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The diversity of Carangidae (Carangiformes) was revealed by DNA barcoding collected from the traditional fish markets in Java and Bali, Indonesia

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Abstract. *Andriyono S, Alam MJ, Sumartiwi L, Mubarak AS, Pramono H, Suciyono, Kartika GRA, Sari AHW, Sektiana SP. 2022. The diversity of Carangidae (Carangiformes) was revealed by DNA barcoding collected from the traditional fish markets in Java and Bali, Indonesia. Biodiversitas 23: 2799-2806.* Biodiversity has been utilized in various ways, including in fulfilling the protein needs of fish for coastal communities. For the island of Java, with the largest population in Indonesia, the intensive fisheries in the Java Sea are sufficient to support domestic food needs. This Carangid fish diversity study in Java is the beginning to identify commercial fish in Indonesia, which have been exploited for a long time. In this study, identification was carried out molecularly in the Cytochrome c oxidase subunit I (COI) gene region with the universal primary set and found a great variety of Carangids fish species. Thirty-three fish specimens have been identified, indicating two suborder groups, Caranginae (31) and Scomberoidenae (2). The Caranginae suborder group is more dominated with the most types of which are *Megalaspis cordyla* (3), *Atule mate* (3), and *Decapterus macarellus* (4). Meanwhile, the Scomberoidinae suborder is *Scomberoides commersonnianus* and *Scomberoides tala*. This study also found two species that have the potential to be ciguatera poisoning agents that need to be watched out for (*Decapterus macarellus* and *Selar crumenophthalmus*). Food safety in the fisheries sector has received considerable attention for a long time. Fishery commodities in highly polluted habitats are among the chains in increasing heavy metals concentration and the other residual chemical compounds that may impact human health.

Keywords: Biodiversity, commercial, conservation, fisheries, genetic study

INTRODUCTION

In Indonesia, an archipelago is distributed in tropical areas called the Indo-Malay Archipelago (IMA), with Incredible biodiversity in marine life. This region has been recognized as one of the centers of marine biodiversity in various taxon fish, molluscs, and various other taxa (Lohman et al. 2011), located between the Pacific Ocean in the North and the Indian Ocean in the South. The island of Java, located in the southern part of Indonesia, has the potential for diversity, strongly influenced by the Indian Ocean compared to the Pacific Ocean region. However, the Java Sea's existence in the middle of the Indonesian archipelago allows the mixing of Pacific waters through the Makassar Strait gap between Kalimantan and Sulawesi Islands. The presence of the South China Sea also enables fish resources to enter the Java Sea through the Riau Islands and into the Java Sea. This considerable fishery potential causes capture fisheries in the Java Sea to be very potential, dominated by the Carangid fish.

The Carangidae family (order Carangiformes) includes fish with varying body sizes ranging from 16 cm-250 cm, with irregular body shapes (elongated, fusiform, and compressed) (Randall 1995). Fish from this family are commonly found in fisheries commodities such as scads, jacks, trevallies, pompanos, and rainbow runners. In addition, Carangids have become essential raw materials in Southeast Asia's processing industry as a food and animal protein source. Even though intensive exploitation continues, taxonomy and scientific naming is still confused because of the similarities in morphology, size, and color pattern, which causes ambiguity in this family taxonomy (Froese 2009). In addition, the juvenile stage to adulthood sometimes undergoes significant morphological changes and pigmentation in the life cycle. As a result, misidentification down to the species level has often happened. The color difference in the *Cichla* species occurs in the *Cichla kelberi* species which has a color similar to that of *Cichla temensis* (Sastraprawira et al. 2020). The implication of misidentification is mislabeling of fisheries products (Guardone et al. 2017). For instance, the type of *Cichla ocellaris* circulating in Indonesia has a higher price than other types of this fish. The *Cichla* fish in the ornamental fish market is known as peacock bass or peacock cichlids with attractive color patterns. Misidentification cases based on morphology also occur in juvenile *Alectis ciliary* long filament but will shorten and eventually disappear as it grows to maturity (Randall et al. 1997).

