Diversity of Scombridae in Cenderawasih Bay, Papua, as Revealed by DNA Barcoding

Bayu Pranata[1]*, Abdul Hamid A. Toha[1], Dwi Muhammad Maulana[1], Robi Binur[2], Muhammad Dailami[3], Esie Mega Wangi[4], Yerri D. Mundoni[5], Sipriyadi[6], Aradea Bujana Kusuma[7]

[1]Department of Fisheries, Faculty of Fisheries and Marine Science, University of Papua
[2]Department of Biology, Faculty of Mathematics and Natural Sciences, University of Papua
Jl. Gunung Salju Manokwari, 98314, West Papua, Indonesia
[3]Study Program of Aquaculture, Faculty of Fisheries and Marine Science, Universitas Brawijaya
Jl. Veteran Malang, 65145, East Java, Indonesia
[4]National Park Office of Teluk Cenderawasih Bay
Jl. Drs Esau Sesa Jl. Sowi Gn., Sowi, Manokwari District, Manokwari Regency, Papua Barat 98315, Indonesia
[5]Department of Fisheries, Waropen Regency Government, Waropen Regency, Papua Province
Jalan Sulawesi, Dok Vii 6-8 99115 Kota Jayapura Papua, Indonesia
[6]Department of Biology, Faculty of Science, Bengkulu University
Jl. WR. Supratman, Kandang Limun, Bengkulu Indonesia
[7]International Doctoral Program in Marine Science and Technology, National Sun Yat-sen University
No.70 Lien-hai Road, Kaohsiung 80424, Kaohsiung, Taiwan
Email: b.pranata@unipa.ac.id

Abstract

The family Scombridae comprises economically important pelagic fishes traded across. However, taxonomic knowledge of Scombridae in Cenderawasih Bay remains remarkably limited. This study applied DNA barcoding of the mitochondrial cytochrome c oxidase subunit I (COI) gene for species taxonomy resolution. A total of 57 specimens were successfully sequenced and identified through BLAST against the NCBI database. We detected 12 species from 9 genera, namely Auxis thazard, Katsuwonus pelamis, Euthynnus affinis, Thunnus albacares, Acanthocybium solandri, Scomberomorus commerson, Gymnosarda unicolor, Grammatorcynus bilineatus, Thunnus obesus, Rastrelliger kanagurta, Rastrelliger faughni, and Rastrelliger brachysoma. Genetic diversity varied among the five focal species analyzed; Thunnus albacares and Auxis thazard exhibited the lowest haplotype diversity, indicating potential population vulnerability. Intraspecific genetic distances were below 2%, while interspecific distances exceeded 2%, confirming the effectiveness of the COI gene for species discrimination. Phylogenetic reconstruction produced well-supported clades consistent with established taxonomic relationships. The observed species composition in Cenderawasih Bay was dominated by widespread Indo-Pacific taxa. Notably, among the five tuna species (Thunnus spp.) known to occur in Indonesian waters, only Thunnus albacares and Thunnus obesus was detected in Cenderawasih Bay, raising the question of what ecological and oceanographic factors limit the presence of other tuna species. The ecological characteristics of Cenderawasih Bay are suggested to contribute to shaping the local Scombridae diversity patterns. These findings provide a fundamental genetic reference for Scombridae fisheries management and highlight the need for further oceanographic and ecological studies to understand the mechanisms driving species composition in the unique marine environment of Cenderawasih Bay.

Keywords: *COI gene, mtDNA, Thunnus, Diversity, Phylogenetic*

Introduction

Cenderawasih Bay is one of the regions within the Bird's Head Seascape (BHS) of Papua. It includes several conservation areas, notably the Cenderawasih Bay National Park (TNTC), which spans 1.45 million hectares (Purwanto *et al*., 2021), and the Padaido Islands marine protected area, covering 185,000 hectares. Cenderawasih Bay falls within Indonesia's Fisheries Management Area (WPPNRI) 717 [PERMEN KKP No. 18/PERMEN-KP/2014]. The region has strong potential for capture fisheries, particularly pelagic resources from the family Scombridae (tunas, skipjack, and mackerels) and demersal fishes such as groupers and snappers. This region also plays important ecological and economic roles in fishery (capture fisheries, aquaculture, conservation, tourism, education, and research).

Marine protected areas in Cenderawasih Bay are regarded as among the most effective strategies for safeguarding species and ecosystem diversity

*) Corresponding author
© Ilmu Kelautan, UNDIP
https://ejournal.undip.ac.id/index.php/ijms
DOI: 10.14710/ik.ijms.31.3.323-332
Received : 02-05-2026
Accepted : 07-07-2026

(Starger *et al*., 2015) while improving community well-being. Despite its conservation status, the area continues to face multiple threats, including climate-change impacts, illegal activities, and unplanned coastal development (Mangubhai *et al*., 2012). Effective management and conservation require accurate taxonomic and biological data; however, taxonomic databases for the region's fishes, particularly those from the family Scombridae, remain limited. This gap hinders stock assessment, population monitoring, and enforcement of protective measures for vulnerable or threatened species.

Taxonomic challenges are not confined to the local scale. Globally, scombrid taxonomy remains dynamic and uncertain. Several genera, such as *Scomberomorus*, have unresolved relationships due to limited species representation in molecular studies (Bayona-Vásquez *et al*., 2018). Prior work across the Indo-Pacific has often focused on a single genus or a few economically valuable species (Habib and Sulaiman, 2016; Astarini *et al*., 2021; Andriyono *et al*., 2022; Widayanti *et al*., 2022; Ramadhaniaty *et al*., 2024; Purba *et al*., 2025), yielding an incomplete picture of diversity across the family. Recent findings, such as cryptic diversity within *Scomberomorus commerson* (Zeng *et al*., 2022) and the description of new genera and species (Bannikov, 2020; 2024), underscore how far we are from a comprehensive understanding of scombrid diversity.

