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Genetic diversity and connectivity of Red Snapper *Lutjanus gibbus* in the Papua Waters, Indonesia

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Abstract. *Pranata B, Sala R, Kusuma AB, Toha AHA, Purbani DC, Mokodongan DF, Sipriyadi. 2024. Genetic diversity and connectivity of Red Snapper* Lutjanus gibbus *in the Papua Waters, Indonesia. Biodiversitas 25: 276-286.* There is limited knowledge regarding the genetic connectivity and diversity of the Red Snapper (*Lutjanus gibbus* Forsskål, 1775) populations that inhabit Papua Waters, Indonesia. Thus, the current study attempted to ascertain the genetic characteristics, level of diversity and genetic connectivity of the *L. gibbus* population. We conducted genetic research on 38 *L. gibbus* specimens from five different places in Papua Waters. We analyzed the *L. gibbus* genetic characteristics, diversity and genetic connectivity using the Cytochrome C Oxidase subunit I (COI) gene as the genetic marker. There were 15 polymorphism sites in the COI gene sequence among *L. gibbus* individuals. Polymorphism occurs due to transversion and transition mutations. The COI gene fragment was translated, producing 188 amino acids composed of 19 different amino acids. A total of 13 distinct haplotypes were detected among the *L. gibbus* population residing in Papua Waters. The haplotype diversity (Hd=0.740) and nucleotide diversity (Pi=0.002) were relatively medium. The genetic structure study indicated that the 5 populations of *L. gibbus* in the Papua Waters had little genetic differentiation, as evidenced by a Fixation Index (Fst) of 0.018 (P-value 0.35386±0.01443). Based on this, the management of Red Snapper resources at these 5 locations must be carried out as a single unit.

Keywords: COI Gene, haplotype, mtDNA, nucleotides, Red Snapper

INTRODUCTION

Based on the Decree of the Minister of Maritime Affairs of the Republic of Indonesia No. 19/2022, the Papua Waters have been designated part of fisheries management area 717. The potential for demersal and pelagic fisheries in these seas is significant. Based on the Decree of the Minister of Maritime Affairs of the Republic of Indonesia No. 19/2022, the estimated capacity for demersal fish and coral fish, including the *Lutjanus* genus, within the fisheries management area is 19,814 tonnes and 69,2010 tonnes, respectively. The *Lutjanus* genus is one of the main targets for fishermen's catches in Papuan bird's headwaters (Sala et al. 2022).

Fish belonging to the genus *Lutjanus* inhabit coral reef aquatic settings (Souza et al. 2019) and are distributed over the subtropical and tropical oceans of the Indo-West Pacific (Allen et al. 2013). The Indo-West Pacific is home to forty-three species of Red Snapper (Lutjanidae) (Allen et al. 2013), and the new species of Red Snapper, *Lutjanus xanthopinnis* Iwatsuki, Tanaka & Allen, 2015 was described by Iwatsuki et al. (2015). One species belonging to the genus *Lutjanus* is *Lutjanus gibbus* Forsskål, 1775. This species inhabits the aquatic environments of Papua's bird's head region (Sala et al. 2022).

The use of fishery resources necessitates careful consideration of the natural stock availability. Overfishing can lead to a decline in the population of specific species. The decline in population size can lead to a reduction in genetic diversity (Pinsky and Palumbi 2014; Petit-Marty et al. 2022). Genetic diversity holds significant importance for organisms. It is the fundamental basis for natural selection, enabling organisms to adjust and thrive in response to environmental alterations effectively. The diminishment of genetic diversity will reduce the adaptive ability of a certain species (Pinsky and Palumbi 2014; Bernatchez et al. 2017; Gandra et al. 2021).

No studies have been conducted on the genetic diversity of *L. gibbus* in the Papuan bird's headwaters. Consequently, this study aimed to offer preliminary data to comprehend the alterations in genetic diversity that have transpired within this species. Petit-Marty et al. (2022) elucidated that to comprehend the influence of fishing on genetic diversity, it is necessary to get samples before and after exploitation. An alternative method that can be used to comprehend the reduction in genetic variety involves utilizing comparative datasets of genetic diversity in closely related species (Petit-Marty et al. 2022)

In addition, comprehending the connectivity between stocks or populations of fishery resources is crucial; population connectivity can be a reference in managing captured fisheries resources and conservation. Connectivity

between populations or fishery resource stocks can be investigated using a genetic approach. Genetic research has significantly contributed to managing fishery resources in the European Union (Casey et al. 2016). This research has helped in identifying species based on the genetic connectivity between different populations of commercially fished stocks (Pita et al. 2014; Druon et al. 2015) or mixed fishery stocks (Ovenden et al. 2015) and in estimating harvest levels and abundance of certain fishery stocks (Bravington et al. 2016).

In the previous study, we employed a morphological approach to understanding the connections among *L. gibbus* populations in the aquatic regions of the Papuan bird's head (Sala et al. 2022). Meanwhile, we used a genetic approach to determine the genetic connectivity between *L. gibbus* populations in Papua Waters. We also undertook a comprehensive investigation by including genetic information on *L. gibbus* specimens collected from some references in the waters of the Philippines, Taiwan, China, and Madagascar (Hubert et al. 2012; Chang et al. 2016; Hou et al. 2018; Limmon et al. 2020; Jaonalison et al. 2022; Bemis et al. 2023). The aim was to get insights into the genetic connections within a broader geographic coverage.

