number	Reference
1	<i>L. decussatus</i>	Fakfak	-	This study
2	<i>L. decussatus</i>	Fakfak	-	This study
3	<i>L. decussatus</i>	Fakfak	-	This study
4	<i>L. decussatus</i>	Kaimana	-	This study
5	<i>L. decussatus</i>	Kaimana	-	This study
6	<i>L. decussatus</i>	Kaimana	-	This study
7	<i>L. decussatus</i>	Filipina	OQ387552	(Bemis <i>et al.</i> 2023)
8	<i>L. decussatus</i>	Filipina	OQ386044	(Bemis <i>et al.</i> 2023)
9	<i>L. decussatus</i>	Indonesia	MN870210	(Limmon <i>et al.</i> , 2020)
10	<i>L. decussatus</i>	Indonesia	GU674161	(Chenery, 2010)
11	<i>L. decussatus</i>	Indonesia	MN869973	(Limmon <i>et al.</i> , 2020)
12	<i>L. decussatus</i>	Indonesia	KC130839	(Allen <i>et al.</i> , 2013)
13	<i>L. decussatus</i>	Indonesia	MN870035	(Limmon <i>et al.</i> , 2020)
14	<i>L. decussatus</i>	Filipina	OQ386916	(Bemis <i>et al.</i> 2023)
15	<i>L. decussatus</i>	Filipina	OQ387088	(Bemis <i>et al.</i> 2023)
16	<i>L. decussatus</i>	Filipina	OR524464	(Huang <i>et al.</i> , 2023)
17	<i>L. decussatus</i>	Indonesia	KC130844	(Allen <i>et al.</i> , 2013)
18	<i>L. decussatus</i>	Indonesia	KC130837	(Allen <i>et al.</i> , 2013)
19	<i>L. vitta</i>	Indonesia	MN870152	(Limmon <i>et al.</i> , 2020)
20	<i>L. vitta</i>	Australia	KT781910	(Iwatsuki <i>et al.</i> , 2015)
21	<i>L. lutjanus</i>	Malaysia	OR918734	(Abidin <i>et al.</i> , 2024)
22	<i>L. lutjanus</i>	Filipina	OR524475	(Nanola <i>et al.</i> , 2024)
23	<i>L. xanthopinnis</i>	Malaysia	OR918767	(Abidin <i>et al.</i> , 2024)
24	<i>L. xanthopinnis</i>	Malaysia	OR918757	(Abidin <i>et al.</i> , 2024)
25	<i>L. lemniscatus</i>	Banglades	PX640665	(Hasan <i>et al.</i> , 2025)
26	<i>L. lemniscatus</i>	Australia	EF609397	(Ward & Holmes, 2007)
27	<i>Pristipomoides multidentis</i>	Filipina	OQ387822	(Bemis <i>et al.</i> , 2023)

## RESULTS AND DISCUSSION

Analysis of primary and secondary sequence data resulted in a sequence length of 644 base pairs, consistent with the standard COI barcoding region established for fish species identification. This sequence length is optimal for species-level discrimination while maintaining sufficient conservatism for reliable sequence alignment and comparison across distantly related taxa (Hebert *et al.*, 2003; Ratnasingham *et al.*, 2024). BLAST analysis of samples presumed to be *Lutjanus decussatus* from the waters of Fakfak and Kaimana showed a percent identity of 100%–99.85% with *L. decussatus* sequences available in the GenBank database, indicating high molecular consistency with previously characterized specimens. DNA barcoding analysis using the BOLD system independently confirmed these results, with sample sequences exhibiting 100% similarity. Further validation through Barcode Index Number (BIN) analysis showed that all samples were affiliated with BIN code BOLD:ADF5202, representing a single, unique classification within the BOLD database. These convergent results obtained through independent molecular identification approaches (BLAST, BOLD system similarity) support the reliability and consistency of molecular identification obtained using the DNA barcoding methodology (Ratnasingham & Hebert, 2013).

The consistency between these multiple independent validation methods is noteworthy and strengthens the confidence in species identification. The BIN represents a code generated from the automatic clustering of highly similar DNA sequences into a single group by the BOLD system, with each BIN being distinct from others. This clustering mechanism provides an objective, reproducible approach for rapid organism identification without requiring prior taxonomic knowledge (Ratnasingham & Hebert, 2013). BIN clustering serves as a proxy for rapid organism identification, particularly in cases where morphological characteristics are unavailable, such as when samples are limited to specific tissues or when dealing with newly discovered species that have not yet been taxonomically described (Ratnasingham & Hebert, 2013; Dahruddin *et al.*, 2016). The consistency between BLAST identity scores (99.85%–100%) and BOLD similarity results (100%) to the same BIN code strongly validates the application of COI barcoding for *L. decussatus* in this region (Stoeckle *et al.*, 2017).

