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Revealing Spanish mackerel's diversity in Indonesian through local commodities in the fish market

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Abstract. *Widayanti R, Nugroho HA, Megarani DV, Widiasih DA, Pakpahan S. 2022. Revealing Spanish mackerel's diversity in Indonesian through local commodities in the fish market. Biodiversitas 23: 624-630.* The objective of this study was to explore the diversity of Spanish mackerel in Indonesian Archipelago based on commodities offered in local fish markets. Eighteen specimens were collected from six different fish markets around the Indonesian archipelago. According to Wallace Line, the cytochrome B sequence was used as a genetic marker to reveal diversity from West Indonesia to East Indonesia. Gene amplification was performed using the polymerase chain reaction followed by Sanger sequencing. Based on DNA sequence analysis, we identified three species of Spanish Mackerel available at various fish markets around the region. The first group is related to *Scomberomorus commerson*, which was sold in both Western and Eastern Indonesian fish markets; the second group is related to *Scomberomorus semifasciatus*, which was sold in Eastern Indonesian fish markets; and the third group is related to *Scomberomorus koreanus*, which was sold in Western Indonesian fish markets. The genetic differences amongst Spanish Mackerel populations ranged from 0 to 17% and the average evolutionary divergence in the overall populations was determined to be 4%. The data collected can be utilized to initiate map mackerel diversity based on CYT B sequence throughout the Indonesian Archipelago, as well as for further study and application in the identification of foods produced from mackerel.

Keywords: Diversity, cytochrome b, Spanish mackerel, *Scomberomorus*, Wallace line

INTRODUCTION

Indonesia is an archipelagic country that consists of approximately seventeen thousand islands. Indonesia has long been considered a mega biodiversity country, especially in maritime sectors (Nuryadin et al. 2016; Setyobudi et al. 2019; Ibrahim et al. 2020). Indonesia's vast marine area has very high potential, for example for food sources such as Mackerel (*Scomberomorus* spp.) (Andriyono 2018). The Spanish mackerel (local name: *Tenggiri*) belongs to the family Scombridae (mackerels, tunas, bonitos), subfamily Scombrinae, and is found across the Indo-Pacific, from the Red Sea and South Africa through Southeast Asia, north to China and Japan, and south to Australia (Randall 1995).

Spanish mackerel is widely distributed in various Indonesian seas (Jackson et al. 2014). Commodities sold in traditional fish markets can roughly represent species diversity in the surrounding seas, Indonesia, the Philippines, Sri Lanka, Yemen, and Pakistan were included as the world's top five biggest producers of mackerel (Widodo 1998). The Spanish mackerel (*Scomberomorus* spp.) is a pelagic fish resource commodity with significant economic value that is utilized as an export product as well as to meet domestic demands (Zulfahmi and Swastawati 2014). This fish has a high concentration of marine lipids, amino acids, minerals, and vitamins, which benefit human health (Corapci and Guneri 2020; Negara et al. 2021). The population growth of mackerel is relatively fast, so it can meet the consumption needs of people all over the world (Newman and Mackie 2012; Lee and Mann 2017; Mallawa and Amir 2019). The mackerel-derived fermented fish oil (FFO) extract and it's component docosahexaenoic acid (DHA) stimulate hair development through the anagen-activating pathways in dermal papilla cells (DPC) (Kang et al. 2018).

Genetic studies revealed *S. commerson* distribution in Indonesian Archipelago (Habib and Sulaiman 2016; Habib and Sulaiman 2017). Several genetic studies (Sulaiman and Overden 2010; Jackson et al. 2014; Habib and Sulaiman 2016; Habib and Sulaiman 2017) on Spanish mackerel in Indonesia were already performed. Sulaiman and Ovenden (2010), revealed two major groups of *Scomberomorus commerson* around Wallace's Line, according to D-loop sequence analysis. Jackson et al. (2014) also studied phylogeography of *S. commerson* based on D-loop sequences in the high sea population. According to CYT B (Habib and Sulaiman 2016) and D-loop genes (Habib and Sulaiman 2017), *S. commerson* population in Java Sea was related to South China population, however the data is limited to Java and Bali Sea. The cytochrome b gene has been used to evaluate the fish genetic diversity and phylogeny. Investigations across closely related species are viable because CYT B markers develop fast (Zhu et al. 2016; Kumar et al. 2017; Megarani et al. 2020; Ibrahim 2021).

