ISSN 2620-570X
P-ISSN 2656-7687
JURNAL ILMU KELAUTAN KEPULAUAN, 4 (2) ; 408-415, DESEMBER , 2021
http://ejournal.unkhair.ac.id/index.php/kelautan

Morphometric and genetic identification of Mackerel (Rastrelliger sp.) collected from Muara Baru Fish Market, Jakarta

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Diterima: 20 Juli 2021; Disetujui: 5 Desember 2021

ABSTRACT
Ecosystems in Indonesia have a lot of potential resources, and one of them is seafood resources. Mackerel is potential catches of fish in the Jakarta Bay (as part of Java Sea area) that has high economic value. The Java Sea has only two mackerel species, Rastrelliger brachysoma and R. kanagurta which have similar morphology. Therefore in this study was conducted to compare morphometric and molecular analysis (DNA barcoding) using COI sequences so that specific species from specimens could be identified. The analyzed fish samples have striking characteristics such as blackline on the back, a black line near the pectoral fins, and a dark body part is extending above the rib line. The dorsal fins have a yellowish-gray color, yellowish pectoral, and pectoral fins.The result showed that measurements characteristic features of mackerels in Muara Angke belonging to Rastrelliger genera. DNA Barcoding analysis showed that the collected sample identified as Rastrelliger kanagurta with 99.25% identification similarity. The DNA code can distinguish between all fish species and identify the eggs, larvae, and carcass pieces of this species. So that fish in fish landings can be identified and tagged according to their species. This study has successfully identified a sample from Muara Angke by using a DNA code and therefore would be beneficial for managing food controlling and food safety concerns.

Keywords: *molecular identification, DNA barcoding, Rastrelligerkanagurta, morphological analysis*

I. Introduction

Indonesia has an abundant ecosystem and has a lot of potential resources. One of them is fishery resources that made Indonesia contributed 9.9 million tons of fish caught in 2016, 60% came from small-scale fishers (Ministry of Marine Affairs and Fisheries, 2016). One of the potential catches of fish in the Jakarta Bay area is the Mackerel, which has high economic value (Indaryanto et al., 2015). Mackerel is a small pelagic fish that schools in large quantities and forages in the surface layer(Solanki et al., 2005). The Java Sea only has two species of Mackerel, *Rastrelliger brachysoma* and *R. kanagurta*(Sujastani, 1976). Both have the same morphological character and can be identified by the ratio depth body and the lines along the sides of the body (Muchlisin et al., 2009). One that distinguishes between these two species is *R. kanagurta* has black spots near the fins that distinguish it from *R. brachysoma*(Saanin, 1984).

Accurate fish identification will assist fisheries management for long-term sustainability and will enhance ecosystem research and conservation (Wang et al., 2018). The classification and identification of fish are not only the subjects of taxonomic studies but also useful for fisheries investigations, assessment of nature reserves, and identification of food ingredients and medicines (Ardura et al., 2013;

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Vartak et al., 2014). In general, the identification of fish species depends on morphometric characters (Triantafyllidis et al., 2011). However, there are problems because traditional determination methods require a large amount of taxonomic expertise and inefficient. Fish have a variety of morphological characteristics, and most of the fish have metamorphism ontogenetic. Many morphometric characteristics change during the ontogenetic development stage (Bingpeng et al., 2018).

Correct naming and determining species are crucial for conducting bioecological studies and other studies. Along with the development of molecular biology, a new method has been found to identify DNA-based species known as DNA Barcoding(Hebert et al., 2003). DNA barcoding has been used widely for the identification of various species, such as sea cucumber (Madduppa et al., 2017), sea turtles (Madduppa et al., 2019), shark (Toha et al., 2016), soft coral (Kusuma et al., 2016), to gastropods (Saleky et al., 2016). DNA barcoding provides speed and accuracy in species identification with the focus of analysis on small segments of mtDNA (Karim et al., 2015). The gene of CO1 (cytochrome c oxidase 1) for vertebrata and it has been shown effective in discriminating fish species (Thu et al., 2019).Several previous studies on mackerel that have been conducted in Indonesia, among others, by Zamroni et al. (2017)andIndaryanto et al. (2015)determined the reproductive biology and genetics of the population in the northern waters of Java.