We analyzed genetic diversity and connectivity among *L. gibbus* populations using the Cytochrome C Oxidase subunit I (COI) gene as a genetic marker. Several studies have utilized the COI gene to ascertain species identification, assess the extent of diversity, and determine genetic relationships across populations (Pranata et al. 2018; Pranata et al. 2020; Dwifajri et al. 2022; Sala et al. 2023). Additionally, past studies used the COI gene as a genetic marker to identify the genetic characteristics (Allen et al. 2013), genetic structure and connection (Muths et al. 2012), and phylogenetics of Red Snapper (Halim et al. 2022). The current study attempted to ascertain the genetic characteristics, diversity, and connectivity within the Papua Waters' Red Snapper (*L. gibbus*) populations.

MATERIALS AND METHODS

Study area

This research was carried out from August 2022 to November 2023. Samples of Red Snapper were obtained from several locations in the Papua Waters, Indonesia with much fishing, namely Raja Ampat, Numfor Island, Biak Island, Nabire and Jayapura (Figure 1). This location has DNA extraction, amplification and electrophoresis were carried out at the Biology Department Laboratory, Universitas Bengkulu, Indonesia and at the Biosystematics and Evolution Research Center laboratory (National Research and Innovation Agency, BRIN, Indonesia.

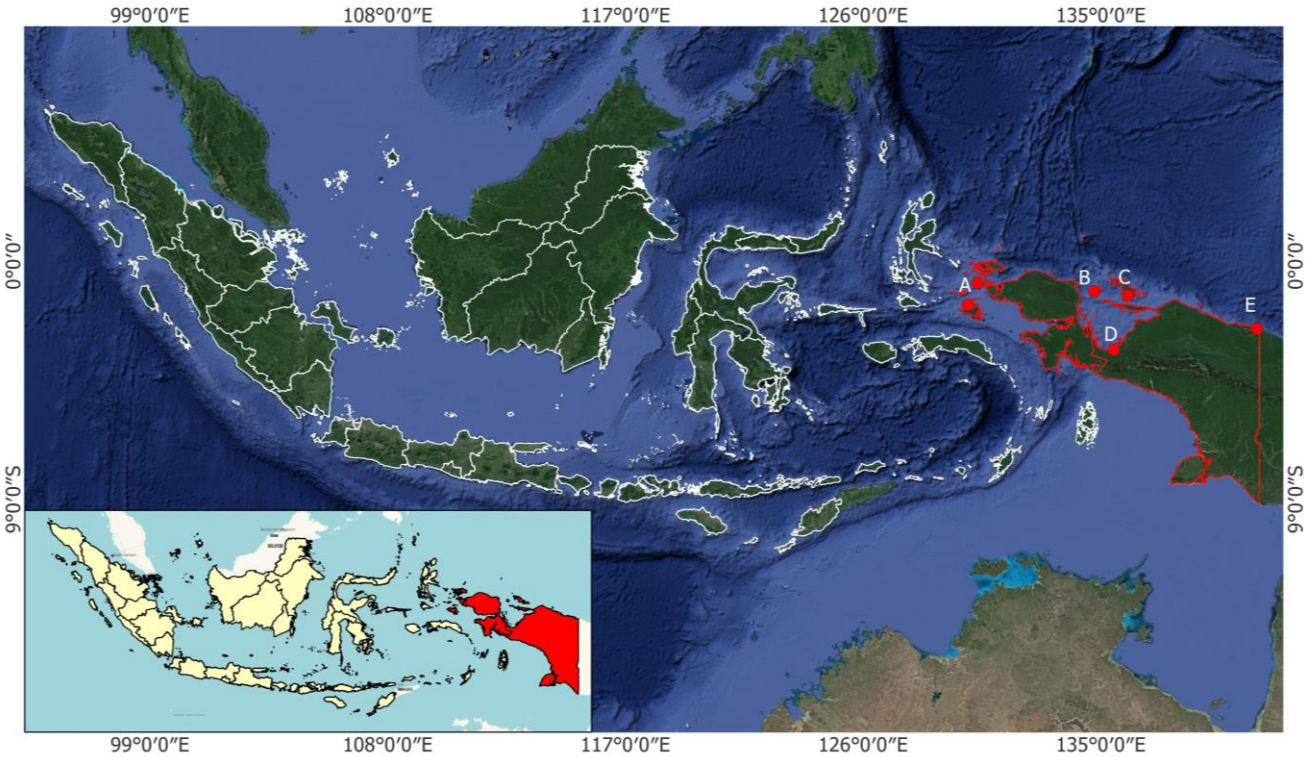

Figure 1. Sampling sites of Red Snapper *Lutjanus gibbus* in Indonesia: A. Raja Ampat; B. Numfor Island; C. Biak Island; D. Nabire; E. Jayapura

Specimen collection

Red Snapper samples were obtained from fish landing ports and markets. The Red Snapper was selected based on its morphology and size. Morphological identification was done based on the identification book by Moore and Boris (2016). One centimeter of Red Snapper dorsal fin tissue was taken and placed in a tube containing 80% ethanol. The tissue was then transported to the laboratory for extraction, amplification, electrophoresis, and sequencing.

Extraction, amplification and electrophoresis

DNA extraction was carried out according to instructions from DNeasy Blood & Tissue Kits - QIAGEN. Amplification of COI gene utilized the 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′.

