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Original Article

Disclosure of genetic diversity of mackerel fish (*Scomberomorus* spp.) in Indonesian waters based on the mitochondrial cytochrome oxidase subunit II (COII) gene

Divulgação da diversidade genética de peixes cavala (*Scomberomorus* spp.) em águas indonésias com base no gene mitocondrial da subunidade II da citocromo oxidase (COII)

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Abstract

Mackerel fish (*Scomberomorus* spp.) represents a significant marine fisheries commodity in Indonesia, characterized by its high commercial value and nutritional content. To understand the intraspecific interactions and genetic variability of *Scomberomorus* spp., a more extensive research of *Scomberomorus* spp. populations, including both cultivated and wild specimens, is required. This study aimed to explore the genetic diversity of mackerel fish in Indonesian waters, focusing on the mitochondrial DNA (mtDNA) cytochrome oxidase subunit II (COII) gene, which encodes the second subunit of cytochrome c oxidase (complex IV), is essential for aerobic respiration and energy transformation. Muscle tissue samples from 18 individual mackerel fish collected from various regions in Indonesia, including Palembang, Cilacap, Rembang, Banjarmasin, Ambon, and Fak-Fak Regencies, were utilized. The genomic DNA was isolated and amplified using specific primers: CO2TF (5'-ACCGCTCTGTCACTTTCTTC-3') and CO2TR (5'-ATGTCACTAAGGGTGGTTGG-3'). Subsequently, the obtained amplicons were subjected to sequencing. The sequence data were then analyzed using the MEGA11 and DnaSP 6 software. Our findings revealed 120 variable sites within the 691 base pairs of mtDNA COII sequences, resulting in a nucleotide diversity (Pi) of 0.07169. Furthermore, we identified eight haplotypes, demonstrating a haplotype diversity (Hd) of 0.8889. Remarkably, all mackerel samples from Palembang and Cilacap clustered into discrete haplotypes, specifically haplotype 1 and haplotype 2, respectively. Our phylogenetic analysis delineated three distinct clades. Clade I, closely related to *Scomberomorus cavalla*, encompassed all individuals from Ambon, Palembang, Rembang, and one from Banjarmasin. Clade II, associated with *Scomberomorus niphonius*, included individuals from Cilacap and two from Banjarmasin. Clade III, linked to *Scomberomorus semifasciatus*, exclusively consisted of individuals from Fak-Fak (Papua). In conclusion, Indonesian waters harbor diverse genetic variations within *Scomberomorus* spp., and population relationships based on the mtDNA COII gene exhibit notable complexities. Future research endeavors should focus on further elucidating the diversity and relationships among *Scomberomorus* spp. in diverse Indonesian populations.

Keywords: COX2 gene, marine biodiversity, mackerel, phylogenetic, *Scomberomorus* spp.

Resumo

O peixe cavala (*Scomberomorus* spp.) representa uma importante *commodity* da pesca marinha na Indonésia, caracterizada pelo seu alto valor comercial e conteúdo nutricional. Para entender as interações intraespecíficas e a variabilidade genética do *Scomberomorus* spp., é necessária uma pesquisa mais ampla das populações de *Scomberomorus* spp. Este estudo teve como objetivo explorar a diversidade genética do peixe cavala nas águas da Indonésia, com foco no gene mitocondrial do DNA (mtDNA) da subunidade II da citocromo oxidase (COII) que codifica a segunda subunidade da citocromo c oxidase (complexo IV), essencial para a respiração aeróbica e a transformação de energia. Foram utilizadas amostras de tecido muscular de 18 peixes cavala individuais, coletados de diversas regiões da Indonésia, incluindo Palembang, Cilacap, Rembang, Banjarmasin, Ambon e Regiões de Fak-Fak. O DNA genômico foi isolado e amplificado utilizando iniciadores específicos: CO2TF (5'-ACCGCTCTGTCACTTTCTTC-3') e CO2TR (5'-ATGTCACTAAGGGTGGTTGG-3'). Posteriormente, os amplicons obtidos foram submetidos a sequenciamento. Os dados de sequência foram então analisados usando o software MEGA 11 e DnaSP 6. Nossos resultados revelaram um total de 120 sítios variáveis dentro dos 691 pares de bases das sequências de mtDNA COII, resultando em uma diversidade de nucleotídeos (Pi) de 0,07169. Além disso, identificamos oito haplótipos distintos, demonstrando uma diversidade haplotípica (Hd) de 0,8889. Notavelmente, todas as amostras de peixe cavala de Palembang e Cilacap se agruparam em haplótipos distintos, especificamente haplótipo 1 e haplótipo 2, respectivamente. Nossa análise filogenética delineou três clados distintos. O Clado I, intimamente relacionado ao *Scomberomorus cavalla*, englobou todos os indivíduos de Ambon, Palembang, Rembang e um de Banjarmasin. O Clado II, associado ao *Scomberomorus niphonius*, incluiu indivíduos de Cilacap e dois de Banjarmasin. O Clado III, ligado ao *Scomberomorus semifasciatus*, consistiu exclusivamente de indivíduos de Fak-Fak (Papua). Em conclusão, as águas indonésias abrigam diversas variações genéticas dentro de *Scomberomorus* spp., e as relações populacionais com base no gene mtDNA COII apresentam complexidades notáveis. Futuros empreendimentos de pesquisa devem se concentrar em elucidar ainda mais a diversidade e as relações entre as populações de *Scomberomorus* spp. em diversas regiões da Indonésia.

