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Phylogenetic study of Genus *Scomberomorus* based on *cyt b* gene in the north and south coast of Java, Indonesia

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ABSTRACT. *Indonesia possessed megabiodiversity especially in marine animals. As the highly commercial commodities, the understanding for species distribution of Spanish mackerel and seerfish (Scomberomorus spp.) plays a key role for their sustainability. Thus, the aim of this study was to map the species distribution of Scomberomorus spp. in Java surrounding waters. We collected 10 specimens from 4 landing sites in northern and southern coast of Java. Using partial cytochrome b gene fragment (~400 bp), we characterized the gene and haplotype diversity as well as the phylogenetic relationship. Three species of mackerel and seerfish were found in Java coastal line. The genetic differences Scomberomorus sp. ranged from 0 to 14%. By this study, we provide useful data that can be utilized as biodiversity monitoring. In addition, we confirmed that cythocrome b were effective for future application in fishery management and product authentication of mackerel in Java and surrounding areas.*

Keywords: *mackerel, coast of Java, cytochrome b gene, fish landing site*

INTRODUCTION

As megabiodiverse country, Indonesia's maritime sector has high economic potential, especially as a food commodity the maritime sector considered to have high potential for economic especially as food commodity [1-2]. Genus *Scomberomorus* belongs to subfamily Scombrinae consisted of 18 species which widely known as Spanish mackerel and seerfish [3]. The fish are distributed from tropical South Africa and Red Sea to temperate China, Japan, and Australia neritic waters, including Indonesia waters [4-5]. Genus *Scomberomorus* is categorized as a large pelagic fish catch widely distributed in all fisheries management areas in Indonesia sea [6]. Thus, the production of tuna-like species (Spanish mackerel) of Indonesia was the second highest in South East Asia region (32,03%) [7]. Genus *Scomberomorus* is important high economical and nutritional values that assessing the species distribution and stock structure are very crucial for its sustainability. However, traditional morphological identification methods face limitations, particularly in distinguishing closely related or cryptic species. Morphological traits might be influenced by environmental factors, ontogenetic variation, and phenotypic plasticity, leading to potential misidentifications. This is especially problematic in fisheries management, where accurate species identification is crucial for sustainable stock assessments and conservation strategies. Thus, genetic studies as DNA barcoding utilizing DNA markers offer a more reliable approach to species differentiation. These methods allow for: Studies of molecular systematics have been conducted for genetic stock identification and phylogeography of species in fishing areas [8-9]. However, most of the species of *Scomberomorus* is still not evaluated for its genetic structure.

As one of the most exploited fisheries commodities, the data regarding the life story and genetic of *Scomberomorus* is very limited especially in Indonesia. The accurate identification and molecular systematics of the species of genus *Scomberomorus* is important for management of aquatic resources utilization. Several genetic studies were successfully identified the genetic and stock structure of *Scomberomorus* especially *Scomberomorus commerson* [10-12]. The recent study revealed that Spanish mackerel in Indonesia consisted of four species *S. commerson*, *S. koreanus*, *S. semifasciatus* based on the sequence of mitochondrial gene *cytochrome b* (*Cyt b*). In addition, using another mitochondrial marker cytochrome oxidase subunit II (*COII*), Widayanti et al., (2024) revealed two species of *Scomberomorus*, *S. niphonius* and *S. cavalla* which caught in Indonesia waters. Due to its high market price, the accurate species identification of *Scomberomorus*, especially caught and sold in big

markets such as Java Island, is urgently needed. Traditionally, morphological classification was used for species identification. However, the development of technology such as PCR and sequencing advances the more accurate and easy identification process. DNA barcoding analysis using mitochondrial DNA has remained the marker of choice in many studies about species identification, population structure, phylogenetic, migration, and biogeographic in marine animals [2,5].