The mitochondrial (mt) DNA is a potentially useful technique for distinguishing species and ensuring characteristics traceability (Mascolo et al. 2019). The demand for accurate and consistent methods for animal species identification has steadily increased over the last few decades, especially in the overall diversity issue (Flynn et al. 2015; Kidd et al. 2015; Ouso et al. 2020). The molecular identification method based on DNA provides a considerable result, allowing animal and plant species to be identified in virtually any organic substrate (Quek et al. 2018; Widayanti et al. 2019; Ibrahim 2021). Currently with the development of technology, in identifying species can be done easily by using DNA barcoding analysis, this is a great technique for identifying and confirming species, with forensics being one of the most essential applications (Vassou et al. 2015; Imtiaz et al. 2017).

The Commodities sold in wet market can be used as sources for rapid assessment of local fish diversity from surrounding areas, as shown in studies performed by Situ and Sadovy (2004); White et al. (2014); Alfian et al. (2020), and Shellem et al. (2021). The information can roughly show the utilization of local fish sold as consumption commodities, as the risk of unmanaged aquatic resources utilization (Alfian et al. 2020; Shellem et al. 2021).

The genetic diversity and phylogenetic linkages of the Spanish mackerel are investigated in this study. These findings will also be used to conduct a genetic marker analysis on Spanish mackerel commonly used as fisheries commodities in various places in Indonesia. The information can be used for conservation purposes and pelagic fisheries management, as well as for further study and application in the identification of foods produced from mackerel.

MATERIAL AND METHODS

Sample collections

Eighteen specimens were collected from several local fish markets in six various locations across Indonesian archipelago (Figure 1). The locations were selected as representation for two divided areas according to Wallace's Line. Samples collected from Western Indonesia were from: Palembang, South Sumatera (3); Cilacap, Central Java (3); Rembang, Central Java (3); Banjarmasin, South Kalimantan (3); and from Eastern Indonesia were from Ambon, Maluku (3); and Fak Fak, West Papua (3). The fish were bought from local fish markets. The fish were obtained from fishing activities in surrounding seas in each respective location. Muscles tissues were obtained from dorsal muscles and then were preserved in RNALater buffer© (Qiagen, Germany).

Isolation of genomic DNA and amplification of the CYT B gene

The total DNA of the Spanish Mackerel was extracted using gSync™ DNA extraction Kit (Geneaid, Taiwan). A set of primer was designed based on mitochondrial genome sequence of Scomberomorus concolor (KX925518) using Primer3Plus (https://www.bioinformatics.nl/cgi-bin/primer3plus/primer3plus.cgi). The primer set was targeting 1329 bp regions containing 52 bp partial ND6 gene, 69 bp tRNA-Glu gene, 4 bp spacer, target 1141 bp CYT B gene, and 63 bp partial tRNA-Thr gene. The primers sequence were as follow: TF: 5' CTACCAGCCCCAAAACTAAA 3' and TR: 5' GAGGATTTTAACCTCCGACA 3'.

The PCR reagent volume was 50 µL, including 25 µL of master mix, 3 µL of DNA template, 1 µL (10 pmol) of each primer, and 20 µL of ddH2O. Amplifications were carried out under optimum PCRcondition as follows: An initial denaturing stage of 5 minutes at 94°C was followed by 35 cycles of 30 seconds at 94°C, 30 seconds at 48°C, and 90 seconds at 72°C, with a final step of 6 minutes at 72°C. The electrophoresis of 0.8% agarose gel with a 1000 bp DNA marker was used to confirm all PCR amplicons. We then submitted the unpurified PCR product to First Base Laboratory (First Base, Selangor MY) for PCR product purification and then Sanger Sequencing Analysis. The obtained sequence data were analyzed for genetic distance and phylogenetic tree.