Palavras-chave: gene COX2, biodiversidade marinha, cavala, filogenia, *Scomberomorus* spp.

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1. Introduction

Indonesia, celebrated as a mega biodiversity nation, boasts abundant natural resources, with its extensive reserves of freshwater and saltwater fish constituting a vital component of the country's wealth (Widayanti et al., 2023). Mackerel, a member of the family Scombridae and classified under the genus *Scomberomorus*, is a pelagic species that is widely distributed in the Indo-Pacific region. There are 18 species of Indo-Pacific king mackerel, with their distribution encompassing two species in the East Pacific, four in the West Atlantic, one in the East Atlantic, and eleven in the Indo-West Pacific. These mackerel species are prevalent in Indonesian waters, spanning across regions including Sumatra, Java, Bali, Nusa Tenggara, Kalimantan, Sulawesi, Maluku, and Papua. Their habitat ranges from the continental slope's edge to coastal waters, encompassing saline and slightly turbid conditions. Indo-Pacific king mackerel are commonly found in coastal waters at depths ranging from 15 to 80 meters (Wujdi et al., 2022; Chodrijah et al., 2020). Mackerel (*Scomberomorus* spp.), occupies a prominent role in Indonesia's marine fisheries commodities owing to its substantial commercial worth and abundant nutritional benefits (Wujdi et al., 2022; Agustina et al., 2021). The influence of mackerel is notably substantial in the production of diverse processed food items in Indonesia. Nonetheless, it's noteworthy that one mackerel species, *Scomberomorus commerson*, has been classified as "Near Threatened" according to the IUCN conservation status (Siahaya et al., 2021).

Preserving the future of mackerel fish populations demands dedicated conservation efforts to avert potential fishery collapses and the risk of extinction. Mitochondrial DNA (mtDNA) has proven to be an accurate and sensitive tool for estimating fish population stocks (Hoolihan et al., 2006). Successful identification of genetic and stock structures of mackerel fish (*Scomberomorus* spp.) has been achieved in various regions worldwide through mitochondrial DNA analysis (Sulaiman and Ovenden, 2010; Nakajima et al., 2013; Welch et al., 2015; Vineesh et al., 2016, 2018; Johnson et al., 2021). In recent years, researchers have increasingly delved into genetic distinctions between mackerel fish and other marine fish species (Mansourkiaei et al., 2016; Habib and Sulaiman, 2017; Johnson et al., 2021). Exploring the genetic connectivity of narrow-barred Spanish mackerel populations across the South China Sea, Bali Sea, and Java Sea has uncovered robust genetic ties. Analyses, including mismatch distribution assessments and tests of evolutionary neutrality, have indicated that *Scomberomorus commerson* populations have not experienced significant recent sudden population expansions (Habib and Sulaiman, 2016).

Scomberomorus spp. holds a pivotal position as the primary target species for artisanal fishers across Southeast Asia, primarily owing to its premium market price, making a substantial contribution to the fishery sector (Habib and Sulaiman, 2016). However, there has been a recent scarcity of this fish in the market, and when available, it commands a significantly higher price than other marine fish varieties. Consequently, there is an urgent need to ascertain the genetic diversity of *Scomberomorus* spp. within Indonesian waters. To unravel the intraspecific relationships and genetic variability of *Scomberomorus* spp., a more comprehensive study of *Scomberomorus* spp. populations, encompassing both cultured and wild specimens, is imperative. The availability of lower sequencing costs further facilitates the feasibility of such an investigation. The cytochrome c oxidase subunit 2 (COII) gene, responsible for encoding the second subunit of cytochrome c oxidase (complex IV), plays a vital role in aerobic respiration and energy transformation in most eukaryotes and numerous microorganisms (Torgunakova et al., 2012). Despite this, limited research has been conducted on the COII gene in marine fish, with only a few studies reported in the literature (Wang and Li, 2004; Zhao et al., 2020). Remarkably, no prior research has been conducted on *Scomberomorus* spp. using the COII gene. This study aims to explore the genetic diversity of mackerel fish (*Scomberomorus* spp.) in several Indonesian water populations based on the mitochondrial cytochrome oxidase subunit II (COII) gene as another alternative in conducting genetic analysis with gene targets that are different from those previously reported. The findings from this research can serve as valuable resources for designing conservation strategies and managing pelagic fisheries effectively. Furthermore, they hold the potential to facilitate advancements in the authentication of food products derived from mackerel fish, ensuring accurate labeling and quality control.