The aims of the research were morphological and genetic identification of *Rastrelliger* so that specific species from fish samples could be identified. The identification of fish using morphometrics is not enough so that it is necessary to use genetic identification (DNA barcoding) to get more accurate results.

II. Research Method

Sample Collection

Samples of fish were collected from the local fish market in Muara Angke, Jakarta Baypada bulan October 2019 and analysis of fish samples was conducted at the Marine Biodiversity and Biosystematics Laboratory. Samples of morphological fish identified were 30 fish. Each specimen is temporarily identified based on the black dot on the body of the fish. Fin tip is taken and put into a sample bottle that contains 96% ethanol for molecular analysis with DNA barcoding.

Morphometric analysis

Samples are identified by measuring total length, fork length, head length, head depth, and body depth, colors, and other special features. Details on measured landmarks are shown in Figure 1. The identification carried out refers to the identification book by Saanin (1984). Each fish sample was photographed using a camera. Then the sample was redrawn using Adobe Illustrator software. The length-weight equation:

$$W = aL^b \quad (1)$$

was used to estimate the relationship between the weight of the fish and its total length (Cren, 1951). Using the linear regression of the log-transformed equation:

$$\log (W) = \log (a) + b \log (L) \quad (2)$$

the parameters a and b were calculated with ‘a’ representing the intercept and ‘b’ the slope of the relationship to describe the LWRs related to periodic variations that can

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affect b (Zargar et al., 2012). Three possible values appear in the measurement of fish length and weight, b <3, b = 3, and b> 3. As for b <3, it shows that weight gain is not as fast as length gain, b = 3 means that weight gain and weight gain are the same. As for the value of b> 3 the length increase is slower than the weight gain (Muchlisin et al., 2010).

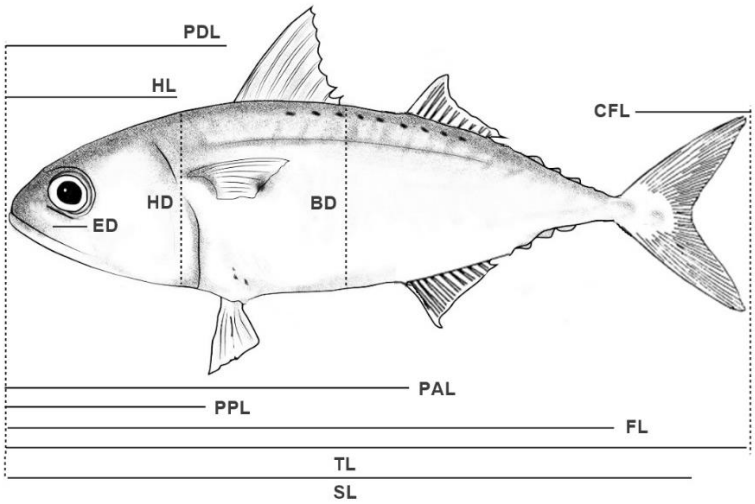

Figure 1.Landmarks measured from fish sample *Rastrelliger sp.* Measures are as follows: total length (TL); standard length (SL); fork length (FL); head length (HL); pre-dorsal length (PDL); pre-pelvic length (PPL); pre-anal length (PAL); head depth (HD); body depth (BD); caudal fin length (CFL); eye diameter (ED).