To address identification and taxonomic issues, DNA barcoding of the mitochondrial cytochrome c oxidase subunit I (COI) gene has emerged as a powerful, standardized tool. This method complements morphology, which often fails with damaged or juvenile specimens (Hebert *et al*., 2003), and can reveal cryptic species (Zeng *et al*., 2022), identify invasive taxa (Iwatsuki *et al*., 2015), and support the description of new species (Allen *et al*., 2013; Iwatsuki *et al*., 2015). Although widely applied in many regions (Sembiring *et al*., 2015; Antil *et al*., 2022), comprehensive COI barcoding studies of Scombridae from Cenderawasih Bay, especially around Manokwari and Wondama, remain limited or absent.

This study aims to establish a reference COI DNA-barcode database for Scombridae captured in the waters of Manokwari and Wondama, Cenderawasih Bay, characterize species-level and genetic diversity of scombrids in the region, and analyze phylogenetic relationships among the identified species. The resulting data are expected to provide a critical scientific foundation for sustainable conservation and fisheries management of Scombridae in Cenderawasih Bay and to contribute to the global understanding of scombrid taxonomy.

Materials and Methods

The current study was conducted in the Bird's Head waters of Papua, Indonesia (Figure 1). Extraction, electrophoresis, and amplification were carried out at the Genetics Laboratory of Universitas Bengkulu.

Specimen collection

Tissue samples of fish were obtained from fish landing ports and markets (The interview method was used to ensure that the samples obtained came from Cenderawasih Bay). Initial morphological identification referred to the identification book of Moore and Cocas (2016). One centimeter of red snapper dorsal fin tissue was taken and put into a tube containing 80% ethanol for genetic analysis.

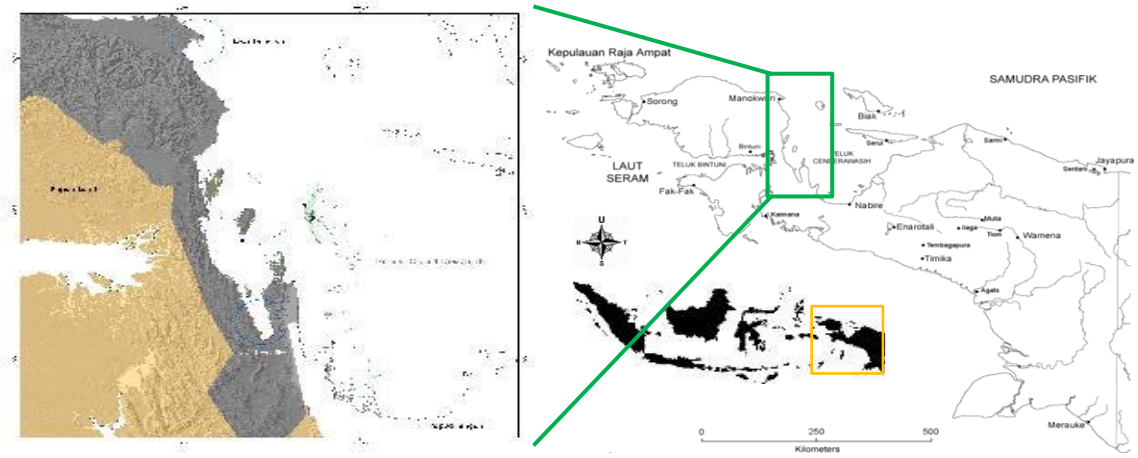

Figure 1. Study Area Map of Cenderawasih Bay (blue circles indicate sampling sites)

Extraction, amplification, and sequencing

DNA extraction was carried out based on the instructions from the Geneaid gSYNCTM DNA extraction kit. COI gene amplification was done using primers developed by Ward *et al.* (2005): F1 5′-TCA ACC AAC CAC AAA GAC ATT GGC AC-3′ and R1 5′-TAG ACT TCT GGG TGG CCA AAG AAT CA-3′.

PCR mix Go Taq Green Master Mix contained Go Taq Green 25 µL, 1.5 µL F1 primer, 1.5 µL R1 primer, 3 µL DNA template and 19 µL nuclease free water. The thermal cycle was set as follows: initial denaturation at 95˚C for 5 min and followed by 35 cycles of denaturation at 95˚C for 30 sec, annealing at 54˚C for 45 sec, elongation at 72˚C for 1 min, post PCR at 72˚C for 7 min. Visualization of DNA fragments in the PCR products was performed via electrophoresis. The result of the PCR was sent to the 1st BASE Sequencing Service Sdn. Bhd. (Malaysia).

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 *et al.*, 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 Scombridae species obtained from NCBI were incorporated with the primary sequence data generated in this study for phylogenetic analysis (Appendix 1).

Results and Discussion

BLAST-based identification

DNA sequence data provided strong verification of specimen identifications for fishes collected from the waters of Cenderawasih Bay. BLAST-based identification compares nucleotide or protein sequences generated in this study against reference databases and evaluates the statistical significance of the matches. The NCBI database serves as a global platform for documenting biodiversity and provides reference sequences for comparison, including in cases of newly discovered species (Halim *et al*., 2022). BLAST results for all 57 specimens are summarized in Table 1.