2. Materials and Methods

2.1. Sample collection

The samples utilized in this study were acquired from the collection curated by Widayanti et al. (2022). We obtained eighteen specimens from diverse local fish markets situated in six distinct locations within Indonesian waters; we collected three specimens from each area as a representative sample. The locations were chosen to depict two split territories (Western and Eastern Indonesia) defined by Wallace's Line, namely Palembang-South Sumatra Province (-2.9950069, 104.7501622), Cilacap-Central Java Province (-7.7425351, 108.992148), Rembang-Central Java Province (-6.7011665, 111.3350358), Banjarmasin-South Kalimantan Province (-3.3309737, 114.5823114), Ambon-Maluku Province (-3.6879875, 128.1810204), and FakFak-West Papua Province (-2.9359145, 132.3126991) (Figure 1). These fish were procured directly from local fish markets and sourced from fishing activities conducted in the vicinity of each respective location.

2.2. Genomic DNA isolation and COII gene amplification

DNA isolation, COII gene amplification, and electrophoresis were carried out at the Laboratory of Biochemistry and Molecular Biology, Faculty of Veterinary Medicine, Universitas Gadjah Mada, while sequencing was carried out at the 1st BASE Laboratory, Malaysia. The samples used in this study were collected by Widayanti et al. (2022).

The samples were taken from muscle tissue from the specimens' dorsal muscles and diligently preserved in RNA later buffer (Qiagen, Germany). Whole DNA extraction from the mackerel specimens was performed using the gSYNC™ DNA Extraction Kit (Geneaid, Taiwan), following the manufacturer's specified protocols. Amplification, electrophoresis, and sequencing were carried out from June to November 2022.

Primers were meticulously designed based on the mitochondrial genome sequence of *Scomberomorus cavalla* (DQ536428), employing the Primer3web version 4.1.0 (University of Tartu, 2023; Kõressaar et al., 2018). The designed primers facilitated the amplification of a 869 bp DNA fragment, comprising 19 bp from the tRNA-Ser gene, 3 bp of spacer, 73 bp of the tRNA-Asp gene, 6 bp of spacer, 691 bp of the COII gene, 74 bp of the tRNA-Lys gene, 1 bp of spacer, and 2 ATP8 gene bp. The primer sequences employed were as follows: forward primer CO2TF 5'-ACC GCT CTG TCA CTT TCT TC-3' and reverse primer CO2TR 5'-ATG TCA CTA AGG GTG GTT GG-3'.

The PCR reagent volume totaled 50 µL, comprising 25 µL of PCR master mix (Bioline, USA), 3 µL of DNA template, 1 µL (10 pmol) of each primer, and 20 µL of ddH_2O. The PCR amplification process is carried out by optimizing the appropriate conditions to obtain the most suitable thick band results. The PCR amplification was conducted using Cleaver® GTC96S (Cleaver Scientific Ltd.) according to the program: initial denaturation step at 94 °C for 5 minutes, followed by 35 cycles involving denaturation at 94 °C for 30 seconds, annealing at 48 °C for 30 seconds, extension at 72 °C for 1.5 minutes, and concluding with a final extension at 72 °C for 5 minutes. Electrophoresis was conducted to confirm the successful amplification of PCR products, employing a 1% agarose gel alongside a 1000 bp DNA ladder (Geneaid, Taiwan). Subsequently, the unpurified PCR product was submitted to the 1st Base Laboratory (1st Base, Malaysia) for purification and sequencing. The obtained sequence data underwent comprehensive analysis to assess genetic diversity, genetic distances, and the construction of a phylogenetic tree.

2.3. Data analysis

The amplicons of the mtDNA COII gene were aligned using ClustalW (Thompson et al., 1994) and fine-tuned based on the sequence chromatograms obtained from both the forward and reverse directions. Multiple sequence alignments were conducted by cross-referencing with GenBank data available at the National Center for Biotechnology Information (NCBI, 2023) within a database encompassing related mackerel species. Amino acids are translated from aligned nucleotides. To discern the relationships between the DNA samples and reference DNA, an analysis of nucleotide diversity (Pi), amino acid diversity, haplotype diversity (Hd) (Nei and Tajima, 1983; Nei, 1987), and genetic distances was undertaken using the two-parameter Kimura method (Kimura, 1980). For constructing a phylogenetic tree, the Maximum Likelihood method (Jones et al., 1992) with a bootstrap of 1000 replicates was employed (Felsenstein, 1985). This tree aimed to elucidate the connections and groupings among mackerel species. The mtDNA COII gene sequences utilized for the construction of the phylogenetic tree were sourced from the GenBank collection. Noteworthy species for comparison were drawn from the NCBI database, including *S. cavalla* (NC008109; DQ536428), *Scomberomorus concolor* (NC033531; KY091265), *Scomberomorus niphonius* (NC016420; KY228987), *Scomberomorus semifasciatus* (NC021391; JX559745), *Scomberomorus sierra* (NC033887; KX925517), *Auxis rochei* (NC005313), *Cottus dzungaricus* (NC024739), *Katsuwonus pelamis* (NC005316), and *Thunnus alalunga* (NC005317). The data analysis was conducted using the Molecular Evolutionary Genetics Analysis (MEGA) version 11.0 software (Tamura et al., 2021) and DNA Sequence Polymorphism (DnaSP) version 6.0 software (Rozas et al., 2017).