Polymerase chain reaction (PCR) mix 25 µL Qiagen kit PCR 2× was reacted with 2 µL primer Forward, 2 µL primer reverse, 4 µL DNA template and 17 µL DNase free water. The thermal cycle consisted of initial denaturation for 4 minutes at 95℃, followed by 35 denaturation cycles at 95˚C (30 seconds), annealing at 54℃ (45 seconds), elongation at 72℃ (1 minute), and post PCR at 72℃ (7 minutes). The amplification result was analyzed using electrophoresis. Electrophoresis was performed to visualize the presence of DNA in the PCR product. The pure PCR results were then sequenced at 1st BASE Sequencing Service Sdn. Bhd. (Malaysia).

Data analysis

DNA sequencing results were edited and aligned using MEGA XI software (Tamura et al. 2021). DNA sequence identification was carried out using the BLAST (Basic Local Alignment Search Tool) method at the online National Center for Biotechnological Information (NCBI) (www.ncbi.nml.nih.gov). BLAST assisted in finding the regions of similarity between DNA sequences. BLAST helped compare the nucleotide sequence to a sequence database and calculate statistical significance. Genetic diversity (haplotype and nucleotide), the average number of nucleotide differences, Tajima's D, Fu's Fs statistic, polymorphism sites and haplotypes were analyzed using Dna SP software. Nucleotide composition, transversion and transition mutations, amino acid composition and genetic distance, were analyzed using Mega XI software (Tamura et al. 2021). Phylogenetic analysis was based on the Neighbor-Joining (NJ) method Kimura 2-Parameter (K2P) model using Mega XI software (Tamura et al. 2021). The outgroup in the present study was *Nemipterus zysron* (Bleeker, 1856). Molecular variance (AMOVA) was analyzed using the Arlequin software package version 3.5 (Excoffier and Lischer 2010). Evolutionary relationships were reconstructed between haplotypes using the NETWORK 10.2 software (Bandelt et al. 1999). Genbank sequences from several countries have been added to compare *L. gibbus* connectivity in Indonesia with global connectivity (Table 1).

RESULTS AND DISCUSSION

Genetic characteristics and genetic diversity

We conducted a genetic analysis on 38 *L. gibbus* individuals from five locations in the Papua Waters. The BLAST results of the COI gene fragment sequence showed that all research samples were identified as *L. gibbus* with a COI gene sequence similarity level of 99.80 - 100% (query cover 97-99% and e-values 0.0). The COI gene fragment sequence length was 564 base pairs (bp). The nucleotide composition and mutation characteristics of *L. gibbus* sequences from various locations are presented in Tables 2 and 3. The average frequency of *L. gibbus* nucleotide was A (adenine base) = 25.2%, T/U (thymine or uracil base) = 29.7%, C (cytosine base) = 26.8%, and G (guanine base) = 18.3%. In general, the nucleotide composition of *L. gibbus* from various locations was consistent, with the highest nucleotide composition being T, followed by C, A and G.

Polymorphisms were observed in the COI gene sequence of *L. gibbus* individuals in the Bird's Head Seascape of Papua. This polymorphism was found at nucleotide positions 66, 108, 124, 129, 133, 144, 201, 210, 270, 324, 345, 381, 393, 504, and 558. The polymorphisms occurred due to substitution-transition mutations from T to C or vice versa, A to G, or vice versa. Furthermore, polymorphisms resulted from substitution-transversion mutations involving the conversion of T to A, A to C, and T to G (Figure 2).

Table 1. Sources of the *Lutjanus gibbus* COI nucleotide sequences deposited in GenBank

Location	Sequence Number	Sequence Length (bp)	Access Code	References
Philippines_Aurora	3	548	OQ387703.1, OQ387116.1, OQ386226.1	Bemis et al. (2023)
China_Nansha Islands	9	634	KY371680.1, KY371681.1, KY371682.1, KY371683.1, KY371684.1, KY371685.1, KY371686.1, KY371687.1, KY371688.1	Hou et al. (2018)
Indonesia_Ambon	5	648	MN870348.1, MN869991.1, MN870401.1, MN870575.1, MN870581.1	Limmon et al. (2020)
Madagascar	9	567	JQ350090.1, JQ350091.1, JQ350092.1, OL410155.1, OL409903.1, OL409831.1, OL409549.1, OL409397.1, OL409259.1	Hubert et al. (2012) Jaonalison et al. (2022)
Taiwan	4	552	KU943923.1, KU943900.1, KU943899.1, KU943895.1	Chang et al. (2016)

Table 2. Characteristics of *Lutjanus gibbus* in the Papua Waters, Indonesia

Location	Sample Number	% T	% C	% A	% G	Tr	Tv	Ps	Neutrality Tests	Source Sequences
Raja Ampat Papua	9	30.3	27.2	24.9	17.6	7	3	10	Tajima's	This study
Numfor Island	8	29.5	26.7	25.3	18.5	2	1	3	D: -2.01297	
Biak Island	11	29.5	26.7	25.3	18.5	4	-	4	Statistical significance: *, P < 0.05	
Jayapura	4	29.6	26.6	25.3	18.5	1	-	1	Fu's Fs statistic: -8.169	
Nabire	6	29.4	26.7	25.3	18.5	5	3	8		

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

Table 3. Characteristics of *Lutjanus gibbus* in the Indo-Pacific and Indian Ocean