BIN clustering is generally based on the COI barcoding region, as this marker demonstrates high effectiveness in distinguishing taxa and minimizes ambiguity due to low intraspecific variation and high interspecific divergence (Kartavtsev, 2018). The principle underlying barcoding discrimination relies on the existence of a species-level barcoding gap, defined as the non-overlapping range between maximum intraspecific and minimum interspecific distances, which enables clear species delineation (Meier *et al.*, 2006). This barcoding gap phenomenon is critical to the success of DNA barcoding as a taxonomic tool, as it ensures that sequences within a species remain sufficiently similar while maintaining clear separation from other species.

A total of 5 polymorphic sites were identified among *L. decussatus* sequences, indicating the presence of genetic variation within the species and enabling the definition of distinct haplotypes based on differences in nucleotide composition at these variable positions. These polymorphic sites resulted in haplotype grouping patterns based on geographic origin, with distinct haplotypes associated with sampling locations from Fakfak, Kaimana, the Philippines, and other Indonesian waters (Table 2). Intraspecific variation can be estimated through the number of polymorphic sites that appear in the sequence results, which represents the raw genetic diversity within a species and serves as a quantitative measure of intraspecific genetic heterogeneity (Zhang *et al.*, 2020; Saleky & Dailami, 2021).

Haplotypes represent clustered DNA variations within a species and are used to assess genetic variation within a single population or among different populations (Gu *et al.*, 2022; Thakur *et al.*, 2025). The haplotype network revealed a marked geographic structure, with particular haplotypes showing strong association with specific sampling regions, suggesting population-level genetic differentiation driven by geographic isolation or restricted gene flow at fine spatial scales (Carpenter

et al., 2011). This geographic structure is important for understanding population connectivity and evolutionary history.

A single population may consist of more than one haplotype with varying levels of diversity, which serves as a key indicator in conservation efforts to evaluate the adaptive capacity of a population to environmental changes (Zhang et al., 2020). The identification of multiple haplotypes within *L. decussatus* populations suggests significant genetic heterogeneity that may underpin adaptive potential and resilience to environmental stressors, including climate change and anthropogenic disturbance (Selkoe & Toonen, 2011). The occurrence of such variation arises from DNA mutations influenced by evolutionary processes as well as demographic factors, including population expansion, bottleneck effects, gene flow, genetic drift, habitat fragmentation, and selection (Rahman et al., 2025; Wakimura et al., 2023).

The analysis revealed five distinct haplotypes, each showing characteristic clustering patterns and geographic associations. Haplotype 1 (11 individuals) exhibited the highest frequency and demonstrated genetic sharing between populations from Fakfak and Kaimana, indicating genetic connectivity between these geographically proximate regions. This widespread haplotype was also shared with reference sequences from the Philippines and multiple Indonesian locations, suggesting a widespread ancestral haplotype distributed across the broader Indo-Pacific region. The sharing of Haplotype 1 between Fakfak and Kaimana populations is particularly significant, as it indicates that despite geographic separation, these local populations are part of a larger, genetically connected meta-population. Genetic sharing was also observed in Haplotype 2 (4 individuals) between sequences from the Philippines and one sample from Fakfak, suggesting ongoing or historical gene flow across the Indo-Pacific Ocean and supporting the observation that oceanic barriers do not substantially restrict *L. decussatus* dispersal.

In contrast, a sample from Fakfak (Haplotype 3) appeared as a singleton (unique haplotype found only in a single individual) similar to samples from Indonesia that formed independent haplotypes (Haplotypes 4 and 5), each represented by single individuals. The presence of singleton haplotypes may reflect private mutations arising through recent mutations in isolated lineages,