This DNA barcode method uses a reasonably short but standardized area of mitochondrial DNA with an identification accuracy up to the species level (Aquilino et al. 2011), which is high enough to approach 100% and has been widely accepted to date. Thus, the identification based on morphological needs to be complemented by other identification methods to ensure identification accuracy. The form of identification currently being developed molecularly reduces errors in identification. This identification is referred to as barcoding DNA. This DNA barcode method uses a reasonably short but standardized area of mitochondrial DNA. That region is Cytochrome c Oxidase subunit I (COI). This gene region has been widely used for a molecular approach-based identification with accuracy up to the species level high enough to be close to 100%. DNA barcoding has been globally accepted (Hebert et al. 2003). Open-access databases are continually being developed in connection with the emergence of misidentification through the molecular approach (Ratnasingham and Hebert 2007). Simplifying the identification process through molecular methods is relatively easy and allows for finding new taxa (Hajibabaei et al. 2007).

This study carried out tests on Carangidae fish from Java’s northern and southern regions and analyzed the COI sequence's genetic diversity. This analysis is essential for the sustainable management of Carangidae fish resources because most of them are not reported and are not well identified.

MATERIALS AND METHODS

Sampling site

Thirty-three fish samples (Table 1) were collected from the five traditional fish markets around Jawa Island in July 2019. In the northern part of Java, samples were obtained from the Banten-BNT (6º0'50.00'S-106º10'21.00"E), Gresik-GRK (6º52'56.65'S-112º12'15.87" E), and Pekalongan-PKL (6º51'32.10"S 109º41'09.52"E). While Southern Java was represented samples from Pelabuhan Ratu-PRT (6º59’20.92”S 106º32’29.91”E), Malang-MLG (8º 26’ 06.65’ S - 112º 40’ 55.31” E), the Banyuwangi-BWI (8º 12’ 07.52’ S - 114º23’07.18” E), and Denpasar-DPS (8º 45’ 23.00’ S - 115º 10’ 05.68” E). Morphological identification was conducted according to the guideline from FAO (Heemstra 1993), and species confirmation was carried out with molecular identification carried out in this study using the COI gene region. No specific permit was required for this study because collection from the local traditional fish market was dead upon purchasing. All samples were photographed for every single specimen using a digital camera.

Table 1. Carangids species list from Jawa, Indonesia

Species	Sampling location						
	GRK	PKL	BNT	PRT	MLG	BWI	DPS
Alectis indica					1		
Alepes melanoptera		1					
Alepes vari	1						
Atropus atropos			1				
Atule mate		1		1	1		
Carangoides armatus		1					
Carangoides chrysophrys						1	1
Carangoides malabaricus			1				
Caranx sexfasciatus				1			
Caranx tille							1
Decapterus macarellus		1			1		1
Decapterus macrosoma			1				
Decapterus maruadsi					1		
Megalaspis cordyla	2	2					
Parastromateus niger	1			1			
Selar boops	1						
Selar crumenophthalmus		1					1
Selaroides leptolepis		3		1			
Scomberoides commersonnianus	1						
Scomberoides tala						1	
TOTAL	6	10	3	4	4	2	4

Note: GRK: Gresik; PKL: Pekalongan; BNT: Banten; PRT: Pelabuhan Ratu; MLG: Malang; BWI: Banyuwangi; DPS: Denpasar

DNA extraction and PCR condition

All samples were preserved in 90% ethanol for further laboratory experiments. In preparation for the genomic DNA extraction process, cutting the tissue in each specimen was done aseptically (around 0.5 cm) and immediately washed to remove ethanol under running water. After the washing process was carried out, the sample was put in a microtube containing 6X lysis buffer and mixed with the TissueLyser II (Qiagen). The use of Accuprep® Genomic DNA extraction Kit (Bioneer) was carried out according to the Kit protocol and followed by measuring the concentration of the extracted with nanoDrop (Thermofisher Scientific D1000). The extract was used in the application process by the PCR or stored at -70oC for further analysis.