Previous study found two species of Spanish mackerel sold in fish market in Java Island namely *S. commerson* and *S. koreanus* based on analysis of the *Cyt b* gene [13]. However, there is still limited data about genetic diversity from caught fish from Java's surrounding waters. Here, we aimed to assessing species identification, genetic diversity, and phylogenetic relationships of *Scomberomorus* spp. in Java waters which caught by fishermen in north and south coastal of Java Island based on *Cytb* gene. This information could be the valuable resources for designing fisheries management and conservation in marine management area in particular MMA 573 and MMA 712.

RESEARCH METHODS

Fish samples collection

This study was conducted from June to September 2024. A total of 10 individuals of Spanish mackerel fish, which caught by local fishermen, were collected at 3 landing sites. One landing site on the south coast (MMA 573 Bantul, Yogyakarta = 6 samples) and two landing sites on north coastal (MMA 712 Batang = 2 samples, Kendal= 1, Sidoarjo= 1 samples) of Java Island. The small sample size is limited which may not fully capture the genetic diversity and population structure of *Scomberomorus* species in the surrounding seas of Java Island. However, despite this limitation, the sample size remains adequate for a preliminary genetic assessment due the high sensitibity of mitochondrial DNA and would provides valuable baseline data for future investigations. Muscle tissues were obtained from pectoral muscles and then preserved in 95% ethanol until muscle tissue submerged and stored until subjected to DNA extraction.

Laboratory analysis

Genomic DNA was extracted from 30-50 mg pectoral fin tissue using GeneAid Genomic DNA Mini Kit (Tissue) (Geneaid, Canada) according to manufacturer's protocol. Prior to DNA extraction, muscle tissues were washed using sterile water to remove the preserving liquid. The *Cyt b* gene region was amplified using universal primers set L14724 (forward: 5'-GACTTGAAAAACCACCGTTG-3') and H15915 (reverse: 5'-CTCCGATCTCCGGATTACAAGAC-3') [14-15]. Amplification of *Cytb* gene fragments were done using MyTaq HS Red Mix (Bioline, UK) with the following steps: pre-denaturation 3 minutes at 94°C 3 menit, followed by 35 cycles of denaturation at 94 °C for 30 second, annealing at 51 °C for 30 second, and extension at 72 °C for 90 second, with a final step of 72 °C for 3 minutes. The electrophoresis of 0.8% agarose gel with 100 bp DNA marker was used for separating the amplicon. These unpurified PCR products were sent for Sanger Sequencing analysis (First Base, Malaysia). The sequence data were analyzed for species identification, genetic distance, and phylogenetic analysis.

Phylogenetic analysis and haplotype network construction

DNA sequences of *Cyt b* gene fragments were displayed and edited using BioEdit 7.0.9.0 software [16]. This step followed by aligning sequence from forward and reverse direction of the same sample using ClustalW embedded in MEGA X version 10.2.6 [17]. After we combined or contig

Table 1. BLAST-N result of *Scomberomorus* sp. from three landing sites in north and south coastal of Java

Sample ID	Organism species of reference	Accession number	Query cover	E-value	Identity Percentage
T1	*S. koreanus*	OM799603.1	98%	0.0	100%
T2	*S. koreanus*	OM799598.1	98%	0.0	99.7%
T3	*S. koreanus*	OM799603.1	98%	0.0	99.7%
T4	*S. commerson*	OM799596.1	98%	0.0	100%
T6	*S. koreanus*	OM799603.1	98%	0.0	99.7%
T7	*S. koreanus*	OM799598.1	98%	0.0	99.7%
T8	*S. commerson*	OM799596.1	98%	0.0	100%
T9	*S. commerson*	OM799596.1	98%	0.0	99.53%
T10	*S. commerson*	OM799595.1	98%	0.0	99.77%
T11	*S. lineolatus*	OM417808.1	96%	0.0	100%

Table 2. Calculation of genetic distance (below diagonal) and the standard error (above diagonal) between individual *Scomberomorus* sp.