Location	Sample Number	% T	% C	% A	% G	Tr	Tv	Ps	Source Sequences
Bird's Head Seascape	38	29.7	26.8	25.2	18.3	12	3	15	This study
Philippines_Aurora	3	30.1	26.6	25.4	17.9	-	-	0	Bemis et al. 2023
China_Nansha Islands	9	29.0	27.0	25.3	18.8	6	-	6	Hou et al. 2018
Indonesia_Ambon	5	29.8	26.4	25.3	18.5	1	-	1	Limmon et al. 2020
Madagascar	9	29.5	26.6	25.4	18.6	2	-	2	Hubert et al. 2012 Jaonalison et al. 2022
Taiwan	4	30.3	26.8	25.2	17.8	-	-	0	Chang et al. 2016

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

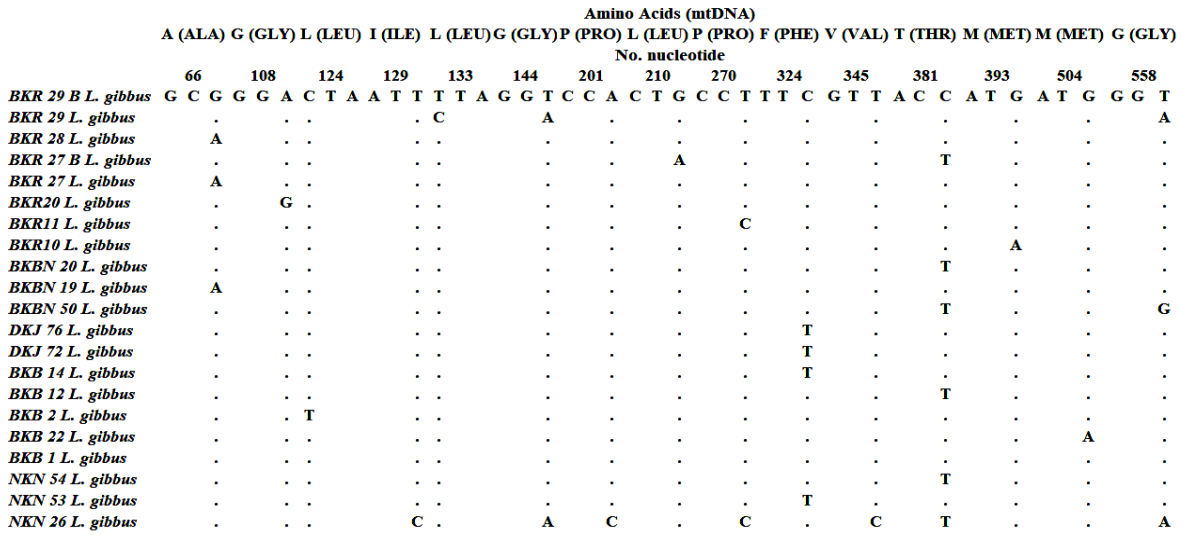

Figure 2. Transition and transversion-substitution mutations of COI Gene

This mutation was categorized as a transition mutation due to substituting a pyrimidine/purine base with another pyrimidine/purine base. It was also classified as a transversion mutation since it involved substituting a pyrimidine base with a purine base or vice versa. Fifteen codons were found to have polymorphisms resulting from mutations, although all of them still encoded the same amino acid despite both transition and transversion mutations. In this study, the COI gene fragment sequence was translated, producing 188 amino acids composed of 19 different amino acids (Figure 3).

The *L. gibbus* populations in the Papua Waters of Papua showed a significant degree of haplotype diversity. The Red Snapper populations in Raja Ampat and Nabire exhibited the highest levels of haplotype diversity, ranging from 0.800 to 0.972, and nucleotide diversity from 0.003 to 0.005. Conversely, the remaining three populations demonstrated haplotype diversity values ranging from 0.618 to 0.667 and a nucleotide diversity value of 0.001 (Table 4). We thoroughly analyzed 38 COI gene sequences obtained from 5 populations in the Papua Waters, resulting in a medium haplotype diversity score of 0.740. We also analyzed the COI gene sequence of *L. gibbus* from GenBank originating from 5 populations in the Indo-Pacific and Indian Ocean. The analysis revealed low genetic diversity in *L. gibbus* global populations, except for the population from Nansha Island, China, which was 0.833 (Table 5).

Genetic connectivity

We analyzed the genetic connectivity within and between Red Snapper natural populations using AMOVA. The result showed a fixation index Statistic (Fst) value of 0.018, Fsc = 0.054, Fct = 0.068, where the highest variance was contributed by within-population diversity of 98.15% (Table 6). This value indicated low or no genetic

differentiation (see the p-value). In other words, genetic differentiation within populations was not significant, with the source of variation for *L. gibbus* being primarily within the Red Snapper aquatic population in the Papua Waters area. Meanwhile, sources of diversity between groups and populations within groups only contributed a variation percentage of 6.89 and -5.04, respectively.

All samples in the phylogenetic tree were classified inside a single group. No notable disparities in genetic distance values were seen among populations, including the Papua aquatic population (Table 7). Figure 4 displays the results of the phylogenetic analysis conducted on all *L. gibbus* specimens collected from the Papua Waters and several other places in the Indo-Pacific and Indian oceans. The tree topologies also indicate no genetic difference among the population or region.