In this study, 57 fish specimens were identified as members of the family Scombridae. BLAST comparisons of COI sequences from these specimens recovered 9 genera comprising 12 species. Statistically, sequence similarity between our data and NCBI references ranged from 99.38% to 100%. Following Bemis *et al*. (2023), sequences were treated as conspecific when percent identity was ≥ 97.5%. Globally, the family Scombridae includes 54 species (www.fishbase.se), 25 of which occur in Indonesian waters (www.fishbase.se). Accordingly, 12 species were detected in Cenderawasih Bay (specifically the waters off Manokwari and Wondama). This total reflects only those two areas and does not represent the entirety of Cenderawasih Bay.

DNA barcoding proved effective for overcoming species-identification challenges in scombrids from mixed fishery landings. In our sampling, fish sizes ranged from juveniles to adults, and some specimens (e.g., tunas) were obtained as fillets. Morphological identification is often unreliable under such conditions due to variations in body color in young and adult fish. By contrast, DNA barcoding enables accurate identification, even for larvae, juveniles, or body parts such as tuna fillets. This can ensure that stock assessments and catch quotas are based on correct species and can reveal cryptic species that may be not identified visually. Although no cryptic species were detected in this study, all specimens were identified with confidence.

DNA barcoding is a sequence-based approach that relies on standardized genetic regions (Toha *et al*., 2020) and has been widely applied in Papua's marine waters to identify diverse marine species (Toha *et al*., 2016; Toha *et al*., 2022; Sala *et al*., 2023; Pranata *et al*., 2024a; Pranata e*t al*., 2024b; Maharani *et al*., 2025). Globally, the mitochondrial cytochrome c oxidase subunit I (COI) gene has been adopted as the standard barcode for animals, including fishes, and has been extensively used across ichthyological studies (Toha *et al*., 2016; 2020; Sala *et al*., 2023; Maharani *et al*., 2025). In food safety, DNA barcoding is a key tool because it can identify both whole and processed fish from which diagnostic morphological characters have been removed (Bemis *et al*., 2023).

Genetic characteristics

COI gene characteristics were evaluated based on nucleotide composition, G+C content, and COI mutations (transitions and transversions); results are presented in Appendix 2. Thymine (T) and cytosine (C) were more abundant than adenine (A) and guanine (G). We detected 15 polymorphic sites across several species, arising from transition and transversion substitutions. Fifteen codons in *Auxis thazard,*

Katsuwonus pelamis, Euthynnus affinis, Thunnus albacares, and *Acanthocybium solandri* experienced mutations; however, these were synonymous and did not alter the encoded amino acids. The amino-acid composition for each species is shown in Appendix 3. Leucine (Leu) had the highest percentage across all species.

The COI gene showed relatively consistent sequence lengths of 600 bp across all species, which is typical for this marker. All species exhibited similar nucleotide-composition patterns, with thymine (T) and cytosine (C) being the most abundant bases, followed by adenine (A) and guanine (G), a pattern common to vertebrate COI. G+C content ranged from 45.5% to 47.6%. Collectively, these features indicate that COI is highly conserved within Scombridae, supporting its utility as a DNA barcode for species identification. The combined proportion of G and C is noteworthy because GC-rich DNA is generally more thermally stable: G–C pairs form three hydrogen bonds, whereas A–T pairs form two. Substitution mutations of both transition and transversion types were detected in *Auxis thazard, Katsuwonus pelamis, Euthynnus affinis, Thunnus albacares,* and *Acanthocybium solandri*. A substitution mutation is a mutation in which one purine is replaced by another (A ↔ G) or one pyrimidine is replaced by another (C ↔ T). Transitions are the most common class of point mutation. Transversions replace a purine with a pyrimidine or vice versa (e.g., A ↔ C, A ↔ T, G ↔ C, G ↔ T) (Aloqalaa *et al*., 2019). *Katsuwonus pelamis* showed the highest total number of substitutions (seven), with a balanced mix of transitions and transversions, suggesting relatively greater genetic differentiation in that sample. By contrast, *Euthynnus affinis* and *Auxis thazard* exhibited only transitions without transversions. Because of nucleotide physicochemical similarities, transitions occur more readily and often do not change the encoded amino acid (Aloqalaa *et al*., 2019). Such mutations can reflect adaptation to local environmental conditions (Fonseca *et al*., 2023). According to Sendell-Price *et al.* (2023), mutation is a fundamental evolutionary mechanism that generates variation within populations.

Neither transitions nor transversions altered the amino-acid sequences. No stop codons were detected, consistent with the role of COI as a structural gene encoding cytochrome c oxidase subunit I (Toha *et al*., 2020). Based on translation, the codons from *Auxis thazard, Katsuwonus pelamis, Euthynnus affinis, Thunnus albacares,* and *Acanthocybium solandri* all yielded identical amino-acid sequences, indicating synonymous (silent) mutations. According to Fonseca *et al*. (2023), silent mutations are unlikely to alter protein structure and may be maintained by natural selection. The large number of invariant nucleotide positions further indicates that portions of this gene are highly conserved across individuals. Overall, the COI gene is stable and conserved throughout Scombridae, making it a reliable identification tool for biology and conservation.

Amino-acid profiles were highly similar across species (see Appendix 3). For example, leucine (Leu) ranged from 14.71% to 15.87%, alanine (Ala) from 10.28% to 11.06%, and glycine (Gly) from 8.33% to 9.48%, underscoring the conserved nature of the COI protein. Leucine (Leu) was the most abundant residue in every species (15%), followed by alanine (Ala) (10.5%) and glycine (Gly) (~9%). This composition is consistent with the structural/functional properties of an inner-mitochondrial-membrane protein such as COI. Notably, cysteine (Cys) was 0.00% in all species—an intriguing signature for this protein in certain animal groups. Although broadly similar, subtle differences can be informative; for instance, *Rastrelliger faughni* showed slightly lower leucine (14.71%) and the lowest glycine (8.33%) among the taxa. When analyzed statistically and integrated with nucleotide data, such small shifts can help resolve finer-scale phylogenetic relationships among these species.