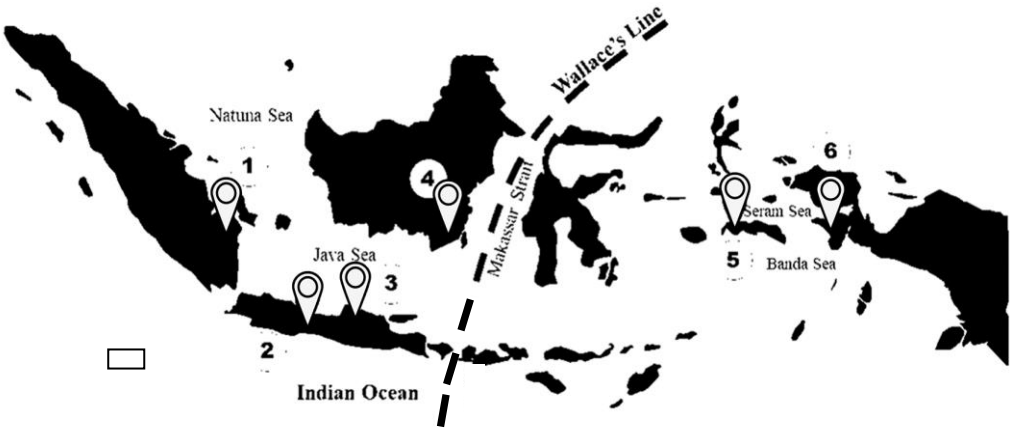

Figure 1. Sampling site locations in Indonesian Archipelago. 1. Palembang, South Sumatera. 2. Cilacap, Central Java. 3. Rembang, Central Java. 4. Banjarmasin, South Kalimantan. 5. Ambon, Maluku. 6. Fak Fak, West Papua. Samples obtained were representing two areas divided by Wallace's Line

DNA sequences and data analysis

The mitochondrial CYT B gene amplicons were aligned using ClustalW software and modified based on sequencing chromatograms of forward and reverse sequences. Multiple alignments were constructed utilizing data from the NCBI database that was correlated to mackerel species from various nations. The mitochondrial CYT B gene sequence was 1140 nucleotides long. Phylogenetic tree was constructed using the Neighbor-Joining (NJ) method to investigate the phylogenetic tree, and the Kimura two-parameter method was used to assess genetic diversity in MEGA X version 10.1 software (Kumar et al. 2018). The bootstrap method, which is used for genetic distance analysis, involves 1000 repeats. To highlight the connection and grouping of Spanish Mackerel, a phylogenetic tree was constructed using the mtDNA CYT B gene sequences and ten sequences from the Genbank collection. The comparison species were obtained from the NCBI database using the following accession numbers: *S. semifasciatus* (JX559746.1), *S. semifasciatus (*JX559745.1*), S. commerson (*EF141176.1*), S. concolor (*KY091265.1*), S. sinensis (*DQ497892.1*), S. sierra (*KX925517.1*), S. koreanus (*DQ497884.1*), S. guttatus (*DQ497878.1*), S. brasiliensis (*DQ080322.1*), Auxis rochei* (DQ080312.1).

RESULTS AND DISCUSSION

Genetic variation of Spanish mackerels collected in several Indonesian fish market

All samples can be amplified successfully, and electrophoresis findings suggest that the amplification length is 1329 bp (Figure 2). According to CYT B gene sequence alignment, our samples were grouped into three clades. Samples from Cilacap (3) and Banjarmasin (1) were grouped in the same clade with *S. koreanus*; samples from Fak Fak (3) were grouped in the same clade with *S. semifasciatus*; and the rest from Palembang (3), Rembang (3), Banjarmasin (2) and Ambon (3) were grouped in the same clade with *S. commerson* (Figure 4).

The Kimura two-parameter model was utilized to conduct the study and the bootstrapping algorithm (1000 repeats) was used to estimate genetic distances. A genetic distance study based on nucleotide sequences was performed on a group of mackerel. The genetic differences amongst Spanish mackerel populations ranged from 0 to 17%. After averaging all sequence pairs among groups, the value of base substitutions at each location in the total populations was determined to be 0.04 (4%) (Table 1). The farthest genetic distance occurred between S. Papua and S. Cilacap, while the closest genetic distance occurred between S. Rembang and S. Palembang.