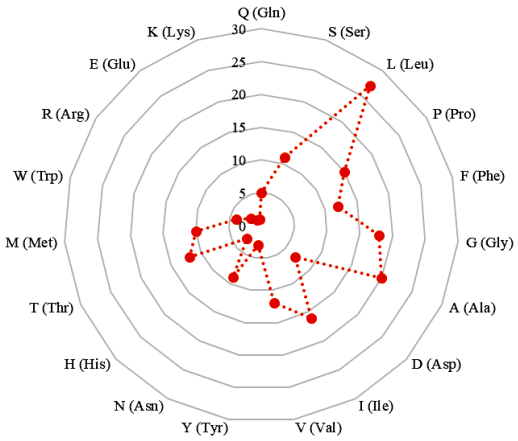

Figure 3. The composition of amino acids relative to proteins coded by COI gene fragments on mitochondrial DNA

Table 4. Comparison of the genetic diversity of *Lutjanus gibbus* populations in the Papua Waters, Indonesia

Location	No. sample	Number of haplotypes	Number of polymorphic (segregating) sites	Haplotype diversity (Hd)	Nucleotide diversity (Pi)	Average number of nucleotide differences (K)
Raja Ampat Papua	9	8	10	0.972	0.003	2.389
Numfor Island	8	4	3	0.643	0.001	0.929
Biak Island	11	5	4	0.618	0.001	0.727
Jayapura	4	2	1	0.667	0.001	0.667
Nabire	6	4	8	0.800	0.005	2.867

Table 5. Comparison of genetic diversity between Papuan *Lutjanus gibbus* and *L. gibbus* from the Indo-Pacific and Indian Ocean regions

Location	No. sample	Number of haplotypes	Number of polymorphic (segregating) sites	Haplotype diversity (Hd)	Nucleotide diversity (Pi)	Average number of nucleotide differences (K)
Bird's Head Seascape	38	13	15	0.740	0.002	1.447
Philippines_Aurora	3	1	0	0.000	0.000	0.000
China_Nansha Islands	9	6	6	0.833	0.002	1.333
Indonesia_Ambon	5	2	1	0.400	0.000	0.400
Madagascar	9	3	2	0.417	0.000	0.444
Taiwan	4	1	0	0.000	0.000	0.000

Table 6. Analysis of Molecular Variance (AMOVA) of *Lutjanus gibbus* populations in the Papua Waters, Indonesia

Source of variation	d.f.	Sum of squares	Variance components	Percentage of variation	Fst	P-value
Among groups	3	2.667	0.05049 Va	6.89		
Among populations Within groups	1	0.377	-0.03692 Vb	-5.04		
Within populations	33	23.720	0.71878 vc	98.15	0.01853	0.35386+-0.01443

Notes: populations and subpopulation grouping are based on geographic distance

Table 7. Estimates of net evolutionary differences between populations of *Lutjanus gibbus*

	Jayapura	Biak	Numfor	Nabire	Raja Ampat	Ambon	Philippines	China	Taiwan
Biak	0.001								
Numfor	0.001	0.001							
Nabire	0.003	0.003	0.003						
Raja_Ampat	0.002	0.002	0.002	0.004					
Ambon	0.001	0.001	0.000	0.002	0.002				
Philippines	0.001	0.000	0.000	0.002	0.001	0.000			
China	0.001	0.001	0.001	0.003	0.002	0.001	0.000		
Taiwan	0.001	0.000	0.000	0.002	0.001	0.000	0.000	0.000	
Madagascar	0.001	0.001	0.001	0.003	0.002	0.000	0.000	0.001	0.000

Note: This analysis involved 68 nucleotide sequences from various countries, including Indonesia, with *L. gibbus* samples from Papua and Ambon, Indonesia