The amplification process of genomic DNA from samples was carried out using a universal primer set, namely FISH-BCL (5'-TCA ACY AAT CAY AAA GAT ATY GGC AC-3 ') and FISH-BCH (5'-TAA ACT TCA GGG TGA CCA AAA AAT CA -3 ') (Baldwin et al. 2009, Handy et al. 2011). The target DNA amplified is the cytochrome c oxidase I (COI) region, about 600 bp in length. The volume of the PCR mixture is 20µL consisting of 11.2 µL ultra-pure water, 1 µL forward and reverse primer (0.5 µM), 0.2 µL Ex Taq DNA polymerase (TaKaRa, Japan), 2 µL 10X ExTag Buffer, 2 µL dNTPs (1 µM, TaKaRa, Japan), and 2 µL genomic DNA as a template. The PCR condition was carried out under the following setting: 95ºC for 5 min in initial denaturation, followed by denaturation at 95ºC for 30 s in 40 cycles, 50ºC for 30 s in annealing, and 72ºC for 45 s in extension step, and a final extension at 72ºC for 5 min. After obtaining the desired band on the electrophoresis gel (1.5% agarose), the PCR products were purified with the AccuPrep® Gel purification kit (Bioneer, Korea).

Data analysis and phylogenetic analysis

All sequences are then aligned with the help of Mega7 software, including the sequences obtained from the GenBank database for comparison. The pairwise evolutionary distance among the species is determined by the Kimura 2-Parameter method. The Neighbor-joining (NJ) tree was constructed, and 1000 bootstrap analysis was carried out by Mega7 (Kumar et al. 2016).

RESULTS AND DISCUSSION

Results

Thirty-three successful identification samples consisted of 2 sub-families within Carangidae, Caranginae (31) and Scomberoidenae (2). In this study, the Caranginae dominated the general catches of fisherman, including *Megalaspis cordyla* (3), *Atule mate* (3), and *Decapterus macarelus* (4). Meanwhile, local fishers' common fish caught in Java and Bali and commercial fish, benefit domestic consumers. In general, the types of fish collected in the Carangids group have low prices and become food sources of protein for the community.

Genetic distance

Genetic distance analysis was carried out using Mega7 compared to the GenBank database. Several sequences have unique, potentially as a haplotype that Indonesia has founded. Within subfamily Scomberoidinae, *Scomberoides tala* is similar to the Indo-Malay (JX261091) than Scomberoides commersonnianus has a very close genetic distance with the Indo-Malay sequence (JX261017) is 0.0019. The smaller the genetic distance, the closer the similarity and may indicate that a particular species may have similar origins (Tapilatu et al. 2021; Dwifajri et al. 2022). In addition, within sub-family of Caranginae, we also found potentially haplotype Indonesia in *Decapterus maruadsi* (0.0079), *Megalaspis cordyla* (0.0019), *Carangoides armatus* (0.0019), *Atropos Atropos* (0.0019), *Alepes vari* (0.0019), *Atule mate* (0.0059), Selar boops (0.019), *Selar crumenophthalmus* (0.0039), *Carangoides malabaricus* (0.0019), *Carangoides chrysophrys* (0.0019), and *Selaroides leptolepis* (0.0019).

Phylogenetic reconstruction

In the phylogenetic tree produced, two clades have been formed: the subfamily Cranginae and Scomberoidinae (Figure 1). Morphologically, Scomberoidinae is closer to the Family of Scombridae with fusiform in body shape (Figure 2), but its body was flattened compared to the torpedo-like Scombridae. Scomberoidinae, in etymology, has a meaning similar to tunny or mackerel. In this study, *Scomberoides tala* and *Scomberoides commersonnianus* were successfully identified from Banyuwangi and Gresik, respectively.