Genetic diversity

Genetic diversity (with an emphasis on haplotypes) was analyzed for species represented by multiple individuals, including *Auxis thazard, Katsuwonus pelamis, Euthynnus affinis, Thunnus albacares,* and *Acanthocybium solandri*. The results show varying levels of diversity, ranging from 0.4 to 1.0 (Table 2). To compare diversity across locations, 10 *Thunnus albacares* individuals from the waters off Fakfak and Kaimana (collected in 2024) were also analyzed.

Genetic diversity within the family Scombridae varied across the five focal species (Table 5). *Katsuwonus pelamis* stood out with the highest diversity haplotype (Hd = 0.762) and nucleotide diversity (Pi = 0.00211), indicating its ability to adapt and survive in changing environments by counteracting the effects of genetic drift (Hughes, 2008). Species with high genetic diversity tend to maintain high genetic diversity, even if they are threatened with extinction, unless their population size decreases due to events such as habitat destruction (Guo *et al*., 2022). By contrast, *Thunnus albacares* showed a concerning pattern despite a sizable sample (17 individuals), haplotype diversity was low (Hd = 0.404). *Thunnus albacares* from the Kaimana and Fakfak waters likewise exhibited low haplotype diversity. A similar pattern was observed in Auxis thazard, which had the lowest diversity (Hd = 0.400), indicating limited genetic variation that could

Table 1. BLAST results for 57 DNA sequences

No	Field ID	Common name	Scientific Name	N	Average Query Cover (%)	Average Per. Ident (%)	Accession
1	BS5	Frigate Tuna, Bullet Tuna	*Auxis thazard*	5	99.6	99.9	KX768120.1
2	BW4						KX768120.1
3	BW5						KX768120.1
4	BS4						MH638710.1
5	BS6						KX768120.1
6	Sar2	Skipjack Tuna	*Katsuwonus pelamis*	15	99.8	99.9	MT645984.1
7	S1						MT645983.1
8	CK1M						MT645983.1
9	CK2M						KF597042.1
10	BW3						KF597042.1
11	CK11M						MH638701.1
12	CK12M						KX863677.1
13	S2						KF597042.1
14	S3						KF597042.1
15	CK4M						KF597042.1
16	CK10M						EU014258.1
17	CK13M						EU014258.1
18	CK14M						KF528381.1
19	CK15M						EU014258.1
20	CK16M						MT645977.1
21	EU2	Little Tuna	*Euthynnus affinis*	11	100	99.93	OQ127663.1
22	EU3						OQ127663.1
23	EU4						OQ127663.1
24	EU5						OQ127663.1
25	EU6						OQ127663.1
26	EU7						OQ127663.1
27	EU8						OQ127663.1
28	BW6	Yellowfin Tuna	*Thunnus albacares*	17	99.9	99.9	OQ135296.1
29	BW8						OQ135296.1
30	MS1						OQ135296.1
31	EU1						OQ135296.1
32	Th.al5						KU168654.1
33	Th.al6						KU168653.1
34	Th.al7						KU168653.1
35	Th.al8						KU168654.1
36	Th.al9						OQ387746.1
37	Th.al10						KU168653.1
38	Th.al11						KU168653.1
39	Th.al12						KU168653.1
40	ThMa						KU168653.1
41	ThM						KU168652.1
42	BW1						MK301244.1
43	Th.al1						KU168654.1
44	Th.al2						KY984984.1
45	Th.al3						MH638705.1
46	Th.al4						KY984984.1
47	Thob1						KX768137.1
48	Thob2						MH638705.1
49	Tc1	Wahoo	*Acanthocybium solandri*	2	100	100	MK301241.1
50	Tc2						KX768119.1
51	BW2	Narrow-barred Spanish Mackerel	*Scomberomorus commerson*	1	99	99.84	KP112397.1
52	Th1	Dogtooth Tuna	*Gymnosarda unicolor*	1	99	99.84	PP406860.1
53	BS8	Double-lined Mackerel	*Grammatorcynus bilineatus*	1	100	100	NC_051931.1
54	Th2	Bigeye Tuna	*Thunnus obesus*	1	100	100	KU168651.1
55	BS1	Indian Mackerel	*Rastrelliger kanagurta*	1	100	99.69	AP012948.1
56	OC1	Faughn's Mackerel, Island Mackerel	*Rastrelliger faughni*	1	100	99.18	KJ202195.1
57	BW7	Short Mackerel, Shortbodied Mackerel	*Rastrelliger brachysoma*	1	100	98.61	OM460828.1

Table caption: N (number of individuals)

Table 2. The level of genetic diversity of the Scombridae family

Study Location	Species	(N)	, (h)	(Hd)	, (Pi)	Reference
Cenderawasih Bay	*Auxis thazard*	5	2	0.400	0.00064	this study
	Katsuwonus pelamis	15	6	0.762	0.00211	
	Euthynnus affinis	11	4	0.491	0.00092	
	Thunnus albacares	17	3	0.404	0.00073	
	Acanthocybium solandri	2	2	1	0.00325	
	Scomberomorus commerson	1	-	-	-	
	Gymnosarda unicolor	1	-	-	-	
	Grammatorcynus bilineatus	1	-	-	-	
	Thunnus obesus	1	-	-	-	
	Rastrelliger kanagurta	1	-	-	-	
	Rastrelliger faughni	1	-	-	-	
	Rastrelliger brachysoma	1	-	-	-	
Fakfak-Kaimana	*Thunnus albacares*	10	2	0.356	0.00054	Unpublished

Table caption: N (number of individuals); h (number of haplotypes); Hd (haplotype diversity); Pi (nucleotide diversity)

increase vulnerability to threats. In general, low genetic diversity occurs due to various factors such as genetic drift or inbreeding (Frankham 2005; avlova *et al*., 2017). In this study, low genetic diversity in *Thunnus albacares* and *Auxis thazard* is assumed to be due to population size decline. Population size decline due to fishing often results in the loss of rare haplotypes, leaving only a few dominant lineages.