	T1	T2	T3	T4	T6	T7	T8	T9	T10	T11
T1_Bantul		0,004	0,002	0,020	0,002	0,004	0,020	0,020	0,019	0,019
T2_Bantul	0,007		0,005	0,020	0,005	0,000	0,020	0,020	0,019	0,019
T3_Bantul	0,002	0,009		0,020	0,003	0,005	0,020	0,020	0,020	0,019
T4_Kendal	0,136	0,133	0,133		0,021	0,020	0,000	0,003	0,006	0,019
T6_Bantul	0,002	0,009	0,005	0,139		0,005	0,021	0,020	0,019	0,019
T7_Bantul	0,007	0,000	0,009	0,133	0,009		0,020	0,020	0,019	0,019
T8_Batang	0,136	0,133	0,133	0,000	0,139	0,133		0,003	0,006	0,019
T9_Batang	0,136	0,133	0,133	0,005	0,139	0,133	0,005		0,007	0,019
T10_Sidoarjo	0,127	0,124	0,130	0,017	0,130	0,124	0,017	0,022		0,018
T11_Bantul	0,130	0,127	0,133	0,121	0,133	0,127	0,121	0,121	0,110	

the two sequences, the *Cyt b* sequence from each sample was identified using GenBank BLAST-N program to determine the species. After got the alignment result, we downloaded the top sequences and used it as reference sequences. We did multiple alignments of our sample and the reference sequences from NCBI database. Genetic distance calculation was done using MEGA X with Kimura-2 parameter. Besides, we calculated nucleotide variation and constructed phylogenetic tree. Phylogenetic tree of *Cyt b* gene was constructed using the Neighbor-Joining (NJ) method (Saitou and Nei 1987) with Kimura-2 parameter (Kimura 1980) for nucleotide substitution model. The choice of the method and parameter driven by the suitability for analyzed the genetic differences in diverse taxa. We used 1000 bootstrap replication for phylogeny test. The comparison species used as reference obtained from the NCBI database (accession number: *S. commerson* (OM799595.1, OM799596.1), *S. koreanus* (OM799598.1, OM799603.1, OM799604.1), *S. lineolatus* (OM417810.1), *S. semifasciatus* (NC021391.1), *S. brasiliensis* (DQ080322.1), *S. concolor* (NC033531.1), *S. sinensis* (DQ497893.1), *S. sierra* (NC033887.1), *S. koreanus* (DQ497884.1), *S. guttatus* (OR357877.1), *S. brasiliensis* (DQ080322.1), *Cottus dzungaricus* (MH638324.1), *Katsuwonus pelamis* (NC005316.1), and *Thunnus albacares* (KM588080.1).

The multisite of haplotype were from sequence data of *Scomberomorus* sp. using DNASP v.5 [18]. The relationship of the haplotypes of *Scomberomorus* sp. from the coast of Java were constructed and rooted with the sequence of *cytb* of other *Scomberomorus* sp. found in the GenBank using the median-joining algorithm implemented in NETWORK v10. [19]. After the haplotype network was obtained, the placement was customized, the circle diameter was adjusted, and the color of each haplotype was modified. The size of the circle was adjusted based on the sequence number of each haplotype while the color of each circle was based on the sample identity.