Figure 1. Sampling locations of mackerel fish populations in Indonesia: (1) Palembang, South Sumatra Province; (2) Cilacap, Central Java Province; (3) Rembang, Central Java Province; (4) Banjarmasin, South Kalimantan Province; (5) Ambon, Maluku Province; and (6) Fak-Fak, West Papua Province.

3. Results and Discussion

3.1. Genetic variations in various mackerel fish populations in Indonesian waters

Successful amplification of the sample yielded a product with an amplicon length of 869 bp. The genetic diversity of various mackerel fish populations in Indonesia, based on mtDNA COII sequences, is summarized in Table 1. This data reveals that among the 691 bp of mtDNA COII gene sequences in Indonesian mackerel fish, there exist 120 variable sites (Figure 2), resulting in a nucleotide diversity (Pi) of 0.07169. Additionally, eight distinct haplotypes were identified, demonstrating a haplotype diversity (Hd) of 0.8889. Specifically, mackerel fish samples from Palembang and Cilacap were classified into haplotype 1 and haplotype 2, respectively. For further insights into genetic relationships, Tables 2 and 3 present the genetic distances between individual mackerel fish and among group populations, respectively. Individual genetic distances ranged from 0.000 to 0.139, while distances between group populations varied from 0.0019 to 0.1359. Notably, the mackerel fish populations from Palembang and Rembang exhibited the closest genetic distance, while the greatest distance was observed between the mackerel fish populations from Cilacap and Fak-Fak (Papua).

Within the 691 bp nucleotides encompassing the COII mtDNA sequence, it can encode a total of 230 amino acids. In this study, we identified 12 amino acid variations among individual samples. When compared with reference data, this number increased to 16 amino acid variations, as summarized in Table 4. Interestingly, each population group exhibited a consistent amino acid pattern, with the notable exception of the sample from Banjarmasin. Individual samples originating from Palembang, Rembang, and Ambon shared identical amino acid compositions, indicative of a common genetic profile within these populations. Conversely, most individual samples from Banjarmasin displayed patterns akin to those observed in the individual samples from Cilacap. However, Fak-Fak (Papua) samples exhibited the most distinctive amino acid patterns, showcasing a striking 100% similarity to those of *S. semifasciatus*.

3.2. Phylogenetic relationship

In this study, a phylogenetic tree was constructed by employing the Maximum Likelihood (ML) approach, complemented by a bootstrap test consisting of 1000 replicates, based on the amino acid sequence. The resulting phylogenetic tree, showcased in Figure 3, elucidated the intricate relationships among various mackerel fish populations in Indonesia, revealing their division into three distinct clades. Within the phylogenetic tree, mackerel fish samples sourced from Ambon, Palembang, Rembang, and a solitary sample from Banjarmasin formed a cohesive clade alongside *S. cavalla* (NC_008109 and DQ536428). This association garnered support through a bootstrap value of 43% ML, underscoring their genetic proximity.

Notably, individual samples from Cilacap and two samples from Banjarmasin were intricately linked within a common clade, sharing genetic affinities with *S. niphonius* (NC_016420 and KY228987). This genetic association was substantiated by a robust bootstrap value of 76% ML, further affirming their genetic coherence. Additionally, individual samples from Fak-Fak (Papua) distinctly clustered within a dedicated clade, closely aligning with *S. semifasciatus* (NC_021391 and JX559745). This particular genetic bond was reinforced by a strong bootstrap value of 91% ML, reinforcing their genetic kinship. The construction of this phylogenetic tree offers valuable insights into the genetic relationships among mackerel fish populations in Indonesian waters, delineating their evolutionary connections and highlighting the distinct clades that emerge within this diverse species.

Table 1. Genetic diversity of various mackerel fish populations in Indonesian waters based on mtDNA COII sequences.

Traits	Value
Sample number	18
Number of sites	691
G+C contents	0.4468
Number of invariable (monomorphic) sites	571
Number of variable (polymorphic) sites (S)	120
Nucleotide diversity (Pi)	0.07169
Number of haplotypes (h)	8
Haplotype diversity (Hd)	0.8889
Haplotype members:	
Haplotype 1 (Hap 1)	5 (Palembang1, Palembang2, Palembang3, Rembang1, Banjarmasin1)
Haplotype 2 (Hap 2)	3 (Cilacap1, Cilacap2, Cilacap3)
Haplotype 3 (Hap 3)	1 (Rembang1)
Haplotype 4 (Hap 4)	2 (Rembang3, Ambon1)
Haplotype 5 (Hap 5)	2 (Banjarmasin2, Banjarmasin3)
Haplotype 6 (Hap 6)	2 (Ambon2, Ambon3)
Haplotype 7 (Hap 7)	2 (Papua1, Papua3)
Haplotype 8 (Hap 8)	1 (Papua2)