Meanwhile, *Euthynnus affinis* displayed moderate diversity (Hd = 0.491). The data for *Acanthocybium solandri* should be interpreted with caution: although its haplotype diversity equaled 1 and its nucleotide diversity was the highest (Pi = 0.00325), these results are likely biased because they are based on only two individuals. Overall, these findings highlight the need for species-specific fisheries management, especially for *Thunnus albacares* and *Auxis thazard*, which merit greater conservation attention due to low genetic diversity. Populations with higher genetic diversity are generally more resilient and have a greater likelihood of persistence in the wild (Toha *et al.,* 2020).

Estimates of net evolutionary differences

Intra and interspecific genetic distances are summarized in Table 3. Intraspecific genetic distances for the five species (*i.e. Auxis thazard, Katsuwonus pelamis, Euthynnus affinis, Thunnus albacares,* and *Acanthocybium solandri*) were 0.00. At the interspecific level, the closest relationship was observed between *Thunnus albacares* and *Thunnus obesus*. The largest distance was found between *Rastrelliger faughni* and *Grammatorcynus bilineatus* (0.222). No significant differences were observed among intraspecific distances across species, whereas all interspecific distances exceeded 2%. The phylogenetic tree reconstruction grouped taxa according to their genetic affinities (Figure 3).

Intraspecific genetic distances for the five species (*i.e. Auxis thazard, Katsuwonus pelamis, Euthynnus affinis, Thunnus albacares,* and *Acanthocybium solandri*) were 0.00, indicating high genetic uniformity among individuals within each species. The remaining seven species had no intraspecific value (n/c) due to limited sample sizes. Based on analyses of 13,320 species across 11 phyla, Hebert *et al*. (2003, 2004) concluded that intraspecific COI distances typically fall below 2%. In general, smaller genetic distances correspond to greater morphological similarity among observed species, and vice versa (Sala *et al.*, 2023).

At the interspecific level, the closest relationship was observed between *Thunnus albacares* and *Thunnus obesus*, with a genetic distance of just 0.004 affirming a very close evolutionary affinity within *Thunnus*. Similar patterns occurred for *Katsuwonus pelamis* and *Auxis thazard* (0.073), *Rastrelliger kanagurta* and *Rastrelliger brachysoma* (0.050), and *Rastrelliger kanagurta* and *Rastrelliger faughni* (0.091). The largest distance was found between *Rastrelliger faughni* and *Grammatorcynus bilineatus* (0.222). Taken together, these patterns not only support existing taxonomic groupings, particularly at the genus level, but also provide a molecular basis for more effective conservation and fisheries-management strategies for Scombridae.

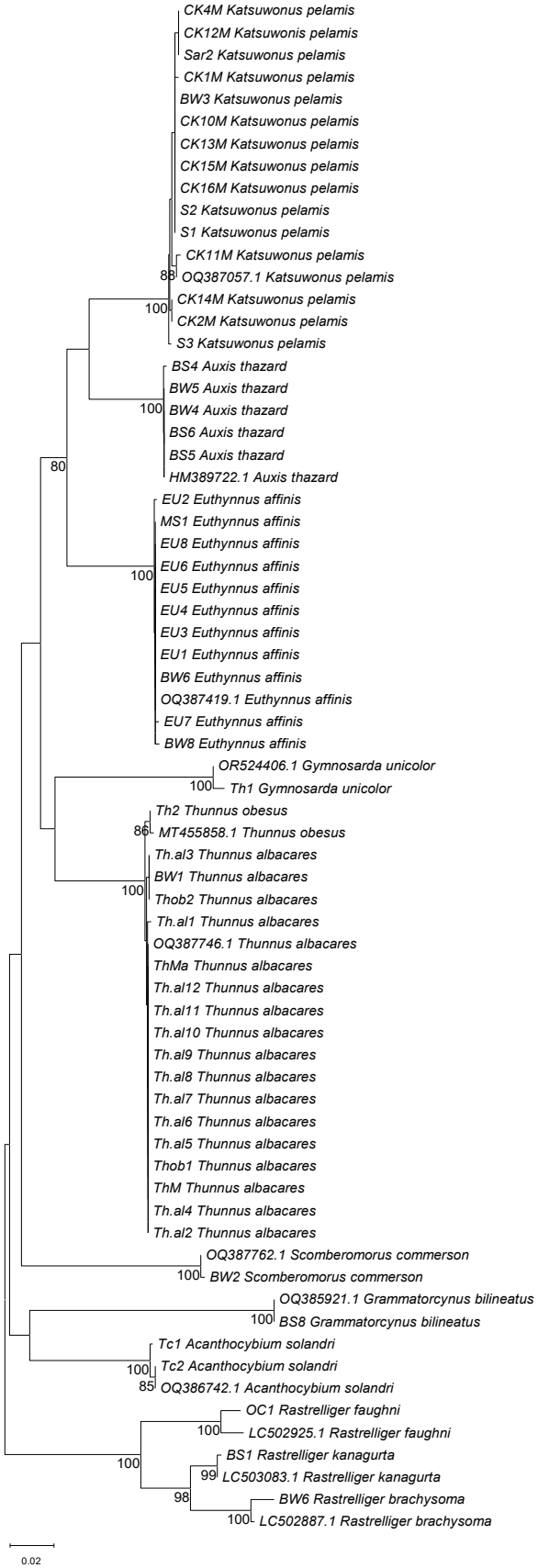

Figure 3. Neighbor-joining phylogenetic relationships (K2P) of combined species in the present study and GenBank. Bootstrap values (1000 replicates) greater than 80% are shown.