Genetic analysis

One sample of fish was carried out DNA isolation by referring to the working procedure of the gSYNC DNA Extraction Kit. The primary used is F15'-TCA-ACCAAC-CAC-AAA-GAC-ATT-GGA-C-3' (forward) and R15'-TAG-ACT-TCT-GGG-TGG-CCA-AAG-AAT-CA-3' (reverse) with target PCR product at 600 bp (Ward et al., 2005). Polymerase Chain Reaction (PCR) was conducted in 25 µL reaction volume containing 1 µL template DNA, 12.5 µLMy taq, 1.25 µL primer, and 9 µL ddH2O. The PCR conditions used were pre-PCR (94 °C for 30 seconds), denaturation (94 °C for 30 seconds), annealing or attachment (50 °C for 1 minute), extension (72 °C for 1 minute), and post-PCR (72 °C for 7 minutes). The extracted DNA was migrated to 1% agarose gel and visualized with the help of UV transilluminator. PCR products were sent to sequencing facility at First Base, Singapore. Sequences were aligned and edited in Mega 6 then sequences are compared with GenBank data using BLAST.

III. Result and Discussion

In general, observations showed that fish have a slim and long body shaped like a torpedo. Parts of the body are covered with fine scales on the back of the pectoral fin. The front and back of the eye have puffy eyelids (adipose). The sample has a turquoise color at the top, and the bottom is yellowish-white. Blackline on the back, a black line near the pectoral fins, and a dark body part is extending above the rib line. The dorsal fins have a yellowish-gray color, yellowish pectoral, and pectoral fins.

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These measurements (Table 1) are characteristic features of mackerels belonging to Rastrelliger genera. The length of the head is almost the same as the body depth in most of the samples shown in Table 1. The typical dark line and black spots on the fins seen in fish samples indicate that the fish samples are *Rastrelliger kanaguta* (Luther, 1973).

Table 1.Morphometric measurements of the *Rastrelliger sp.*

Morphometric characters	Average Length (cm)	Standard Deviation
Total length (TL)	16.23	0.72
Standard length (SL)	12.81	0.67
Fork length (FL)	14.34	0.43
Head length (HL)	3.81	0.34
Pre-dorsal length (PDL)	4.90	0.33
Pre-pelvic length (PPL)	4.56	0.45
Pre-anal length (PAL)	8.71	0.50
Head depth (HD)	3.82	0.31
Body depth (BD)	3.95	0.35
Caudal fin length (CFL)	3.23	0.31
Eye diameter (ED)	1.14	0.12

The average value of fish size caught in Muara Angke is 16.23 cm, the size at which mackerel is in its first adult stage. According toCollette & Nauen (1983)the length at first maturity of Rastrelliger kanagurta is about 16 cm. If this condition occurs continuously and there is no good governance in fishing activities, it will have the potential to become over-catch status.The results of observations of the teeth in the upper and lower jaws are small and conical also head longer than body depth. Morphometric measurements (Table 1) of fish showed that the head was 23.5% and body depth was 24.3% of total length. Pre-dorsal length, pre-pelvic length, and pre-anal length was 30.1%, 28.0%, and 53.6% of total length. The eye diameter was 29.9% of head length. The ratio of fork length to body depth is 4:1 and head length to greatest depth is 1: 1.