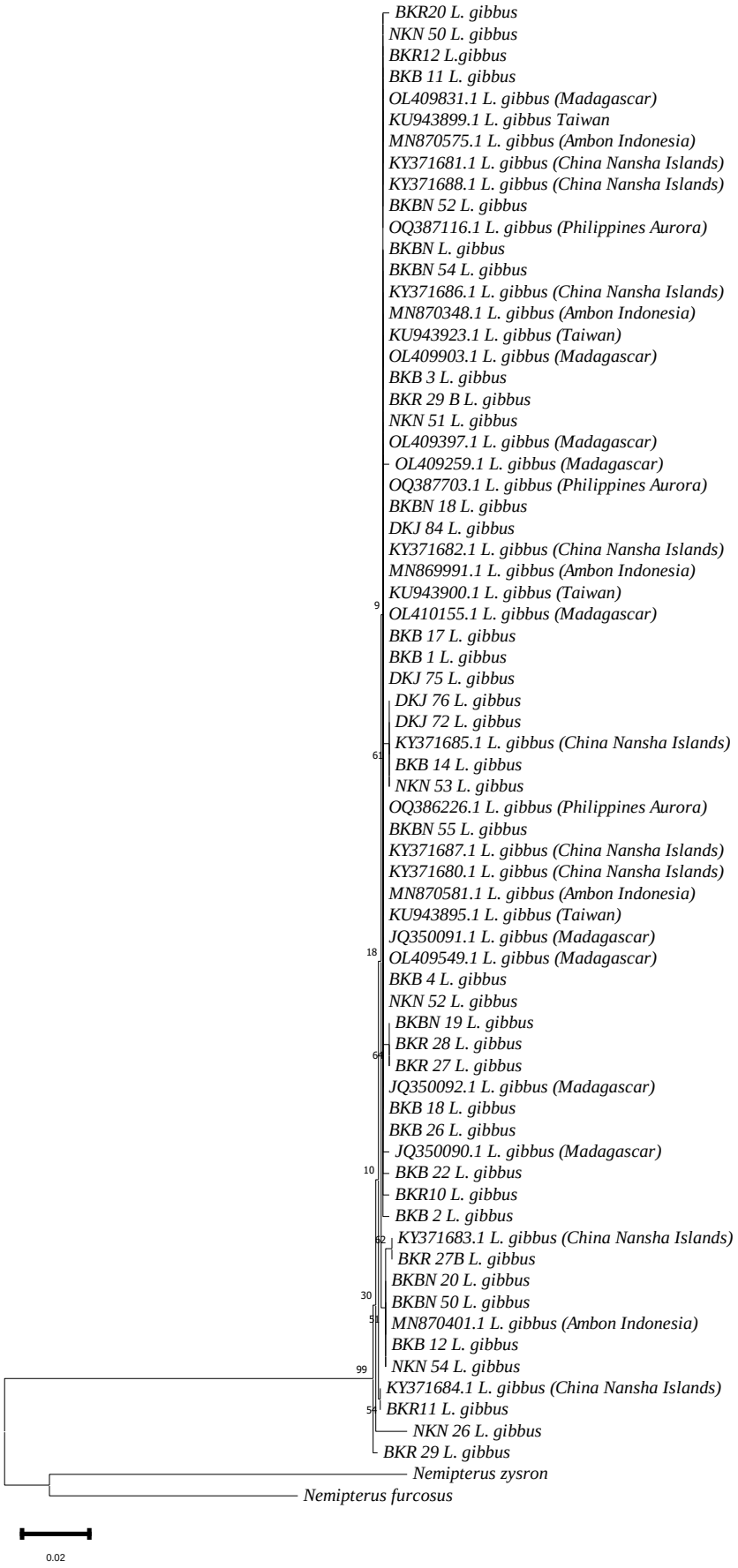

Figure 4. Reconstruction of the phylogenetic tree of Red Snapper of *Lutjanus gibbus* by using the Neighbor-joining method, with 1000x bootstrap and *Nemipterus zysron* as an outgroup

Evolutionary relationships were reconstructed between haplotypes using NETWORK (Figures 5 and 6). According to these figures, haplotype (H1) was the most dominant (central haplotype). All haplotypes were closely related and connected to the central haplotype (H1). H1 indicates that the Papua population has similar haplotypes to all Indo-Pacific populations (Figure 5). Circles represent different individual haplotypes, and the circles' size indicates each haplotype's relative frequency. The color indicates population location. The length of the branches of some haplotypes reflects the number of changes that have occurred in the DNA sequence to form various haplotypes. The longer the branch is formed, the more changes will occur in the DNA sequence and vice versa. Red rectangle (mv) = median vector represents unsampled or extinct ancestral sequences. The numbers indicate the position of the nucleotide that has undergone changes or mutations, thus forming a haplotype that is different from the central haplotype. Figure 5 shows the evolutionary relationship of haplotypes found among the *L. gibbus* populations in the Papua Waters. Meanwhile, Figure 6 shows the relationship between the evolution of *L. gibbus* haplotypes in the Papua Waters and other regions in the Indo-Pacific and the Indian Ocean.