Table 3. Intraspecific and interspecific genetic distances

	1	2	3	4	5	6	7	8	9	10	11	12
Auxis thazard	0.00											
Katsuwonus pelamis	0.073	0.00										
Euthynnus affinis	0.087	0.086	0.00									
Thunnus albacares	0.104	0.110	0.100	0.00								
Acanthocybium solandri	0.131	0.143	0.136	0.127	0.00							
Scomberomorus commerson	0.156	0.152	0.148	0.142	0.169	n/c						
Gymnosarda unicolor	0.154	0.151	0.148	0.119	0.126	0.176	n/c					
Grammatorcynus bilineatus	0.162	0.170	0.167	0.190	0.169	0.188	0.187	n/c				
Thunnus obesus	0.104	0.110	0.102	0.004	0.127	0.146	0.119	0.193	n/c			
Rastrelliger kanagurta	0.185	0.176	0.177	0.159	0.190	0.202	0.184	0.214	0.158	n/c		
Rastrelliger faughni	0.180	0.189	0.187	0.176	0.191	0.202	0.201	0.222	0.176	0.091	n/c	
Rastrelliger brachysoma	0.204	0.204	0.205	0.184	0.196	0.216	0.208	0.216	0.183	0.050	0.097	n/c

Note: Boldface values indicate intraspecific genetic distances; non-bold values indicate interspecific genetic distances. n/c = not computed (only one individual was available for that species, so intraspecific genetic distance cannot be estimated).

All samples in the phylogenetic tree formed coherent groups: *Auxis thazard, Katsuwonus pelamis, Euthynnus affinis, Thunnus albacares, Thunnus obesus,* and *Gymnosarda unicolor* formed a monophyletic clade*; Rastrelliger kanagurta, Rastrelliger faughni, and Rastrelliger brachysoma* formed another monophyletic clade*;* and *Acanthocybium solandri* grouped with *Grammatorcynus bilineatus* on the same branch. Bootstrap values of 80–100 on the phylogenetic tree branches indicate node support. In this context, the bootstrap value represents the percentage confidence for a given clade in the tree analysis; higher values correspond to greater confidence (accuracy) in the grouping. No ambiguous clustering was observed, consistent with the high sequence similarity. Correcting misidentified sequence groupings is one of the significant outcomes of the phylogenetic tree analysis.

Conclusion

This study identified 12 scombrid species from Cenderawasih Bay (specifically the waters off Manokwari and Wondama). DNA barcoding proved highly effective for species identification and for distinguishing morphologically similar taxa. COI characteristics demonstrate that this gene is a reliable evolutionary marker for identifying scombrid species across the Bird's Head Seascape (BHS), Indonesia. Genetic diversity varied among species, underscoring the importance of species-specific fisheries management, particularly for *Thunnus albacares* and *Auxis thazard*, which warrant heightened conservation attention due to their low genetic diversity.

Acknowledgement

We extend our sincere thanks to the Directorate of Research and Community Service, Directorate General of Research and Development, Ministry of Higher Education, Science, and Technology (Kemdiktisaintek), for funding this study under the Fundamental Research scheme, pursuant to Decree No. SP DIPA-139.04.1.693320/2025 (Revision 04; April 30, 2025), Agreement/Contract No. 095/C3/DT.05.00/PL/2025, and the derivative contract No. SP/03/N42.15/PG/2025 (June 6, 2025).

References

Pavlova, A., Beheregaray, L.B., Coleman, R., Gilligan, D., Harrisson, K.A., Ingram, B.A., Kearns, J., Lamb, A.M., Lintermans, M., Lyon, J., Nguyen, T.T.T., Sasaki, M., Tonkin, Z., Yen, J.D.L. & Sunnucks, P. 2017. Severe consequences of habitat fragmentation on genetic diversity of an endangered Australian freshwater fish: A call for assisted gene flow. *Evol. Appl.,* 10: 531–550. https://doi.org/10.1111/eva.12484

Allen, G.R., William, T.W. & Mark, V.E. 2013. Two new species of snappers (Pisces: Lutjanidae: Lutjanus) from the Indo-West Pacific. *J. Ocean Sci. Found*, 6: 33-51. https://doi.org/10.5281/zenodo.1036813

Aloqalaa, D.A., Kowalski, D.R., Bła˙zej, P., Wnetrzak, M., Mackiewicz, D. & Mackiewicz, P. 2019. Ratio on the Optimal Genetic Code Graph Partition. Conference: 10th International Conference on Bioinformatics Models, Methods and Algorithms, Volume 3. https://doi.org/10.5220/0007381000550065

Astarini, I.A., Ningsih, E.Y., Simanungkalit, D., Ardiana, S.A., Al Malik, M.D., Yusmalinda, N.L.A., Sembiring, A., Pertiwi, N.P.D., Cahyani, N.K.D. & Collins, A. 2021. Genetic variation of longtail tuna *Thunnus tonggol* landed in four fish markets in Indonesia based on mitochondrial DNA. *Biodiversitas*, 22: 1644–1651. https://doi.org/10.13057/biodiv/d220408

Andriyono, S., Damora, A. & Kim, H. 2022. Molecular Identification and Phylogenetic Tree

Reconstruction of Marine Fish Species from the Fishing Port of Kutaradja, Banda Aceh. *J. Trop. Biodivers. Biotechnol.*, 7(3): 1-17. https://doi.org/10.22146/jtbb.71955