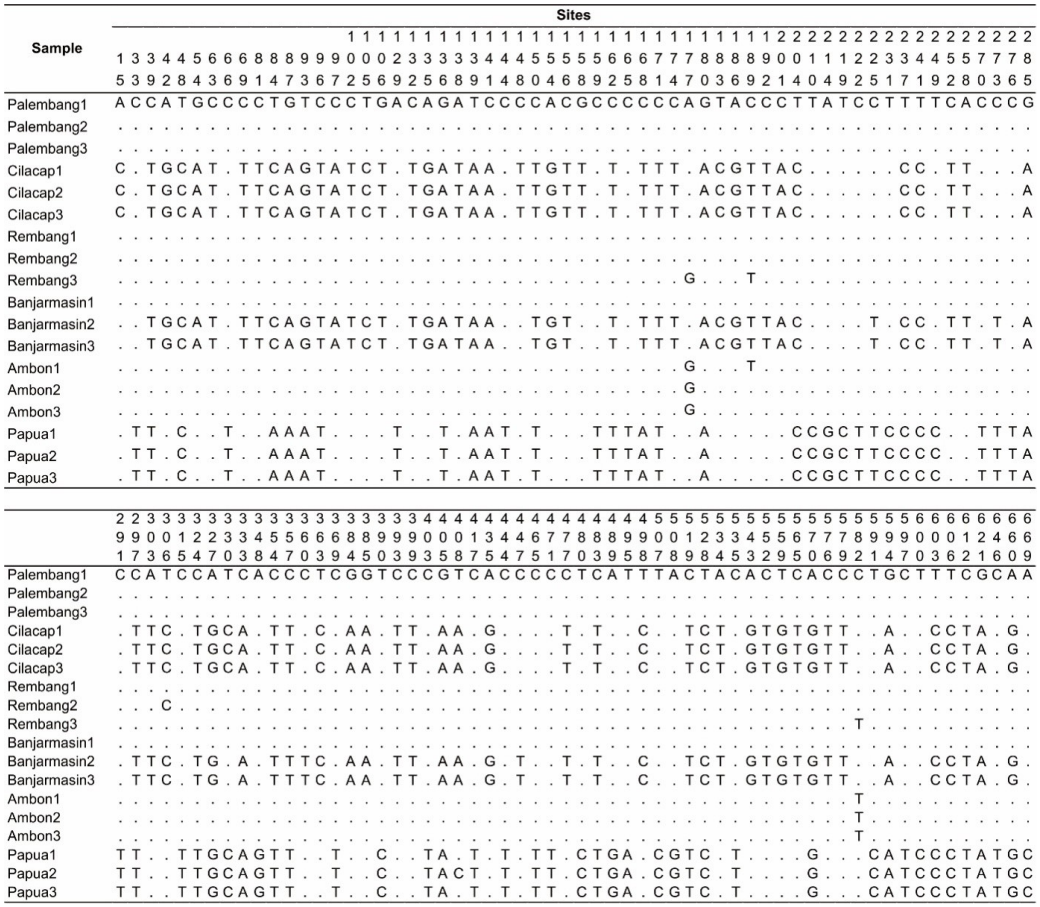

Figure 2. Polymorphic sites of mitochondrial DNA COII gene in the mackerel fish populations in Indonesia waters. Dots indicate identical nucleotides.

Table 2. Calculation of genetic distance (below diagonal) and standard error (above diagonal) between individual mackerel fish based on sequence pairings.

Sample	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Palembang1		0.000	0.000	0.015	0.015	0.015	0.000	0.001	0.003	0.000	0.015	0.015	0.003	0.002	0.002	0.014	0.014	0.014
Palembang2	0.000		0.000	0.015	0.015	0.015	0.000	0.001	0.003	0.000	0.015	0.015	0.003	0.002	0.002	0.014	0.014	0.014
Palembang3	0.000	0.000		0.015	0.015	0.015	0.000	0.001	0.003	0.000	0.015	0.015	0.003	0.002	0.002	0.014	0.014	0.014
Cilacap1	0.130	0.130	0.130		0.000	0.000	0.015	0.015	0.015	0.015	0.004	0.004	0.015	0.015	0.015	0.016	0.016	0.016
Cilacap2	0.130	0.130	0.130	0.000		0.000	0.015	0.015	0.015	0.015	0.004	0.004	0.015	0.015	0.015	0.016	0.016	0.016
Cilacap3	0.130	0.130	0.130	0.000	0.000		0.015	0.015	0.015	0.015	0.004	0.004	0.015	0.015	0.015	0.016	0.016	0.016
Rembang1	0.000	0.000	0.000	0.130	0.130	0.130		0.001	0.003	0.000	0.015	0.015	0.003	0.002	0.002	0.014	0.014	0.014
Rembang2	0.001	0.001	0.001	0.128	0.128	0.128	0.001		0.003	0.001	0.015	0.015	0.003	0.003	0.003	0.014	0.014	0.014
Rembang3	0.004	0.004	0.004	0.132	0.132	0.132	0.004	0.006		0.003	0.015	0.015	0.000	0.001	0.001	0.015	0.015	0.015
Banjarmasin1	0.000	0.000	0.000	0.130	0.130	0.130	0.000	0.001	0.004		0.015	0.015	0.003	0.002	0.002	0.014	0.014	0.014
Banjarmasin2	0.130	0.130	0.130	0.012	0.012	0.012	0.130	0.128	0.131	0.130		0.000	0.015	0.015	0.015	0.016	0.016	0.016
Banjarmasin3	0.130	0.130	0.130	0.012	0.012	0.012	0.130	0.128	0.131	0.130	0.000		0.015	0.015	0.015	0.016	0.016	0.016
Ambon1	0.004	0.004	0.004	0.132	0.132	0.132	0.004	0.006	0.000	0.004	0.131	0.131		0.001	0.001	0.015	0.015	0.015
Ambon2	0.003	0.003	0.003	0.134	0.134	0.134	0.003	0.004	0.001	0.003	0.133	0.133	0.001		0.000	0.014	0.014	0.014
Ambon3	0.003	0.003	0.003	0.134	0.134	0.134	0.003	0.004	0.001	0.003	0.133	0.133	0.001	0.000		0.014	0.014	0.014
Papua1	0.117	0.117	0.117	0.139	0.139	0.139	0.117	0.119	0.123	0.117	0.135	0.135	0.123	0.121	0.121		0.001	0.000
Papua2	0.119	0.119	0.119	0.139	0.139	0.139	0.119	0.121	0.125	0.119	0.135	0.135	0.125	0.123	0.123	0.001		0.001
Papua3	0.117	0.117	0.117	0.139	0.139	0.139	0.117	0.119	0.123	0.117	0.135	0.135	0.123	0.121	0.121	0.000	0.001	