There were 395 nucleotides that were replaced out of the total of 1140 nucleotides in the CYT B sequences, which encoded 380 amino acids. The 237 nucleotide substitutions were subsequently translated into 21 amino acid variant sites (Table 1). The substitutions of nucleotides resulted in nucleotide or amino acid variation and based on the 21 amino acid changes, there was no amino acid variation in each population of mackerel S. Papua, S. Rembang, S. Palembang, S. Cilacap, and S. Ambon, except S. Banjarmasin. The mackerel population of S. Papua differs by 9 amino acids from S. Rembang, 21 amino acids from S. Banjarmasin, 9 amino acids from S. Palembang, 21 amino acids from S. Cilacap, and 9 amino acids from S. Ambon. S. Rembang, S. Palembang, and S. Ambon mackerel had no variations in amino acids. The arrangement of amino acid variations showed that S. Papua had 100% similarity to *S. semifasciatus*, while S. Rembang, S. Palembang, and S. Ambon had 100% similarity to *S. commerson*, S. Banjarmasin and S. Cilacap had similarity with *S. koreanus*.

Philogenetic relationships of taxa

The Neighbor-Joining approach was used to infer the evolutionary history of Indonesian mackerel. Next to the branches are the percentages of duplicate trees in which the related taxa are grouped together in the bootstrap test (1000 replicates). There were a total of 1140 positions in the final dataset. The samples of Spanish Mackerel were divided into three clades. The samples from Maluku, Rembang, and one from Banjarmasin were classified as *S. commerson* (EF141176.1), which was supported by bootstrap 99% NJ. The samples from Papua were classified as *S. semifasciatus* (JX559745.1), which was supported by bootstrap 99% NJ.; and samples from Cilacap and two from Banjarmasin were classified as *S. koreanus* (DQ497884.1), which was supported by bootstrap 86% NJ (Figure 4).

Discussion

The disclosure and utilization of diversity is critical for the advancement of science and the well-being of human life. Because of its critical function in precisely identifying specimens, diversity has grown in relevance in other branches of biology, including ecology, evolutionary, molecular biology, and biotechnology (Simberloff et al. 2013). According to Shen et al. (2013), erroneous sequences are uncommon in GenBank, and DNA barcoding can be used to confirm sequencing correctness and uncover issues such as misidentified species, incorrect taxonomy, contamination, and probable sequencing errors. Although DNA barcoding is currently the most popular method of identification, it can also be used to identify species complexes within populations when combined with traditional taxonomy (Iwatsuki et al. 2015; Imtiaz et al. 2017). In the identification of market replacement in seafood, DNA barcoding remains a viable method. The identification of market replacement in seafood, DNA barcoding remains a viable method. In Canadian seafood, there was a 23 percent mislabeling of market replacement, revealing instances of substitution with potential economic consequences (Naaum and Hanner 2005). DNA Barcoding mitochondrial cytochrome c oxidase 1 (COI) revealed mislabeling of imported fish products in the Nansha new port of Guangzhou (China), and this result was confirmed by the morphological analysis (Yan et al. 2016). The COI gene sequence can distinguish closely related species because the sequence is conserved among each other (Indrayani et al. 2021.

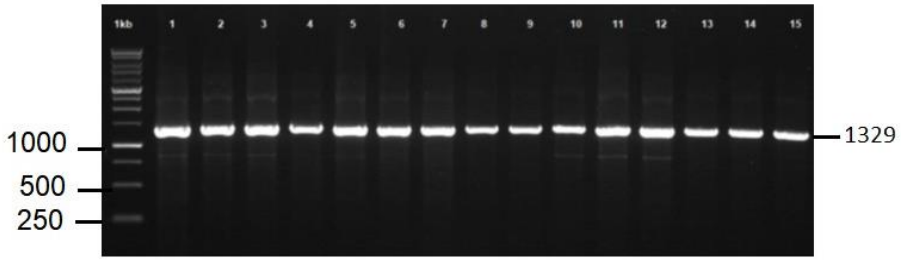

Figure 1. PCR products were electrophorized using 0,8% agarose gel, then visualized using sYBR safe under UV light. The PCR amplification produced amplicons that were approximately 1329 bp in length