Antil, S., Abraham, J.S., Sripoorna, S., Maurya, S., Dagar, J., Makhija, S., Bhagat, P., Gupta, R., Sood, U., Lal, R. & Toteja, R. 2022. DNA barcoding, an effective tool for species identification: a review. *Mol. Biol. Rep.*, 50: 761–775. https://doi.org/10.1007/s11033-022-08015-7

Bayona-Vásquez, N.J., Glenn, T.C., Domínguez-Domínguez, O., Uribe-Alcocer, M. & Díaz-Jaimes, P. 2018. Mitochondrial genomes of the Pacific sierra mackerel *Scomberomorus sierra* and the Monterey Spanish mackerel *Scomberomorus concolor* (Perciformes, Scombridae). *Conserv. Genet. Resour.*, 10: 471–474. https://doi.org/10.1007/s12686-017-0851-9

Bannikov, A.F. 2020. A New Genus and Species of Scombrid Fish (Perciformes, Scombroidei, Scombridae) from the Lower Oligocene of the Caucasus. *Paleontol. J.*, 54: 59–67. https://doi.org/10.1134/S0031030120010037

Bemis, K.E., Girard, M.G., Santos, M.D., Carpenter, K.E., Deeds, J.R., Pitassy, D.E., Flores, N.A.L., Hunter, E.S., Driskell, A.C., Macdonald, K.S. & Weigt, L.A. 2023. Biodiversity of Philippine marine fishes: A DNA barcode reference library based on voucher specimens. *Sci. Data*, 10: 411. https://doi.org/10.1038/s41597-023-02306-9

Bannikov, A.F. & Erebakan, I.G. 2024. A New Genus for the Sarmatian (Uppermost Middle Miocene) Mackerels (Scombridae) from the North Caucasus. *Paleontol. J.*, 58: 315–323. https://doi.org/10.1134/S0031030124700102

Frankham, R. 2005. Genetics and extinction. *Biol. Conserv.,* 126: 131–140. https://doi.org/10.1016/j.biocon.2005.05.002

Fonseca, E.M., Pelletier, T.A., Decker, S.K., Parsons, D.J. & Carstens, B.C. 2023. Pleistocene glaciations caused the latitudinal gradient of within-species genetic diversity. *Evol. Lett.*, 7: 331–338. https://doi.org/10.1093/evlett/qrad030

Guo, X.Z., Chen, H.M., Wang, A.B. & Qian, X.Q. 2022. Population genetic structure of the yellow catfish (*Pelteobagrus fulvidraco*) in China inferred from microsatellite analyses: Implications for fisheries management and breeding. *J. World Aquac. Soc.*, 53: 174–191. https://doi.org/10.1111/jwas.12844

Hebert, P.D.N., Cywinska, A., Ball, S.L. & DeWaard, J.R. 2003. Biological identifications through DNA barcodes. *Proc. R. Soc. Lond. B Biol. Sci.*, 270: 313–321. https://doi.org/10.1098/rspb.2002.2218

Hebert, P.D.N., Stoeckle, M.Y., Zemlak, T.S., Francis, C.M. & Godfray, C. 2004. Identification of birds through DNA barcodes. *PLoS Biol.*, 2: e312. https://doi.org/10.1371/journal.pbio.0020312

Habib, A. & Sulaiman, Z. 2016. High genetic connectivity of narrow-barred Spanish mackerel (*Scomberomorus commerson*) from the South China, Bali and Java Seas. *Zool. Ecol.*, 26: 93–99. https://doi.org/10.1080/21658005.2016.1161121

Halim, L.J., Rahim, I., Mahboob, S., Al-Ghanim, K.A., Amat, A. & Naim, D.Md. 2022. Phylogenetic relationships of the commercial red snapper (Lutjanidae sp.) from three marine regions. *J. King Saud Univ. Sci.*, 34: 101756. https://doi.org/10.1016/j.jksus.2021.101756

Iwatsuki, Y., Fumiya, T. & Gerald, R.A. 2015. *Lutjanus xanthopinnis*, a new species of snapper (Pisces: Lutjanidae) from the Indo-West Pacific, with a redescription of *Lutjanus madras* (Valenciennes 1831). *J. Ocean Sci. Found.*, 17: 23–42. https://doi.org/10.5281/zenodo.1051774

Librado, P. & Rozas, J. 2009. DnaSP v5: A software for comprehensive analysis of DNA polymorphism data. *Bioinformatics*, 25: 1451–1452. https://doi.org/10.1093/bioinformatics/btp187

Mangubhai, S., Erdmann, M.V., Wilson, J.R., Huffard, C.L., Ballamu, F., Hidayat, N.I., Hitipeuw, C., Lazuardi, M.E., Muhajir, Pada, D., Purba, G., Rotinsulu, C., Rumetna, L., Sumolang, K. & Wen, W. 2012. Papuan Bird's Head Seascape: Emerging threats and challenges in the global center of marine biodiversity. *Mar. Pollut. Bull.*, 64: 2279–2295. https://doi.org/10.1016/j.marpolbul.2012.07.024

Moore, B. & Colas, B. 2016. Identification guide to the common coastal food fishes of the Pacific Island region. New Caledonia: Pacific Community.

Maharani, A.N.M.S., Sala, R., Toha, A.H.A., Purbani, D.C., Mokodongan, D.F., Kusuma, A.B. & Pranata, B. 2025. Morphological and genetic characteristics of red snapper (Lutjanidae) in Nabire Waters. *Ilmu Kelautan Indonesian Journal of Marine Science.*, 30: 83–91. https://doi.org/10.14710/ik.ijms.30.1.83-91

Pranata, B., Sala, R., Kusuma, A.B., Toha, A.H.A., Purbani, D.C., Mokodongan, D.F. & Sipriyadi.