Carangidae status in IUCN and CITES

Almost all Carangidae species in this study have the status of Least Concern (LC), and the international trade (CITES) status is not evaluated (Table 2). From several reports, the fishing of Carangids species is carried out intensively, and most of them turn it into fish for processed fishery products such as surimi or only as raw material for fish meal. For example, research on fish catches in the Madura Strait found that Carangidae fish catch was 8% (Purwangka and Mubarok 2018), while other studies found Carangidae species of 38.51% of the catch (Khatami et al. 2018). Apart from Java, the Carangidae species that traditional fishers catch in Kalimantan also entirely dominate around 16.67% (Alfian et al. 2020).

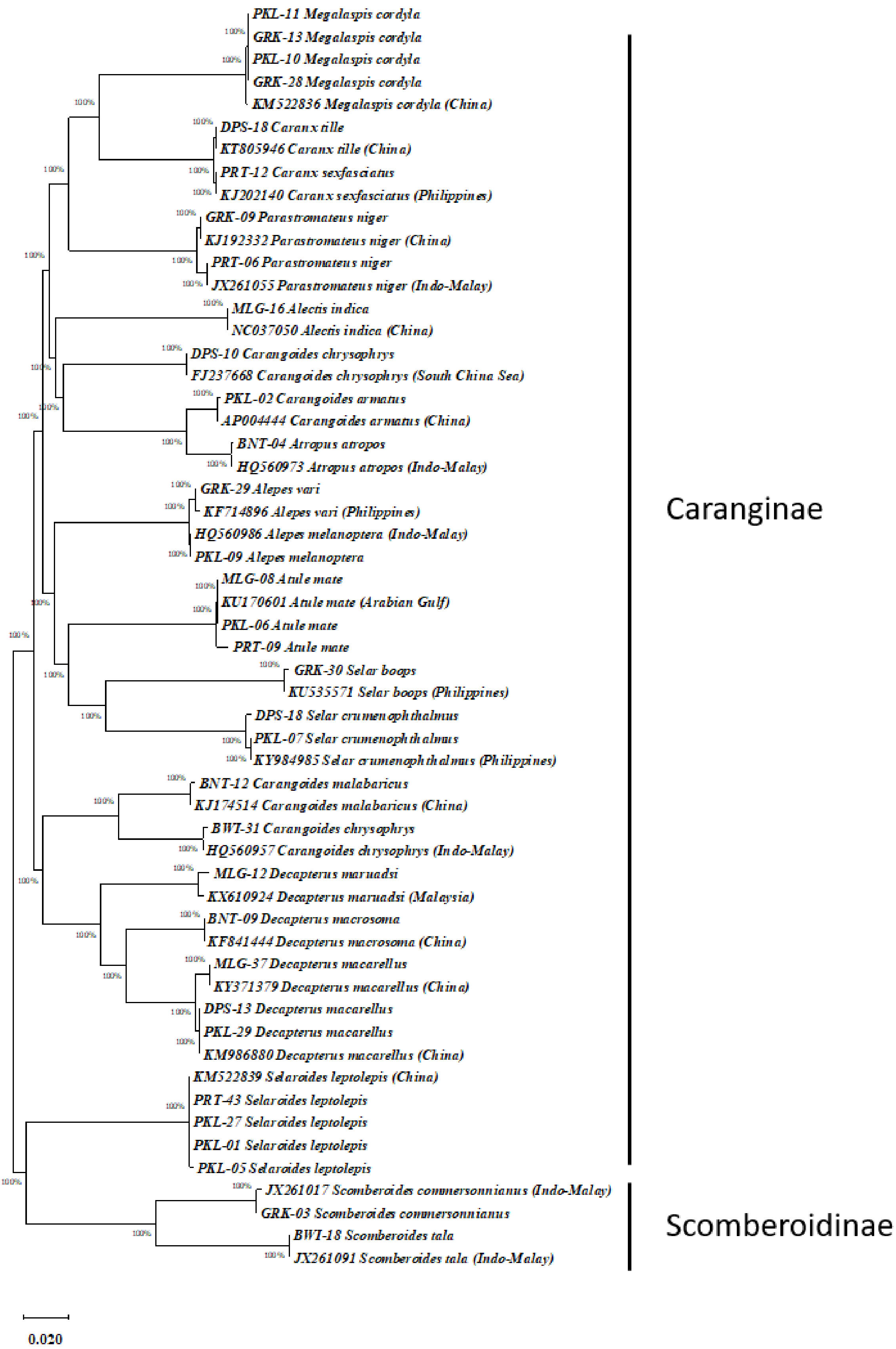

Figure 1. Phylogenetic tree of Carangidae using the neighbor-joining algorithm by Mega7