Table 1. Calculations of genetic distance between groupings based on sequence pairings

Sample	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
S. Papua1																		
S. Papua2	0,001																	
S. Papua3	0,000	0,001																
S. Rembang1	0,129	0,129	0,129															
S. Rembang2	0,130	0,130	0,130	0,004														
S. Rembang3	0,136	0,136	0,136	0,017	0,015													
S. Banjarmasin1	0,127	0,127	0,127	0,002	0,004	0,017												
S. Banjarmasin2	0,174	0,173	0,174	0,153	0,152	0,158	0,153											
S. Banjarmasin3	0,174	0,173	0,174	0,153	0,152	0,158	0,153	0,000										
S. Palembang1	0,129	0,129	0,129	0,000	0,004	0,017	0,002	0,153	0,153									
S. Palembang2	0,129	0,129	0,129	0,000	0,004	0,017	0,002	0,153	0,153	0,000								
S. Palembang3	0,129	0,129	0,129	0,001	0,004	0,018	0,003	0,152	0,152	0,001	0,001							
S. Cilacap1	0,175	0,174	0,175	0,153	0,154	0,160	0,153	0,013	0,013	0,153	0,153	0,152						
S. Cilacap2	0,178	0,176	0,178	0,156	0,154	0,160	0,156	0,014	0,014	0,156	0,156	0,154	0,004					
S. Cilacap3	0,178	0,176	0,178	0,156	0,154	0,160	0,156	0,014	0,014	0,156	0,156	0,154	0,004	0,000				
S. Ambon1	0,136	0,136	0,136	0,017	0,015	0,000	0,017	0,158	0,158	0,017	0,017	0,018	0,160	0,160	0,160			
S. Ambon2	0,131	0,131	0,131	0,016	0,014	0,004	0,016	0,153	0,153	0,016	0,016	0,017	0,158	0,156	0,156	0,004		
S. Ambon3	0,131	0,131	0,131	0,016	0,014	0,004	0,016	0,153	0,153	0,016	0,016	0,017	0,158	0,156	0,156	0,004	0,000	

Table 2. Amino acids variation in Spanish mackerel

Sample	26	58	64	96	159	215	234	238	240	314	320	327	329	333	353	364	369	370	376	378	380
S. Papua1	S	D	N	F	T	A	V	A	V	G	A	A	I	V	V	V	A	G	V	E	N
S. Papua2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
S. Papua3	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
S. Rembang1	N	.	S	.	M	.	.	.	.	A	.	.	.	A	.	I	.	.	L	K	Y
S. Rembang2	N	.	S	.	M	.	.	.	.	A	.	.	.	A	.	I	.	.	L	K	Y
S. Rembang3	N	.	S	.	M	.	.	.	.	A	.	.	.	A	.	I	.	.	L	K	Y
S. Banjarmasin1	N	.	S	.	M	.	.	.	.	A	.	.	.	A	.	I	.	.	L	K	Y
S. Banjarmasin2	N	N	A	L	M	L	I	T	T	A	V	G	V	A	L	I	T	A	L	D	P
S. Banjarmasin3	N	N	A	L	M	L	I	T	T	A	V	G	V	A	L	I	T	A	L	D	P
S. Palembang1	N	.	S	.	M	.	.	.	.	A	.	.	.	A	.	I	.	.	L	K	Y
S. Palembang2	N	.	S	.	M	.	.	.	.	A	.	.	.	A	.	I	.	.	L	K	Y
S. Palembang3	N	.	S	.	M	.	.	.	.	A	.	.	.	A	.	I	.	.	L	K	Y
S. Cilacap1	N	N	A	L	M	L	I	T	T	A	V	G	V	A	L	I	T	A	L	D	P
S. Cilacap2	N	N	A	L	M	L	I	T	T	A	V	G	V	A	L	I	T	A	L	D	P
S. Cilacap3	N	N	A	L	M	L	I	T	T	A	V	G	V	A	L	I	T	A	L	D	P
S. Ambon1	N	.	S	.	M	.	.	.	.	A	.	.	.	A	.	I	.	.	L	K	Y
S. Ambon2	N	.	S	.	M	.	.	.	.	A	.	.	.	A	.	I	.	.	L	K	Y
S. Ambon3	N	.	S	.	M	.	.	.	.	A	.	.	.	A	.	I	.	.	L	K	Y
S. commerson (EF141176)	N	.	S	.	M	.	.	.	.	A	.	.	.	A	.	I	.	.	L	K	Y
S. semifasciatus (JX559745)	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
S. koreanus (DQ497884)	N	N	A	L	M	L	I	T	T	A	V	G	V	A	L	I	T	A	L	D	P