RESULTS AND DISCUSSIONS

Genetic variation of mackerel fish in coast of Java

We successfully amplified and sequenced *Cyt b* gene fragment from 10 *Scomberomorus* samples. After editing and combining forward and reverse sequence, the length of *Cyt b* fragment was 426 bp (142 amino acids). The similarity analysis using BLAST-N result showed the similarity of our sample with three species of Genus *Scomberomorus* namely *S. commerson*, *S. koreanus*, and *S. lineolatus* (Table 1). The result of BLAST-N in all sample sequences above 95% in "Identity Percentage" value. Within the same species, the range of genetic distance was 0-9.5%, while between different species was more than 0.1 (10%) (Table 2). In contrast, the interspecific genetic distance exceeded 10% (0.1 genetic distance), suggesting clear genetic differentiation among *Scomberomorus* species [20]. The farthest genetic distance occurred between T4_Kendal (*S. commerson*) and T6_Bantul (*S. koreanus*). Species identifications for Spanish mackerel were made from the phylogenetic tree with NJ approach (Fig. 1). The identifications were *S. commersoni* (4 individuals), *S. koreanus* (5), and *S. lineolatus* (1). The bootstrap value (percentage) next to the branches showed duplicate trees in which the related taxa are grouped together. The samples of mackerel were divided into three clades showed the respective species. All samples from nort coast of Java were classified as *S. commerson* which was supported by 100% bootstrap value. Whereas four samples from the south coast were classified as *S.*

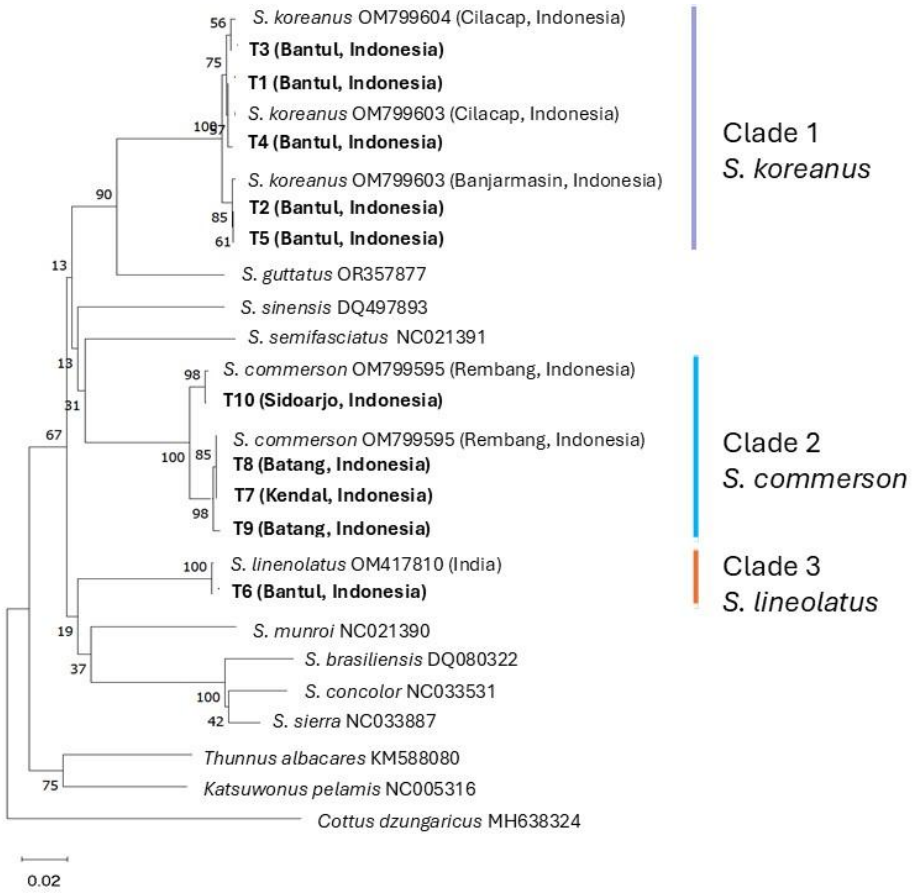

Figure 1. Neighbor-joining tree of *Scomberomorus* spp. showing relationships between fish caught in surrounding waters of Java supplemented with some mackerel species in GenBank. Units of nucleotide divergence of *Cyt b* gene were measured by the Kimura two-parameter method. Bootstrap values (%) are shown for branch points.

koreanus with bootstrap 100% NJ while one sample is clustered with *S. lineolatus* with 100% bootstrap.