Table 3. Calculation of genetic distance (below diagonal) and standard error (above diagonal) between location/population group.

Location	1	2	3	4	5	6
Palembang		0.0148	0.0009	0.0099	0.0021	0.0137
Cilacap	0.1270		0.0147	0.0059	0.0148	0.0154
Rembang	0.0019	0.1270		0.0098	0.0015	0.0138
Banjarmasin	0.0849	0.0501	0.0855		0.0100	0.0130
Ambon	0.0034	0.1301	0.0031	0.0881		0.0139
Papua	0.1175	0.1359	0.1199	0.1275	0.1218	

Table 4. Amino acid variation (dots indicate identical amino acids) in various mackerel fish populations in Indonesian waters and references.

Individual	Sites															
										1	1	1	1	1	1	2
	3	3	3	4	4	5	5	5	7	2	4	5	7	8	8	3
	3	6	7	6	7	0	2	3	2	9	4	2	0	7	9	0
Palembang1	L	A	L	V	S	L	N	S	I	A	I	I	I	S	P	A
Palembang2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Palembang3	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Cilacap1	M	S	.	I	T	F	D	I	.	T	.	.	.	A	S	.
Cilacap2	M	S	.	I	T	F	D	I	.	T	.	.	.	A	S	.
Cilacap3	M	S	.	I	T	F	D	I	.	T	.	.	.	A	S	.
Rembang1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Rembang2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Rembang3	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Banjarmasin1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Banjarmasin2	M	S	.	I	T	.	D	.	.	T	.	.	.	A	S	.
Banjarmasin3	M	S	.	I	T	.	D	.	.	T	.	.	.	A	S	.
Ambon1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Ambon2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Ambon3	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Papua1	.	.	.	L	T	.	.	.	V	.	.	.	V	.	.	.
Papua2	.	.	.	L	T	.	.	.	V	.	.	.	V	.	.	.
Papua3	.	.	.	L	T	.	.	.	V	.	.	.	V	.	.	.
NC 008109 *S. cavalla*	.	.	.	.	.	.	.	.	.	.	.	.	V	.	.	.
NC 033531 *S. concolor*	.	T	.	.	T	.	.	.	.	.	.	.	V	.	.	.
NC 016420 *S. niphonius*	.	.	F	I	T	.	.	.	.	T	V	V	.	.	.	T
NC 021391 *S. semifasciatus*	.	.	.	L	T	.	.	.	V	.	.	.	V	.	.	.
NC 033887 *S. sierra*	.	T	.	.	T	.	.	.	.	.	.	.	V	.	.	.

Note: A: alanine; D: aspartic acid; F: phenylalanine; I: isoleucine; L: leucine; M: methionine; N: asparagine; P: proline; S: serine; T: threonine; V: valine.