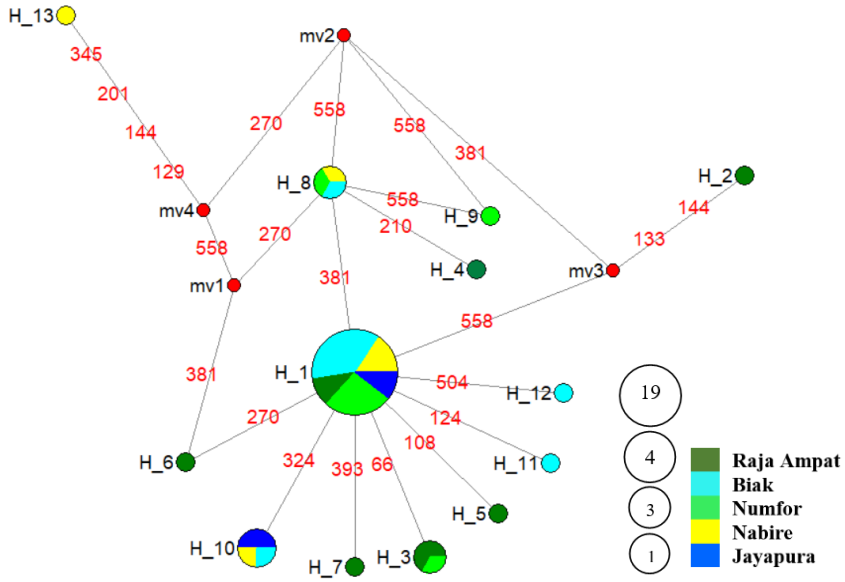

Figure 5. Haplotype network of *Lutjanus gibbus* in the Bird's Head Seascape

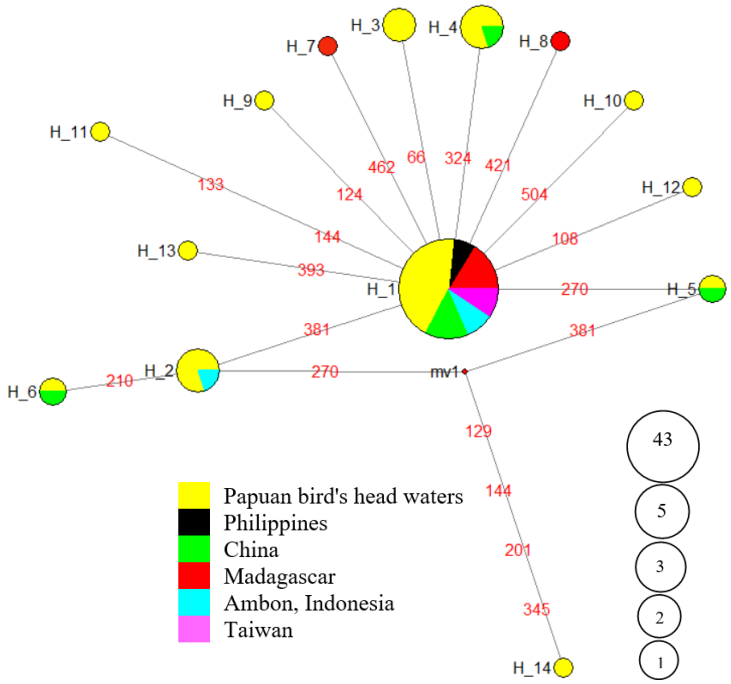

Figure 6. Haplotype network of *Lutjanus gibbus* in the bird's head seascape and the Indo-Pacific and Indian Ocean

Discussion

Genetic characteristics and diversity of Red Snapper in Papua Waters

The cytochrome c oxidase subunit I (CO1) mitochondrial gene is highly effective in species identification and is particularly valuable for reconstructing the phylogenetic relationships of a species (Kartavtsev et al. 2016). This gene has been extensively utilized in many studies, including those focused on fish taxa (Toha et al. 2016, 2020; Sala et al. 2023). DNA barcoding is a method of identification that relies on genetic sequences from certain gene areas (Toha et al. 2020). Identification of Red Snappers using genetic information can also be based on microsatellite markers (Klangnurak et al. 2016). The BLAST findings indicated that all 38 Red Snapper specimens collected from the Papua Waters region belonged to a single species (*L. gibbus)*. The similarity level among these Red Snapper individuals was high (99.80-100%).

The total length of the nucleotide sequence of the *L. gibbus* COI gene fragment from the Papua Waters was 573 bp. The length of the COI gene fragment sequence of *L. gibbus* from all the Indo-Pacific and Indian regions stored in GenBank is listed in Table 1. There were differences in the length of the *L. gibbus* COI gene fragment from various locations in the Indo-Pacific and the Indian Ocean. Variations in gene fragment length are mainly caused by differences in PCR primers and PCR amplicon concentrations (Toha et al. 2020). In this study, the nucleotide composition of T, C, A and G found in the COI gene fragment of *L. gibbus* in Papua Waters resembled that of *L. gibbus* from other locations in the Indo-Pacific region, such as Nansha Island, China, Taiwan, Philippines and Indian Ocean such as Madagascar (Table 3). The G+C content in the *L. gibbus* sequence from several locations in the Indo-Pacific and Indian Ocean was between 44.5% and 45.8 %, and the A+T content was between 54.3% and 55.5%. The average G+C content of all our samples was 45.1%, smaller than the average A+T content, which was 54.9%. In line with this, the percentage of G+C content was 47.2%, and A+T content was 52.8% for several species in the genus *Lutjanus* (Chu et al. (2013).

This study identified the presence of polymorphisms in the COI gene segment of *L. gibbus* populations from the Papua Waters. The polymorphisms in the gene were determined by the occurrence of transition and transversion mutations (Figure 2). According to Sendell-Price et al. (2023), mutations are the fundamental mechanism of evolution that generates diversity within populations. Mutations facilitate evolutionary changes within a population. However, this nucleotide mutation cannot entirely alter its encoded amino acid. All DNA sequences have been translated to obtain the corresponding amino acid sequences of *L. gibbus*. The absence of a stop codon is attributed to the COI gene's role as a structural gene responsible for encoding the cytochrome oxidase I protein (Toha et al. 2020). According to the translation, these codons, which differ by 15 nucleotides, all result in the same amino acid, indicating a silent mutation. Leatherback Turtles (*Dermochelys coriacea* (Vandelli, 1761)) and Green Turtles (*Chelonia mydas* (Linnaeus, 1758)) were also discovered to have silent mutations (Bentley et al. 2023). According to Fonseca et al. (2023), silent mutations are unlikely to alter the protein's structure and may be caused by natural selection.

It is important to understand that there are two distinct categories of nucleotide changes: transitions and transversions. In the event of a transition, a purine nucleotide, namely adenine or guanine, undergoes a mutation to another purine (A↔G), or a pyrimidine nucleotide, specifically cytosine or thymine, transforms into another pyrimidine (C↔T). Transversion refers to the occurrence of mutations where purines are changed to pyrimidines or vice versa (A↔C, A↔T, G↔C, G↔T) (Aloqalaa et al. 2019). Our findings indicate that the rate of transition mutations in *L. gibbus* is higher than that of transversion mutations. This discovery is possible because of the resemblances in the physicochemical properties of *L. gibbus* nucleotides. Furthermore, it is uncommon for transition mutations to result in amino acid alterations within the encoded protein (Aloqalaa et al. 2019). Adaptation to aquatic environmental circumstances can lead to nucleotide alterations in the COI mtDNA gene of *L. gibbus* from the bird's head seascape of Papua (Fonseca et al. 2023).