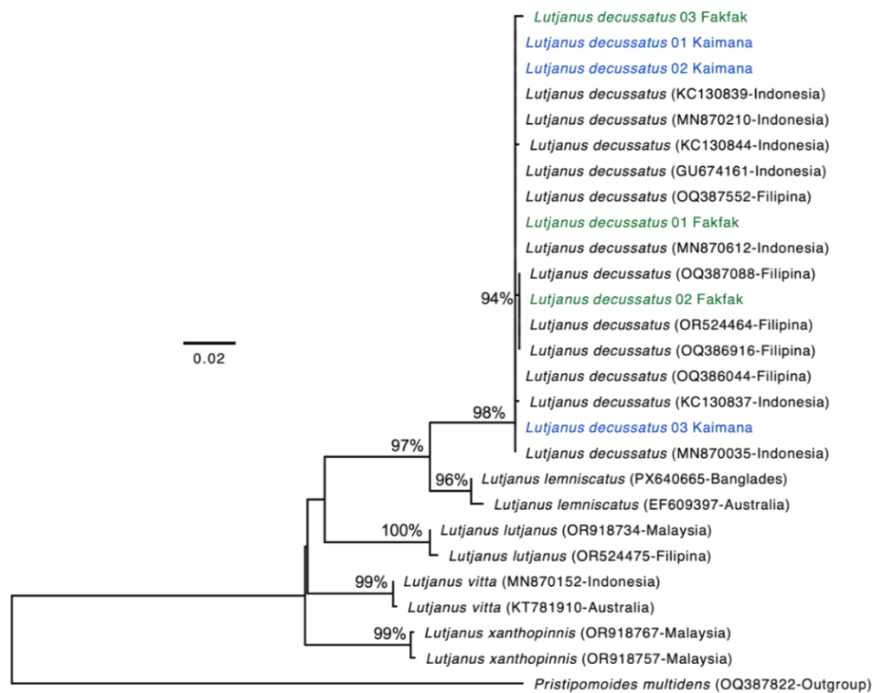
**Table 2.** Haplotype distribution of *L. decussatus* specimens from Fakfak and Kaimana waters (Indonesia), the Philippines, and other Indonesian locations, showing geographic patterns and relative frequencies of distinct haplotypes

No	Haplotype	Number of samples	Sample ID
1	Haplotype 1	11	<i>Lutjanus decussatus</i> -01-Fakfak <i>Lutjanus decussatus</i> -01-Kaimana <i>Lutjanus decussatus</i> -02-Kaimana <i>Lutjanus decussatus</i> -03-Kaimana <i>Lutjanus decussatus</i> _(MN870612-Indonesia) <i>Lutjanus decussatus</i> _(OQ387552-Filipina) <i>Lutjanus decussatus</i> _(OQ386044-Filipina) <i>Lutjanus decussatus</i> _(MN870210-Indonesia) <i>Lutjanus decussatus</i> _(GU674161-Indonesia) <i>Lutjanus decussatus</i> _(KC130839-Indonesia) <i>Lutjanus decussatus</i> _(MN870035-Indonesia)
2	Haplotype 2	4	<i>Lutjanus decussatus</i> -02-Fakfak <i>Lutjanus decussatus</i> _(OR524464-Filipina) <i>Lutjanus decussatus</i> _(OQ386916-Filipina) <i>Lutjanus decussatus</i> _(OQ387088-Filipina)
3	Haplotype 3	1	<i>Lutjanus decussatus</i> -03-Fakfak
4	Haplotype 4	1	<i>Lutjanus decussatus</i> _(KC130844-Indonesia)
5	Haplotype 5	1	<i>Lutjanus decussatus</i> _(KC130837-Indonesia)

cryptic population structure, or rare dispersal events that established isolated lineages in specific geographic regions (Carpenter *et al.*, 2011). These singleton haplotypes could represent locally adapted lineages or transient variants that have not yet become established in other populations. The coexistence of common haplotypes like Haplotype 1 with rare singleton haplotypes within the same geographic region suggests complex demographic history and possibly recent colonization events or population mixing.

Phylogenetic tree reconstruction of *Lutjanus decussatus* sequences was conducted to visualize evolutionary relationships and validate species-level clustering patterns. The analysis employed bootstrap analysis to assess the statistical support for inferred branching patterns. In phylogenetic reconstruction, bootstrap values represent the percentage of times a given branch is recovered after thousands of resampling replicates, with higher values indicating greater statistical support and consistency of the inferred branching patterns (Russo & Selvatti, 2018; Shi, 2024). All sample sequences clustered into a single, well-defined clade that was consistently associated with reference sequences of *L. decussatus* from GenBank, demonstrating robust phylogenetic concordance and supporting the accuracy of species identification across all examined specimens. Figure 2 presents the phylogenetic tree reconstruction showing distinct branching patterns and clade composition.

The phylogenetic tree reconstruction formed all reconstructed branches with high bootstrap support values ( $\geq 95\%$ ), indicating strong statistical support for the inferred branching patterns and clade topology. This high bootstrap support demonstrates that the phylogenetic relationships are robust and not artifacts of the analysis. Secondary *L. decussatus* sequences from the Philippines clustered together with several samples from Fakfak within the main species clade, exhibiting a nested pattern characteristic of recent population divergence or ongoing gene flow (Tovar *et al.*, 2023). These phylogenetic patterns are consistent with the results of genetic distance analysis and haplotype distribution, supporting the presence of gene flow among regions, especially in the Indo-Pacific Ocean where larval dispersal and seasonal fish migration facilitate population connectivity across oceanic basins (Crandall *et al.*, 2019; Shanks *et al.*, 2003).