Figure 2. Scomberoidinae: *Scomberoides commersonnianus* from Gresik and *Scomberoides tala* from Banyuwangi (Bar = 1 cm)

Tabel 2. IUCN and CITES status of all Carangids species

Species	Sub-family	Habitat distribution	Common name	IUCN Red List Status	CITES	Threat to humans
Alectis indica	Caranginae	Indo-Pacific	Indian threadfish	LC	NE	Harmless
A. melanoptera	Caranginae	Indo-Pacific	Blackfin scad	LC	NE	Harmless
A. vari	Caranginae	Indo-West Pacific	Herring scad	LC	NE	Harmless
Atropus atropos	Caranginae	Indo-West Pacific	Cleftbelly trevally	LC	NE	Harmless
Atule mate	Caranginae	Indo-Pacific	Yellowtail scad	LC	NE	Harmless
C. armatus	Caranginae	Indo-West Pacific	Longfin trevally	LC	NE	Harmless
C. chrysophrys	Caranginae	Indo-Pacific	Longnose trevally	LC	NE	Harmless
C. malabaricus	Caranginae	Indo-West Pacific	Malabar trevally	LC	NE	Harmless
Caranx sexfasciatus	Caranginae	Indo-Pacific	Bigeye trevally	LC	NE	Harmless
Caranx tille	Caranginae	Indo-West Pacific	Tille trevally	LC	NE	Harmless
Decapterus macarellus	Caranginae	Circumglobal.	Mackerel scad	LC	NE	Ciguatera poisoning
D. macrosoma	Caranginae	Indo-Pacific and Southeast Atlantic	Shortfin scad	LC	NE	Harmless
D. maruadsi	Caranginae	Indo-West Pacific	Japanese scad	LC	NE	Harmless
Megalaspis cordyla	Caranginae	Indo-West Pacific	Torpedo scad	LC	NE	Harmless
Parastromateus niger	Caranginae	Indo-West Pacific	Black pomfret	LC	NE	Harmless
Selar boops	Caranginae	Pacific Ocean	Oxeye scad	LC	NE	Harmless
S. crumenophthalmus	Caranginae	Circumtropical	Bigeye scad	LC	NE	Ciguatera poisoning
Selaroides leptolepis	Caranginae	Indo-West Pacific	Yellow stripe scad	LC	NE	Harmless
Scomberoides tala	Scomberoidinae	Indo-West Pacific	Barred queenfish	LC	NE	Harmless
S. commersonnianus	Scomberoidinae	Indo-West Pacific	Talang queenfish	LC	NE	Harmless

Discussion

Shallow marine fishery resources, especially in the Java Sea, have provided benefits for meeting animal protein needs in the Java Island region, the most populous island area in Indonesia. Indonesian capture fisheries production in Indonesia until 2017 reached 6,603,630.58 tonnes/year with a value of USD 185,798,801 (Nainggolan et al. 2019). From several ports on Java, the Java Sea fishery shows quite intensive fishery activity with a fairly large catch value. There are potential ports with very diverse catches along the north coast of the island of Java. However, studies conducted by Java sea capture fisheries in the Central Java region show that the Carangids group, such as *Decapterus* spp., is a caught fish species that dominates, especially during the peak season in September-November each year (Chodrijah and Hariati 2017). Another study stated that as a migratory pelagic fish, *Decapterus* spp. into the waters of the Java Sea from the Indian Ocean. The migration occurred in the western season (January-March) and increased during the southeast monsoon (June-July), including two species, *Decapterus macrosoma* and *Decapterus russelli* (Prasetyo et al. 2018).