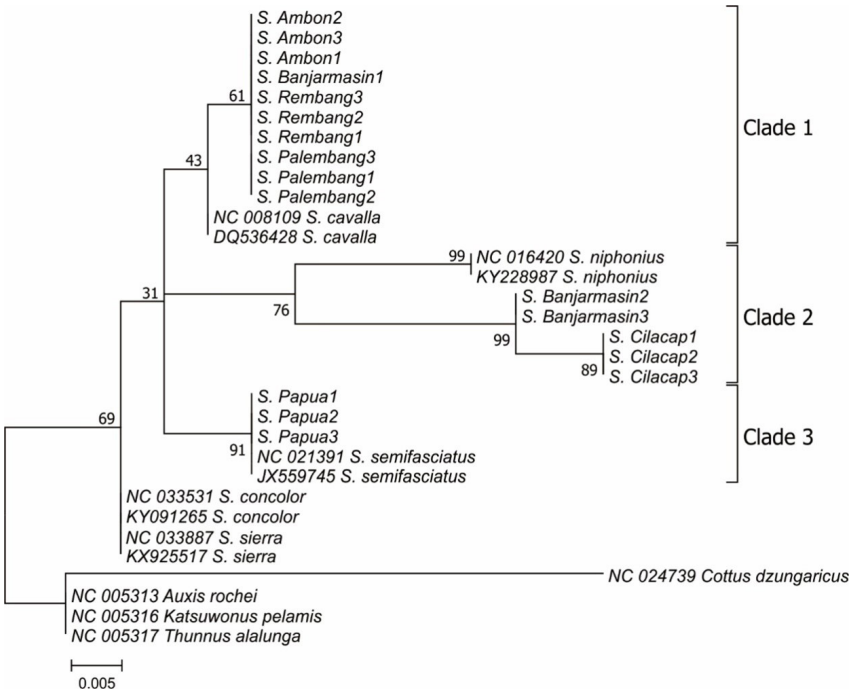

Figure 3. Phylogenetic tree of mackerel fish: comparative analysis of Indonesian populations and GenBank database using Maximum Likelihood method with mtDNA COII sequences.

4. Discussion

Our study focused on analyzing mtDNA COII partial gene fragments, spanning 691 base pairs within multiple mackerel fish populations inhabiting Indonesian waters. The primary aim was to uncover the extent of genetic diversity among these populations and ascertain their genetic affinities with mackerel species found in other geographical regions. The findings of our research brought to light a noteworthy level of genetic diversity, evidenced by the high values of both haplotype diversity (Hd = 0.8889) and nucleotide diversity (Pi = 0.07169) (Table 1). As far as we know, there has never been a scientific publication that traces the COII gene in mackerel, mainly in Indonesian waters. Therefore, our study is the first to report the genetic diversity of mackerel based on the mtDNA COII gene. Research that has been reported to observe the genetic diversity of marine fish using the COII gene includes 15 populations of common carp (*Cyprinus carpio*) in China, which obtained overall results identified as many as 22 haplotypes with Hd = 0.761±0.023 and Pi = 0.0024±0.0002 (Zhao et al., 2020). Other studies with mtDNA COII gene targets are in Koi carp (Hd = 0.4000 and Pi = 0.0006), Oujiang color carp (Hd = 0.8000 and Pi = 0.0026), and Long-fin carp (Hd = 0.8000 and Pi = 0.0033) (Wang and Li, 2004). We were judging from other gene targets in *Scomberomorus* spp. these values are in concordance with the genetic diversity observed in *Scomberomorus commerson* populations dwelling in the Arabian Sea, Bay of Bengal, and Indo-Malay Archipelago, as assessed using the mtDNA D-loop gene (Hd = 0.900-1.000 and Pi = 4-9.5%) (Habib and Sulaiman, 2017). Remarkably, our recorded values surpass those found in *Scomberomorus plurilineatus* sampled from Tanzania, which was studied using the mtDNA cytochrome oxidase subunit I (COI) gene (Hd = 0.111-0.4069 and Pi = 0.0172-0.0689) (Rumisha et al., 2022).

Furthermore, an examination of genetic variation in the mtDNA COI sequence of *S. commerson* populations in the South China Sea unveiled relatively high haplotype diversity (Hd = 0.808±0.040). In contrast, nucleotide diversity was substantially lower, recorded at Pi = 0.004±0.0006 (Yan et al., 2016). These elevated values of Hd and Pi signify a significant level of genetic diversity among haplotypes within the sampled mackerel fish populations. Table 1 provides an overview of haplotype distribution, with haplotype 1 being the most prevalent. Haplotype 1 encompasses all individual samples from Palembang, while individual samples from Cilacap fall under haplotype 2. These findings shed light on mackerel fish populations' genetic composition and distribution within Indonesian waters.

Moreover, our study delved into the genetic distance between sample populations based on the mtDNA COII sequence, revealing values ranging from 0.0019 to 0.1359 (Table 3). The closest genetic distance was observed between the mackerel fish populations from Palembang and Rembang, while the furthest distance existed between Cilacap and Fak-Fak (Papua) populations. It's worth noting that a previous study using mtDNA Cyt b gene sequences reported genetic distances of 0.000-0.018 between mackerel fish populations from Palembang and Rembang, and 0.175-0.178 between populations from Cilacap and Papua (Widayanti et al., 2022). Our research also delved into the amino acid patterns among population groups.

Each location population generally exhibited similar amino acid patterns, except for samples from Banjarmasin (Table 4). Notably, individual samples from Fak-Fak (Papua) displayed distinct patterns and were found to be 100% similar to *S. semifasciatus*.

The phylogenetic analysis in this study yielded a noteworthy discovery of three major clades, as visually represented in Figure 3. Within Clade 3, individual mackerel fish from Fak-Fak, Papua, formed a close cluster with *S. semifasciatus* (NC021391 and JX559745). This finding aligns harmoniously with earlier research utilizing mtDNA Cyt gene sequences, establishing the close relationship between mackerel populations from Fak-Fak (Papua) and *S. semifasciatus* (Widayanti et al., 2022). It's important to note that *S. semifasciatus*, commonly known as gray mackerel, is an indigenous mackerel species found in the southern regions of Papua New Guinea and Northern Australia (Welch et al., 2015; Chaves and Birnfeld, 2023).