**Figure 1.** Phylogenetic tree reconstruction of checkered snapper *L. decussatus*, Fakfak sequences highlighted in green and Kaimana sequences highlighted in blue

The outgroup taxa outside the *L. decussatus* clade consisted of interspecific comparative species sequences within the genus *Lutjanus*, including *L. lemniscatus*, *L. lutjanus*, *L. vitta*, and *L. xanthopinnis*, all supported by high bootstrap values ( $\geq 95\%$ ), indicating robust phylogenetic structure and a clear barcode gap between *L. decussatus* and its closely related congeners (Bakar *et al.*, 2018). The marked phylogenetic separation between *L. decussatus* and other Lutjanidae species at the COI locus confirms the high discriminatory capacity of this marker for species identification, even among closely related species sharing similar morphological characters. The outgroup *Pristipomoides multidens* clearly separated from all *Lutjanus* groups and serves to root the phylogenetic tree, providing an appropriate reference point for evaluating divergence times and evolutionary distances. The overall phylogenetic tree structure provides multiple independent lines of evidence confirming that all samples examined in this study belong to *L. decussatus* and reinforces the effectiveness of DNA barcoding as a method for species-level taxonomic confirmation (Darmawan *et al.*, 2026).

Genetic distance analysis is used to quantify the degree of genetic divergence among individual sequences or populations, providing a quantitative framework for assessing species boundaries and evaluating intraspecific versus interspecific variation (Srivathsan & Meier, 2012). Genetic distance is commonly calculated using the Kimura 2-Parameter (K2P) model, which estimates genetic divergence by accounting for different types of nucleotide substitutions and corrects for multiple substitutions at the same locus (Kimura, 1980; Kumar *et al.*, 2018). This model is particularly well-suited for DNA barcoding applications because it provides more accurate distance estimates than simple uncorrected distances when comparing sequences that may have undergone substantial evolutionary divergence (Xing *et al.*, 2021).

The intraspecific genetic distances of *L. decussatus* ranged from 0.000 to 0.003 among samples from Fakfak and Kaimana and other sequences from various countries, falling well below the commonly accepted threshold of genetic difference of less than 2% (or K2P distance  $< 0.02$ ) that is considered indicative of intraspecific variation rather than interspecific divergence (Hebert *et al.*, 2003; Meier *et al.*, 2006). This clear separation between intraspecific distances and the interspecific threshold (0.000–0.003 versus  $< 0.02$ ) supports robust species delineation and confirms that the samples from Fakfak and Kaimana represent true *L. decussatus* rather than cryptic congeners. The low intraspecific variation observed reflects the recent divergence and ongoing gene flow among populations, which prevents substantial sequence divergence within the species. Smaller genetic distance values indicate higher genetic similarity among individuals, which is typically observed within the same species, reflecting the low mutational divergence that occurs within populations over recent evolutionary timescales (Naz *et al.*, 2022).

In contrast, the genetic distances between *L. decussatus* and other species within the same genus consistently show higher values (0.05–0.09), exceeding the intraspecific threshold by 2.5 to 4.5 times and thereby confirming clear interspecific separation at the COI marker (Bakar *et al.*, 2018; Ward *et al.*, 2005). These interspecific distances are consistent with distances observed between other congeneric fish species, validating the effectiveness of COI barcoding for genus-level discrimination in the Lutjanidae family (Hubert & Hanner, 2015). The ability of genetic distance to clearly discriminate between taxa at different hierarchical levels forms the basis of species identification and is particularly important for detecting cryptic species diversity and preventing misidentification of morphologically similar taxa (Bingpeng *et al.*, 2018).

The molecular results provide strong evidence for gene flow among *L. decussatus* populations across the Indo-Pacific region. The presence of shared haplotypes (particularly Haplotype 1) between Fakfak, Kaimana, and Philippine populations, combined with the nested phylogenetic pattern and relatively low intraspecific genetic distances, all indicate that there are no significant barriers in these waters that restrict genetic mixing among populations. This observation suggests oceanographic connectivity across the broader Indo-Pacific Ocean region (Crandall *et al.*, 2019).