Mitochondrial DNA fragments such as *COI* or *Cyt b* can be used for the identification of unknown or closely related species and variations between populations of vertebrates including fish. . *Cytochrome b* is one of the mitochondrial oxidative phosphorylation (OXPHOS) genes [20]. We found no insertion or deletion of nucleotides in the alignment of *Scomberomorus* sp. This gene is encoding protein that plays a crucial role as the component of electron transport chain in the inner mitochondrial membrane [22]. Thus, in the future, the examination of the population variability of species in *Scomberomorus* sp., especially that globally distributed such as *S. commerson*, might contribute to better understanding in their biological adaptation.

Genetic analysis of *Cyt b* gene sequence in Spanish Mackerel three groups of mackerel: S. *koreanus,* S. *commerson*, and S. *lineolatus. These gorup* weredistributed in coastal of Java Island. In addition to *S. koreanus* which was reported before [13], this study also found *S. lineolatus* in south coast of Java Island or India Ocean. Based on our results, *S. commerson* was only found on the north coast of Java or Java Sea while *S. koreanus* and *S. lineolatus* were found in the south coast. Korean mackerel (S. *koreanus*) is mainly distributed in Indo-Pacific Region from East Coastal of India, India Ocean, Kalimantan waters and up north to Korea and Wakasa Bay in Sea of Japan. In this study, the samples from southern coast of Java were clustered with *S. koreanus* from Banjarmasin and Cilacap [13]. In addition, in the southern coast, we also found *S. lineolatus* which clustered with *S. lineolatus* from India [5]. This species also distributed in west coast of India to Thailand, Malaysia, and Java. The study of genetic diversity and population structure of *S. lineolatus* are still limited in Indonesia. In the other hand, *S. commerson* were reported to be distributed in India Ocean [4]. However, this study not provide the data about the species from the fish landing in the south coast of Yogyakarta. However, compared to other species of genus *Scomberomorus* found in Indonesia waters, *S. commerson* is the most widely spread species that found both in western and eastern part of Wallace's line [13].

Haplotype analysis

Haplotype network constructed to show the relationship among DNA sequence and haplotype found in the population or species [23]. The structure of haplotype network could suggest the