2024a. Genetic diversity and connectivity of red snapper *Lutjanus gibbus* in the Papua Waters, Indonesia. *Biodiversitas*, 25: 276–286. https://doi.org/10.13057/biodiv/d250132

Pranata, B., Sala, R., Kusuma, A.B., Purbani, D.C., Mokodongan, D.F., Sipriyadi, S. & Azhar, M.I. 2024b. Morphological characteristics and genetic relationship of red snappers (*Lutjanus timoriensis*, *Lutjanus malabaricus*, *Lutjanus erythropterus*) in Papuan Waters. *Ilmu Kelautan Indonesian Journal of Marine Science*, 29: 191–200. https://doi.org/10.14710/ik.ijms.29.2.191-200

Purwanto, Andradi-Brown, D.A., Matualage, D., Rumengan, I., Awaludinnoer, Pada, D., Hidayat, N.I., Amkieltiela, Fox, H.E., Fox, M. & Mangubhai, S. 2021. The Bird's Head Seascape Marine Protected Area network—Preventing biodiversity and ecosystem service loss amidst rapid change in Papua, Indonesia. *Conserv. Sci. Pract.*, 3: e393. https://doi.org/10.1111/csp2.393

Purba, L.H.P.S., Sutan, D.K., Simanjuntak, M. & Kusumaningrum, F.V. 2025. Phylogenetic study of genus *Scomberomorus* based on cyt b gene in the north and south coast of Java, Indonesia. *J. Smart Bioprospect. Technol.*, 6: 11–17. https://doi.org/10.21776/ub.jsmartech.2025.006.01.11

Ramadhaniaty, M., Manurung, V.R., Khairunnisa, Lubis, F. & Susetya, I.E. 2024. The first report of DNA barcoding of commercially important fish in Nias Islands, Indonesia. *Biodiversitas*, 25: 742–753. https://doi.org/10.13057/biodiv/d250234

Sembiring, A., Pertiwi, N.P.D., Mahardini, A., Wulandari, R., Kurniasih, E.M., Kuncoro, A.W., Cahyani, N.D., Anggoro, A.W., Ulfa, M., Madduppa, H. & Carpenter, K.E. 2015. DNA barcoding reveals targeted fisheries for endangered sharks in Indonesia. *Fish. Res.*, 164: 130–134. https://doi.org/10.1016/j.fishres.2014.11.003

Starger, C.J., Erdmann, M.V., Toha, A.H.A., Baker, A.C. & Barber, P.H. 2015. Strong genetic structure among coral populations within a conservation priority region, the Bird's Head Seascape (Papua, Indonesia). *Front. Biogeogr.*, 7(3): 91–106.

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

Sendell-Price, A.T., Tulenko, F.J., Pettersson, M., Kang, D., Montandon, M., Winkler, S., Kulb, K., Naylor, G.P., Phillippy, A., Fedrigo, O., Mountcastle, J., Balacco, J.R., Dutra, A., Dale, R.E., Haase, B., Jarvis, E.D., Myers, G., Burgess, S.M., Currie, P.D., Andersson, L. & Schart, M. 2023. Low mutation rate in epaulette sharks is consistent with a slow rate of evolution in sharks. *Nat. Commun.*, 14(1): 6628. https://doi.org/10.1038/s41467-023-42238-x

Toha, A.H., Widodo, N., Subhan, B., Himawan, M.R., Tania, C., Noor, B.A., Stewart, B.S. & Madduppa, H.H. 2016. Close genetic relatedness of whale sharks, *Rhincodon typus* in the Indo-Pacific region. *AACL Bioflux*, 9(3): 458–465.

Toha, A.H.A., Dailami, M., Anwar, S., Setiawan, J.B., Jentewo, J., Lapadi, I., Sutanto, S., Aryasari, R., Ambariyanto, Runtuboi, F. & Madduppa, H. 2020. The genetic relationships and Indo-Pacific connectivity of whale sharks (*Rhincodon typus*) with particular reference to mitochondrial COI gene sequences from Cendrawasih Bay, Papua, Indonesia. *Biodiversitas*, 21(5): 2159–2171. https://doi.org/10.13057/biodiv/d210544

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

Toha, A.H.A., Ambariyanto, A., Widodo, W., Hakim, L., Sumitro, S.B. & Aminin, A.L.N. 2022. Genetic diversity and connectivity of sea urchin *Tripneustes gratilla* in region surrounding Cenderawasih Bay, Papua-Indonesia and Indo-Pacific. *Hayati J. Biosci.*, 29(2): 155–163. https://doi.org/10.4308/hjb.29.2.155-163

Ward, R.D., Tyles, S.Z., Bronwyn, H.I., Peter, R.L. & Paul, D.N.H. 2005. DNA barcoding Australia's fish species. *Philos. Trans. R. Soc. Lond. B Biol. Sci.*, 360(1462): 1847–1857. https://doi.org/10.1098/rstb.2005.1716

Widayanti, R., Nugroho, H.A., Megarani, D.V., Widiasih, D.A. & Pakpahan, S. 2022. Revealing Spanish mackerel's diversity in Indonesian through local commodities in the fish market. *Biodiversitas*, 23: 624–630. https://doi.org/10.13057/biodiv/d230202

Zeng, X-S., Sun, C-H., Huang, X-Y., Lao, Y-L., Huang, J-L., Li, S. & Zhang, Q. 2022. DNA barcoding of *Scomberomorus* (Scombridae, Actinopterygii) reveals cryptic diversity and misidentifications. *ZooKeys*, 1135: 157–170. https://doi.org/10.3897/zookeys.1135.93631