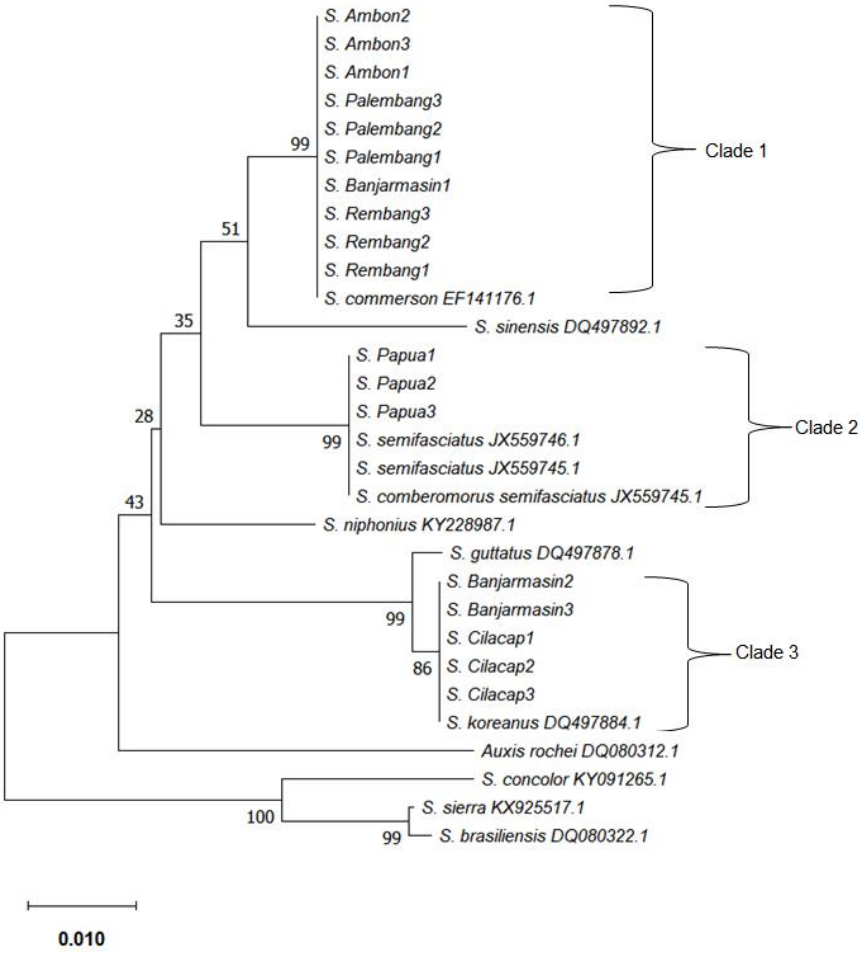

Figure 4. Phylogenetic tree of Spanish mackerel constructed from CYT B sequences and some mackerel from GenBank database