Ocean currents and hydrographic features of the Indo-Pacific play a critical role in mediating marine population structure by either facilitating or restricting larval dispersal and establishing connectivity corridors among geographically separated populations. The relatively short geographic distances between Fakfak and Kaimana, together with ocean current movements in the Coral Triangle region, facilitate gene flow among regions through fish activities such as migration and larval dispersal (Jahnke & Jonsson, 2022). This oceanographic connectivity is particularly important for species with pelagic larvae, such as *Lutjanus* species, which exhibit extended larval periods allowing transport across considerable oceanic distances before settlement (Selkoe & Toonen, 2011).

The results of this study demonstrate the reliability of the COI region marker for not only species identity confirmation but also for providing insights into genetic connectivity among fish populations across their distribution ranges (Saleky & Dailami, 2021). The molecular data presented here support the hypothesis that *L. decussatus* represents a genetically cohesive species with high connectivity across the Indo-Pacific, consistent with its known wide geographic distribution and migratory ecology. However, the presence of geographically structured haplotypes (Haplotypes 3–5 as singletons from specific regions) suggests that fine-scale population structure may exist despite overall high gene flow, potentially reflecting recent demographic events, localized adaptation, or cryptic population subdivisions (Carpenter *et al.*, 2011). These fine-scale patterns indicate that while broad-scale connectivity homogenizes the population, localized processes may maintain genetic variation at smaller geographic scales.

The molecular identification results presented in this study provide multiple independent lines of evidence confirming that the specimens from Fakfak and Kaimana belong to *L. decussatus*. This multi-faceted molecular confirmation strengthens confidence in species identification and demonstrates the robustness of DNA barcoding as a taxonomic tool for species-level resolution in commercially important reef fish species (Ratnasingham & Hebert, 2013; DeSalle & Goldstein, 2019).

The successful application of DNA barcoding to *L. decussatus* has important implications for fisheries management and conservation in Indonesian waters. Rapid, reliable species identification is essential for accurate catch monitoring, preventing mislabeling in fish trade, and ensuring that fishing regulations are applied to the correct species (Vecchioni *et al.*, 2019). In cases where morphological identification is ambiguous or impossible (such as in fish processing facilities, fish markets, or from degraded tissue samples) DNA barcoding provides an objective, standardized method for species verification that can support enforcement of species-specific protection measures and improve the efficacy of fisheries management (Odah, 2023). The COI barcode sequences generated in this study for *L. decussatus* from Fakfak and Kaimana waters expand the available reference database for this species and provide regional molecular baseline data that can facilitate future monitoring efforts and support conservation planning in the Bird's Head Seascape.

The haplotype structure identified in this study suggests that *L. decussatus* populations in the Indo-Pacific exhibit genetic heterogeneity that may be important for long-term population persistence and adaptive capacity (Selkoe & Toonen, 2011). The presence of multiple haplotypes and the observation of population-specific or region-specific genetic variants support the maintenance of within-species genetic diversity as a key conservation priority. Management strategies that preserve population connectivity and maintain genetic diversity across the distribution range of *L. decussatus* are likely to enhance population resilience to environmental change and anthropogenic stressors (Mašanović *et al.*, 2024). The findings suggest that protecting multiple populations across the geographic range, rather than focusing on a single location, will better maintain the genetic diversity and adaptive potential necessary for long-term species persistence. Additionally, the evidence of ongoing gene flow across the Indo-Pacific suggests that conservation measures must consider regional connectivity when designing marine protected areas or implementing fisheries restrictions.

## CONCLUSION

This study successfully applied DNA barcoding to *Lutjanus decussatus* specimens from Fakfak and Kaimana waters, generating the first genetic baseline data for this economically important snapper species in Indonesia's Bird's Head Seascape region. Our analysis of the cytochrome c oxidase subunit I (COI) gene produced standardized 644 bp sequences that were validated through multiple independent approaches (BLAST similarity 99.85%–100%, BOLD analysis with BIN code BOLD:ADF5202, phylogenetic reconstruction with  $\geq 95\%$  bootstrap support, and genetic distance analysis), confirming that DNA barcoding provides a highly reliable tool for species-level taxonomic confirmation. The identification of five haplotypes, with close genetic relationships to populations from the Philippines and other Indonesian waters, reveals both regional-scale population connectivity reflecting Indo-Pacific oceanographic patterns and fine-scale genetic structure indicating localized population subdivisions. These molecular data have direct applications for fisheries management and conservation in Indonesian waters: the established DNA barcode sequences enable rapid species identification in processing facilities, markets, and trade surveillance where morphological identification is unreliable; the evidence of regional genetic connectivity indicates that sustainable management strategies must adopt coordinated, ecosystem-based approaches across multiple jurisdictions; and the availability of molecular baseline data enables future population genetic monitoring to detect changes in genetic diversity and connectivity that may signal population decline or responses to environmental stressors.

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