Clade I encompasses all individuals (100%) sourced from Ambon, Rembang, and Palembang and a single individual from Banjarmasin. Clade I is notably associated with *S. cavalla* (NC_008109 and DQ536428) within the phylogenetic tree. It's worth mentioning that the mackerel fish samples collected from Rembang for this study originated from the Java Sea. These results diverge somewhat from the outcomes of a study conducted by Habib and Sulaiman (2017), which used mtDNA D-loop sequences and concluded that mackerel fish from the Java Sea exhibited close ties to *S. commerson*. In our investigation, the lack of available mtDNA COII gene data for *S. commerson* in the GenBank repository necessitated a BLAST analysis, ultimately revealing the closest match to be *S. cavalla*. Notably, *S. cavalla*, also known as the King mackerel, is a pelagic predator frequently found along the Atlantic Coast, ranging from Northern California to Brazil, encompassing the Gulf of Mexico. Characterized by its sharp, non-serrated, laterally compressed teeth, this species is well-suited for capturing soft-bodied prey (Ferguson et al., 2015). Further research and confirmation are warranted to definitively determine the genetic proximity of mackerel fish to Clade I, whether it leans closer to *S. commerson* or *S. cavalla*, explicitly employing the mtDNA COII gene sequence.

As delineated in this study, Clade II comprises 100% of individuals from Cilacap and two from Banjarmasin. Clade II exhibits clustering with *S. niphonius* (NC016420 and KY228987) within the phylogenetic tree. Notably, the mackerel fish samples from Cilacap were collected from the Indian Ocean, while those from Banjarmasin were obtained from regions surrounding the Java Sea or the Makassar Strait. In a prior study using mtDNA Cyt b, results indicated that mackerel samples from these two locations displayed close affinities with *Scomberomorus koreanus* (Widayanti et al., 2022). *S. koreanus*, also known as the Korean mackerel, is predominantly distributed across the Indo-Pacific Region, ranging from the East Coast of India, along the Sumatra coast, Kalimantan, and extending northward to Korea and Wakasa Bay in the Sea of Japan. It's important to note that detailed taxonomic studies on *S. koreanus* remain limited, resulting in frequent misidentifications in the field (Roul et al., 2022). In parallel with our previous findings, the mtDNA COII gene sequence for *S. koreanus* was unavailable in the GenBank repository. Conversely, the Japanese mackerel (*S. niphonius*), a substantial carnivorous fish, predominantly inhabits the western coastal regions of Japan and represents a pivotal fishery resource, particularly in the Seto Inland Sea (Nakajima et al., 2013). It's essential to underscore that the study of genetic diversity within *Scomberomorus* spp. using the mtDNA COII gene remains relatively scarce. Consequently, the outcomes of this study provide novel insights and a deeper understanding of the genetic profiles of *Scomberomorus* spp. within the Indonesian archipelago. Future research endeavors should consider more extensive and comprehensive investigations involving a more substantial number of *Scomberomorus* spp. samples, especially those indigenous to the Indonesian region.

This study has indeed contributed valuable insights into the genetic diversity and relationships within *Scomberomorus* spp. populations inhabiting the Indonesian archipelago. While the research provides essential information, it is crucial to acknowledge certain limitations, including sample size, marker availability, and the distribution of populations, which could potentially affect the precision of the relationships established among the species. It is evident that further studies with larger sample sizes, additional markers, and more advanced methodologies are imperative to obtain a more accurate representation of species relationships within the *Scomberomorus* spp. group. The limited availability of mtDNA COII gene data for *Scomberomorus* spp. in the GenBank repository underscores the pressing need for additional research and comprehensive data collection efforts.

Nevertheless, this study shines a light on the extraordinary biodiversity of Indonesia, emphasizing the significance of preserving and comprehending this unique natural wealth. Despite its limitations, this research provides crucial information regarding genetic diversity, genetic distances, and relationships among individuals and populations of *Scomberomorus* spp. in the waters of Indonesia. The identification of three distinct clades, each displaying varying genetic affinities with known mackerel species, serves as a compelling call to action for more in-depth and extensive research endeavors. Such efforts can potentially unravel the complexities of Indonesia's rich and diverse biodiversity, contributing to our understanding and conservation of this invaluable natural heritage.

5. Conclusion

This extensive study analyzed six distinct mackerel fish populations in Indonesia using the COII mtDNA gene. Our findings revealed substantial genetic diversity, with high nucleotide and haplotype diversity. We observed varying genetic distances between samples and identified three distinct Clades. Clade I exhibited a close relationship with *S. cavalla*, Clade II with *S. niphonius*, and Clade III with *S. semifasciatus*. This study highlights the complex genetic diversity within *Scomberomorus* spp. populations in Indonesia, emphasizing the need for further research to fully understand these relationships.

Such efforts can improve conservation and management strategies for Indonesia's unique biodiversity.

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