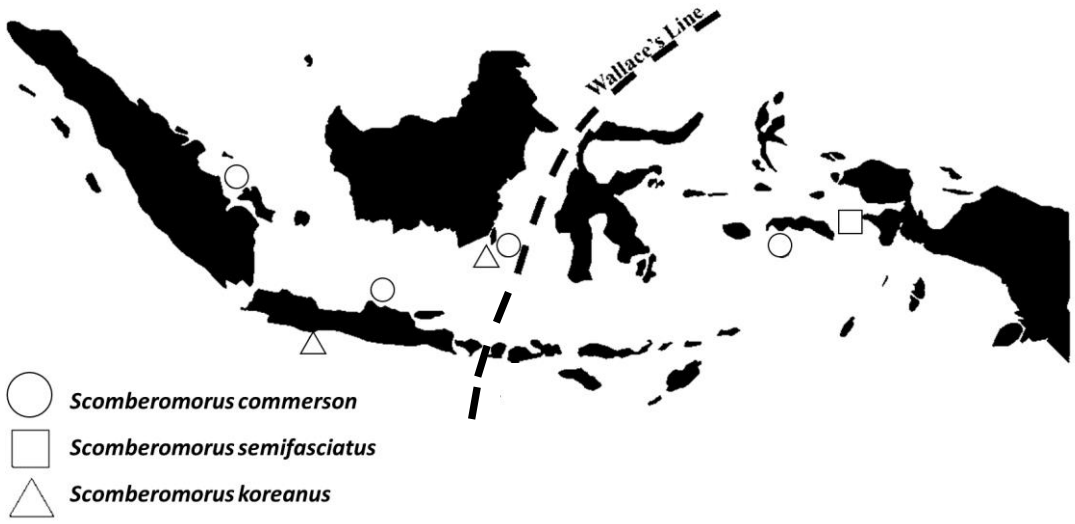

Figure 5. Mackerel's distribution in Indonesia according to genetic study conducted on local commodities sold in several fish markets across Indonesian archipelago

Genetic analysis of Spanish Mackerel based on CYT B sequences, we found that there are three major groups of mackerel in Indonesia: The Western, The Eastern and The Widespread Fish. The Division of the group was based on Wallace's Line. The Line divides biogeographical zone in Indonesia into two areas, with western zone being influenced by Asia mainland, and the eastern zone being influenced by Australian Continent (Mayr, 1944). We found that samples collected from The Western Zone were related to *S. koreanus*, from The Eastern Zone were related to *S. semifasciatus*, meanwhile the widespread group was related to *S. commerson* (Figure 5). The average evolutionary divergence in Spanish Mackerel populations was determined to be 4%. Jackson et al. (2013) have reported the genetic diversity of tuna and mackerel in the Indonesian archipelago using a control region sequence, the nucleotide diversity showed in the range of 0.2-9%. Due to their enormous effective population numbers and propensity for long-distance dispersal, pelagic fishes are predicted to display low genetic differences across wide geographic distances (Habib and Sulaiman 2016; Kumar et al. 2012).

Three species were collected in our study. *S. koreanus* were only collected in western part of Wallace's Line, *S. semifasciatus* were collected in eastern part of the line, meanwhile *S. commerson* were collected in both *regions*. Korean mackerel (*S. koreanus*) is mainly distributed in Indo-Pacific Region, from East Coast of India, along Sumateran coastal, Kalimantan then up north to Korea and Wakasa Bay in Sea of Japan (Luna 2018). The result was resemblance to the references since our samples were collected in fish market in Cilacap and Banjarmasin, western side of The Wallace's Line. Broad-barred mackerel (*S. semifasciatus*) were collected from fish market in Fak Fak, Papua, despite it mainly distributed in western pacific, from South Papua to along Australian northern coast (Collette et al. 2011a; Northern Territory Government 2016). Further study needs to be done to clarify whether the distribution of the fish is going up north or the local fisherman's fishing spots were in southern part of Papua Island. The wide-ranging area Narrow-Barred Spanish Mackerel (*S. commerson*) is centering in Southeast Asia, but also can be found from west coast of Africa, along the northern coastal area in Indian Ocean, to southwest Pacific Ocean (Collette et al. 2011b). Since the fish has wide-ranging distribution from Southern Africa to Pacific, we found the fish from various places in Indonesia, both in western and eastern parts of Wallace's Line. Three species of mackerels were collected in our study. The species have corresponded with their respective known distribution area. It is critical for conservation and fisheries management to understand the distribution of fish frequently utilized as fisheries commodities samples collected from local fish market provides superficial information about fish diversity in the surrounding seas. The results of this study can be used for further research to identify types of mackerel from Indonesia and food products derived from mackerel.

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