Carangidae fishery is a fishery potential that is the mainstay of fulfilling domestic consumption compared to tuna fisheries which are exported products (Yusuf et al. 2018). Carangidae commodity, processed products are an effort to increase the product's added value besides being sold in fresh fish. One of the most famous processed products with Carangids fish raw materials is the scavenging with the main fish species in steam fish (pindang) from the genus Decapterus spp and Atule spp (Lubis et al. 2019).

The surveys in some fish landing areas on the north coast of Java and the south coast illustrate that the Javanese marine fishery makes Carangids a relatively high catch (Table 1). On the other hand, in the southern region of Java, it is the result of large pelagic fisheries such as tuna and skipjack (Firdaus 2019). Previous research has also confirmed that the Java sea fishery with the Carangids commodity is becoming quite popular (Chodrijah and Hariati 2017). Meanwhile, in the Indian sea fishery or the southern coast of Java, tuna fisheries are the mainstay and are the mainstay in Malang (Wiadnya et al. 2018). Pelabuhan Ratu (Mertha et al. 2017) is well known as a center for tuna fish catches in Southern Java (Nurani et al.

2017). Besides, Bali Island, directly facing the Indian Ocean, also has a potential tuna landing center in Benoa (Ahmad et al. 2019).

However, Carangids fish's diversity has provided benefits even though, economically, it is only developed into a domestic product with traditional processing technologies such as dried salted fish and pindang. Phylogenetic tree analysis shows that the carangid diversity caught in the waters around Java is mainly in the Carangidae suborder group. However, a small proportion is included in the Scomberoidinae suborder group. In the Caranginae group, *Megalaspis cordyla* and *Selaroides leptolepis* dominate Java's northern region (Gresik and Pekalongan). In this phylogenetics, two distinct clade sub-orders between the Caranginae and Scomberoidinae sub-orders (Figure 1).

All carangid fish status in the IUCN Red list status and CITES classify all species in this order into the Least Concern (LC) category and Not Evaluated (NE). Attention and regulation are needed in the management of capture fisheries in particular. For instance, the northern part of Java reported having experienced overfishing (Sadhotomo and Atmadja 2016). Another interesting point is that apart from being an essential source of animal protein for coastal communities, two types of fish have been reported to be the causes of Ciguatera poisoning that need to be watched out for in *Decapterus macarellus* (Lange et al. 1992), and *Selar crumenophthalmus* (Rongo and van Woesik 2012). The type of *Decapterus macarellus*, suspected of containing poison is smoked fish imported from the Philippines. The possibility of smoked fish meat has palytoxin, which causes ciguatera poisoning (Kodama et al. 1989). Ciguatera poisoning is a form of ichthyo-sarcotoxism, which means the content of some toxin in that body. This condition occurs when marine fishes inadvertently ingest particular dinoflagellates that produce ciguatoxins (Rongo and van Woesik 2012). Until now, no identified reports about ciguatera poisoning in Indonesia. Food safety in the fisheries sector has received considerable attention for a long time. Fishery commodities that harvested from highly polluted habitats are among the chains in increasing heavy metals concentration that may impact humans (REF?). Furthermore, fish that live in waters with a reasonably complex food chain, allowing food to enter the fish's body, would trigger the cause of ciguatera that has been reported in several other countries outside Indonesia (Chan 2016). Although there have been no reports of ciguatera cases in Indonesia (including Brunei, Cambodia, Myanmar, and North Korea) (Chan 2015a), there needs to be serious attention to Indonesian fishery products, which are also exported commodities. A report states that Indonesian fishery products are reported to cause ciguatera from snapper fish on the commercial market in Germany (Friedemann 2019). However, only China (Chan 2015b) and Hongkong (Chan 2014b) received reports of ciguatera due to fishery products on hump-head wrasse (Chan 2013) and tiger grouper (Chan 2014a), which caused some people to receive medical care.