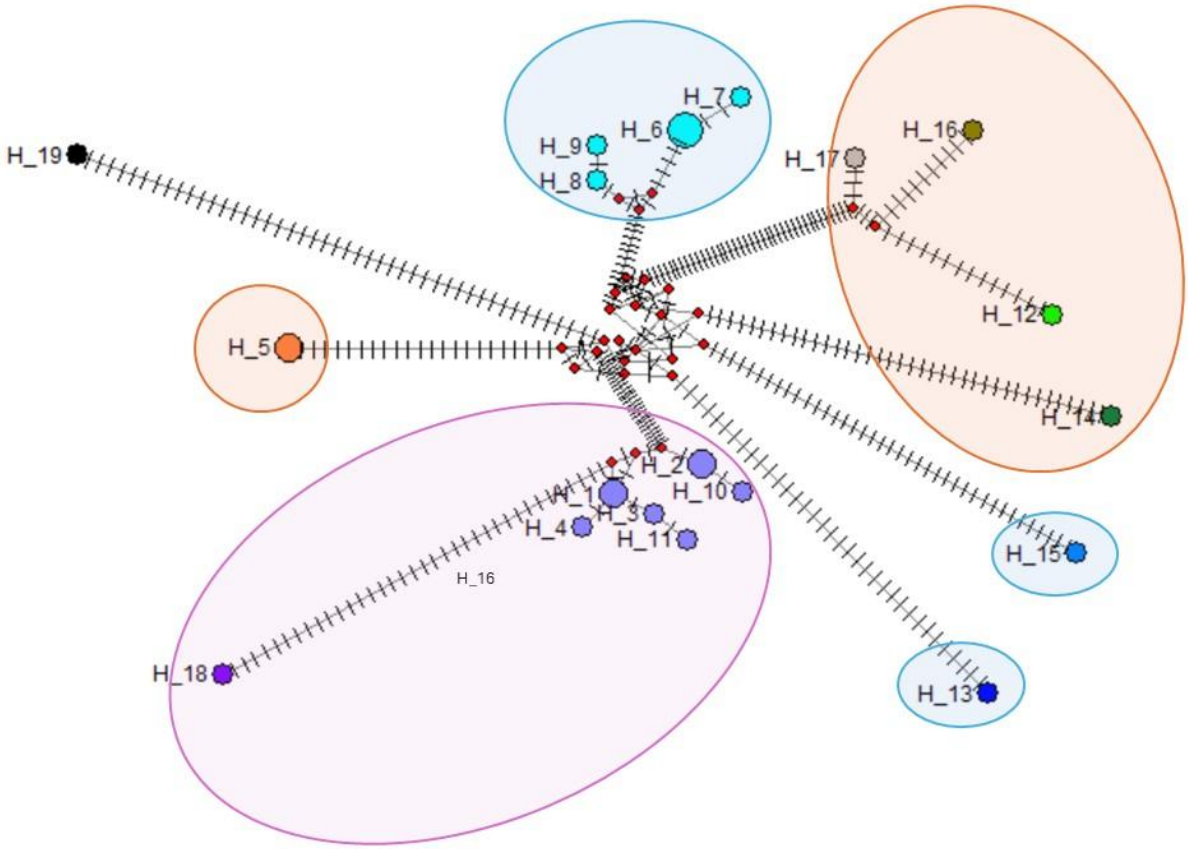

Figure 2. Haplotype network among *Scomberomorus* sp. Each circle represented clade of *Scomberomorus* species. Purple circle consisted of *S. koreanus* and *S. guttatus.* Blue circles consisted of *S. commerson, S. semifasciatus*, and *S. sinensis.* Orange circles consisted of *S. lineolatus, S. sierra, S. brasiliensis, S. munroi*, and *S. concolor*.

population connectivity or isolation. Analyzing DNA sequences of individual fish from multiple geographic distributions allows for answering various questions about the microevolutionary processes [24]. Network methods can be classified according to different criteria. The median joining network (MJN) method is analysis based on similarity of the character [25]. The median joining network describes the variation in mackerel species found in the coast of Java into 9 haplotypes (H_1 to H_8: this study; H_11: [13]) (Fig 2). Each labeled colored circle (H_1 to H_18) represents individual haplotypes of Genus Scomberomorus. The small red dots coded as mv represent the nucleotide mutations or variations between haplotypes. The genetic distance between haplotypes indicated by the number of striped line that separated between the haplotype circles. For example, there were three haplotypes (H_1, H_2, H_4, H_11) of *S. koreanus* from south coast of Java that closely related with H_10 from Banjarmasin [13]. All of the haplotypes of *S. koreanus* are closely related that are connected through one to five mutation steps. Thus, this indicated that the shared haplotypes from different sampling location suggests strong population connectivity facilitated by gene flow [26]. In this study, the possible ancestral haplotype or common haplotype of genus Scomberomorus could not defined. Usually, the ancestral haplotype was located at the center of the network and connected to several other haplotypes through various mutation steps [27].

CONCLUSION

In this study, we identified three species of Spanish mackerel and seerfish namely *S. commerson*, S. guttatus, and *S. koreanus* in surrounding seas of Java Island based on *cytb* gene marker. Our findings provides essential data to validate the species distribution, ensuring that fisheries regulations align with actual species distribution area; thus it might help to support conservation and fisheries management effort. To strengthen further research, we recommend expanding sample size and geographic coverage to improve the accuracy of species distribution pattern. Additionally, incorporating multiple genetic markers, such as *COI* or microsatellite, could enhance species identification and population structure analysis. Furthermore, integrating environmental factor would also

provide insights into the distribution of the fish. Since these species had high commercial value, this information would help for further research to identify types of mackerel from Indonesia and its derived food products.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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