The current study revealed a medium genetic diversity (haplotype) level between *L. gibbus* populations in the Papua Waters. In this study, 13 distinct haplotypes were discovered from 38 *L. gibbus* specimens collected from the waters. The examination of *L. gibbus* genetic diversity in the Indo-Pacific region, namely Taiwan, Philippines, and Ambon-Indonesia, revealed comparatively low values. We also have comparable findings in *L. gibbus* from the Indian Ocean. The data obtained from *L. gibbus* specimens collected from Nansha Island, China, exhibited much higher genetic diversity values than the current findings. The variation in sample size across various locations does not impact the degree of haplotype and nucleotide diversity (Pereira et al. 2004). Populations exhibiting considerable genetic diversity have an increased likelihood of survival, rendering them superior (Toha et al. 2020).

Genetic connectivity

Genetic connectivity can be understood by analyzing genetic structure using analysis of molecular variance (AMOVA). In the current study, we analyzed the genetic structure of *L. gibbus* populations in Papua Waters using Arlequin 3.1 software. The results of this analysis revealed that there was no genetic structure between the 5 populations of *L. gibbus* found in the waters (a Fixation Index (Fst)) value of 0.018). Fst analysis was done to measure genetic differentiation among populations and identify the presence of gene flow. According to DeBoer et al. (2014), a large Fst value (close to 1) indicates the existence of genetic structure between populations. This study identified higher sources of variance within populations (98.15%) than between groups (6.89%). However, the findings of this study differ from those study findings on *Taenioides cirratus* (Blyth, 1860) (Zhang et al. 2023) and the aquatic plant *Batrachium bungei* (Steud.) L.Liu (Yu et al. 2022), which showed that sources of

variance were higher between populations within groups than within populations. We also analyzed the Tajima's D values and Fu's Fs statistic (Table 2). Negative Tajima's D and Fu's prove population size expansion (Ashfaq et al. 2015; Fonseca et al. 2023). Population expansion is characterized by an increase in the population's rare variants (i.e., polymorphic sites present in only one variant-one-or in a few individuals) (Figure 2). Population size expansion is a temporal variation in dispersal that significantly influences the ecology and evolution of acoustic organisms (Peniston et al. 2023).

The genetic connectivity analysis provides additional insight into the fact that *L. gibbus* populations in the Papua Waters were closely genetically related. There is no genetic structure between populations that indicates genetic relatedness between *L. gibbus* populations in these waters. The results of haplotype network analysis between populations in Papua Waters support this finding. The haplotype network did not show any grouping (Clade) of species originating from different geographical locations, and there were no populations genetically isolated from others.

Reconstruction of the haplotype network between populations of *L. gibbus* from the Papua Waters and several regions of the Indo-Pacific and Indian Ocean informed that each population had related haplotypes. The haplotype reconstruction did not reveal any clustering (Clade) of species originating from distinct geographic areas, and no population exhibited genetic isolation. DeBoer et al. (2014) research yielded the same findings, indicating that the *Tridacna crocea* Lamarck, 1819 population in Cenderawasih Bay shared genetic similarities with giant clam (*T. crocea*) populations in the Philippines and central Indonesia. The *T. crocea* population is composed of haplotypes belonging to a single clade. The findings of this study provide more evidence that individuals of *L. gibbus* from five populations in the Papuan bird's headwaters had the same haplotype. Most Red Snapper populations in Raja Ampat exhibited a significant abundance of haplotypes distinct from the center haplotype (Figure 5). DeBoer et al. (2014) found that a high genetic structure score between populations suggested little genetic linkage. The absence of genetic connectivity implies a deficiency in demographic connectivity caused by environmental factors such as water currents (Hedgecock et al. 2007). The duration of the planktonic larval stage and the larvae's behavior are crucial elements that can influence the patterns of dispersion and the degree of connection across marine species populations (Jenkins and Hawkins 2003; Pineda et al. 2007).

The current research results showed that the *L. gibbus* populations in the Papua Waters and several regions of the Indo-Pacific and Indian Ocean were closely related, with genetic distance values ranging from 0.000 - 0.004. These values are small because these populations come from the same species. Halim et al. (2022) revealed that 5 species of the genus *Lutjanus* exhibited a low genetic distance between species, namely 0.1% - 0.7%. The genetic distance between *Lutjanus* species might also range from 10.2-11.4%. Past studies confirmed that species from the genus *Lutjanus* could demonstrate small genetic distances, namely 18.3% (Afriyie et al. 2020), 8.2% (Halim et al. 2022) and 11.3% (Sala et al. 2023).

Phylogenetic analysis was carried out to determine the relationship of *L. gibbus* from several populations. This study showed that *L. gibbus* populations in the Papua Waters, Indo-Pacific, and Indian Ocean could form a monophyletic clade. In other words, no genetic structure was found between populations (Figure 4). Fish behavior (life cycle, planktonic larval period, migration patterns) or physical factors, such as currents and the geographic location where the species originates, can influence connectivity between populations. The current pattern in the Papuan bird's head seascape allows the planktonic larvae to spread widely between regions. No genetic structure was observed in *L. gibbus* populations in Papuan Papua Waters. However, *L. gibbus* populations in the five research locations exhibited strong genetic connectivity. Genetic connectivity can occur due to the long-distance ocean currents (Xuereb et al. 2018; Kling and Ackerly 2020) and distance between populations (Aguillon et al. 2017; Snead et al. 2023).

The results of this study indicated that 5 populations of *L. gibbus* in the Papua Waters had relatively high genetic diversity. High genetic diversity indicates favorable and optimal population conditions. We found no genetic structure between these waters' 5 L. gibbus populations. The *L. gibbus* populations showed a close genetic relationship, indicated by low genetic distance values, grouping on the phylogenetic tree and haplotype similarity.

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