Sub-Family Caranginae

In this study, the sub-family Caranginae dominates with 31 species identified from 31 species (94%), while the remaining groups are Scomberoidinae (4%). In the Caranginae group, it can be found both in the Java Sea and the Indian Ocean. In the Java Sea area, this study found the types of *Megalaspis cordyla* (GRK and PKL), *Alepes vari* (GRK), *Alepes melanoptera* (PKL)), *Selar boops* (GRK), *Carangoides malabaricus* (BNT), and *Selaroides leptolepis* (PKL and PRT). Based on the phylogenetic tree (Figure 1), the species Megalaspis cordyla from Gresik and Pekalongan has close related and probably in the same population and share a habitat in Jawa Sea (Maskur et al. 2020). However, the Indian Ocean and Java Sea regions also share other types of Caranginae, such as *Decapterus macarellus* (DPS, PKL and MLG), and *Decapterus macrosoma* (BNT), *Decapterus maruadsi* (MLG), *Carangoides chrysophrys* (BWI) and *Carangoides malabaricus* (BNT). In brief, the two species of *Decapterus* (*D. macarellus* and *D. macrosoma*) are pretty difficult to distinguish morphologically. The primary and key characteristic in the scattered scales on the lineal lateralis part. Research on the morphological and molecular characteristics of these two species of *Decapterus* has been carried out clearly (Zhang et al. 2020). These types of fish have become one of the targets of artisanal fisheries in Java, both in the southern and northern regions including Bali. Research in the Java Sea waters states that several fish species, such as small pelagic, dominate the catch, including the type of *Selaroides leptolepis*, *Selar crumenophthalmus*, *Dussumieria acuta*, *Rastrelliger brachysoma*, and *Atule mate* (Khatami et al. 2019).

Many studies on artisanal fisheries in Java have been carried out, and Selaroides (Wijayanto et al. 2019a, Wijayanto et al. 2019b) is one of the catch targets for medium-sized nets such as the use of Danish seine fishing gear, which is considered an environmentally friendly fishing gear compared to trawling (Adhawati et al. 2017a, Adhawati et al. 2017b). This artisanal fishery has driven the economy and even continues today. For example, capture fisheries in Pekalongan with mini purse seine fishing gear that get catches belonging to the Caranginae group, such as the type of *Decapterus* sp (Maulana et al. 2017). In addition, artisanal fisheries in the northern waters of Java are also highly developed, such as in Banten (Barlian et al. 2020; Barlian et al. 2021), Tegal (Wijayanto et al. 2019a), Lamongan (Syamsuddin et al. 2020), and Madura (Yonvitner et al. 2021).

Sub-Family Scomberoidinae

Although this group of fish is only 4% of this study, economically, it is included in the fish group with a fairly reasonable price. This fish of the Scomberoidinae group has morphological characteristics similar to the Family Scombridae, which includes tuna and mackerel with a streamlined body shape to become a group of fast swimming fish. In this study, Scomberoides commersonnianus is a group of fish that is quite attractive and becomes potential raw material for fishery products. The method of catching this type of fish varies considerably; the use of blue LEDs is beneficial and increases the catch of this species (Mawardi and Riyanto 2017). Processed products from the Scoberoides fish species are Talang-Talang salted fish in Aceh (Melawati et al. 2019; Riski 2017), fillets (Farsanipour et al. 2020), Surimi, and fish nugget (Moosavi-Nasab et al. 2019, Oujifard and Morammazi 2020). However, the types of processed fishery products from these fish groups in Indonesia are limited.

In conclusion, this research successfully identified the diversity of Carangid fish landed in several fishery centers in Java. Of the 33 carangids fish, the most extensive composition obtained from these locations was *Megalaspis cordyla* (4), *Selaroides leptolepis* (4), *Atule mate* (3), and *Decapterus macarelus* (3). Generally, these fish are consumed locally to meet people's protein needs in coastal areas of Java. Phylogenetic analysis shows that the suborder Caranginae and Scomberoidinae are separated. Fish in the Caranginae suborder dominate compared to other sub-orders. Scomberoidinae in this study has unique morphological characteristics that are sometimes confused with the Scombridae group. This reinforces the use of molecular identification to complement morphological identification.

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