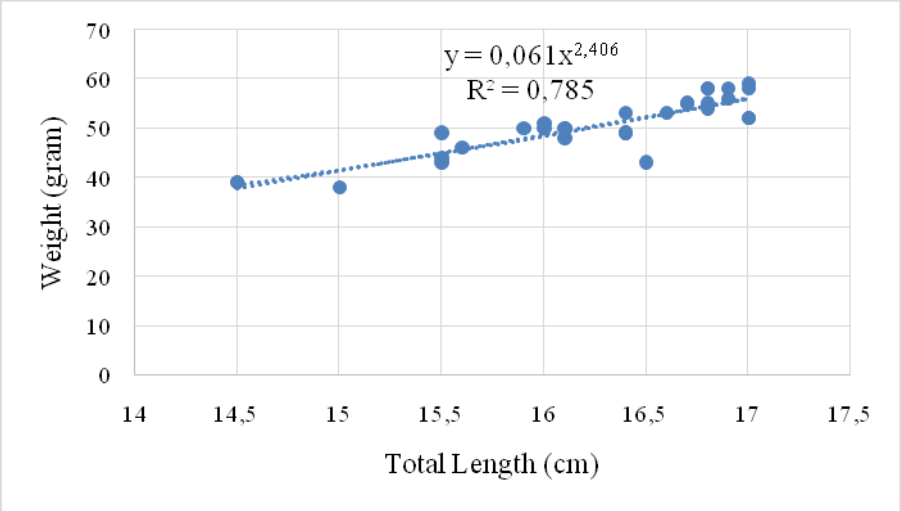

Figure 2. Length-weight relationships of fish sample *Rastrelliger* sp.

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Weight of fish samples ranged from 38-59 gram, with an average of 50.46±5.95 gram. The simulation of the relationship of length-weight shows that there is a negative allometric growth with value of b = 2.4069 with correlation R^2 = 0.7851 (Figure 2). The value of R^2 shows that there is a correlation between length and body weight of fish.

Electrophoresis results (Table 2) showed that the samples match the primers at 600 bp. Sequencing results were analysed and validated using BLAST via the USA National Center for Biotechnology Information (NCBI) website. Validation is used to determine the suitability of homologous gene nucleotide sequences from the sample. Based on the COI gene DNA barcode analysis, the highest level of similarity was found in Kanagurta's Rastrelliger species with the same Max score and total score of 1208, 100% query cover, E-value 0.0 and Ident 99.25%. The most similar gene bank sequences characterized by the same Max Score and Total Score, Query Coverage is close to 100%, E-value is close to 0, and Ident is close to 100% in each database. It was identified that the sample fish were *Rastrelliger kanagurta* or locally referred to as 'Kembung Lelaki' Fish. DNA Barcoding solves problems in identifying fish species.

Based on a molecular approach, DNA Barcoding is a method in molecular taxonomy using short DNA sequences to identify a species. Generally, the target DNA code for higher animals is the sequence in the mitochondrial cytochrome oxidase subunit 1 otherwise known as CO1(Madduppa et al., 2017). This COI sequence is often used for fish identification and has been proven to have been able to identify ten species of the genus Rastrelliger obtained from several regions in Indonesia (Indaryanto et al., 2015; Zamroni et al., 2017). Meanwhile, species that are morphologically similar to *Rastrelliger brachysoma* and *Rastrelliger neglectus* species have an identification similarity percentage of approximately 85% with fish samples.

Table 2.Results of nucleotide base BLAST on GenBank

Description	Max Score	Total Score	Query Score	E-Value	Percentage Identificatio	Accession
Rastrelliger kanagurta mitochondrial DNA com sequence,specimen_vouc NSMT:P:76071	1208	1208	100%	0.0	99.25%	AP012948.1
Rastrelliger kanagurta mithochondrion, complete genome	1208	1208	100%	0.0	99.25%	JX524134.1

Fish samples taken at Muara Angke landing fish have not yet reached maximum length. Based on fish fork length, adult *Rastrelliger kanagurta* has an average fork length between 20-25 cm (Collette and Nauen, 1983). Hariati et al. (2205) found that the *Rastrelliger kanagurta* in the Straits of Malacca reached gonad maturity for the first time at a 17 cm of fork length, but the sample found in Muara Angke is only 14.34 ± 0.43 cm. This shows that morphometric identification is not enough. The DNA code can distinguish between all fish species and identify the eggs, larvae, and carcass pieces of this species. So that fish in fish landings can be identified and tagged according to their species. This is crucial because, as important food fish, *R. kanagurta* is related to food

safety concerns, including uncorrected food labeling (Faisal et al., 2012), food substitution, or recent food contamination.

IV. Conclusion

The morphometry results showed that the fish samples from Muara Baru were identifiead as genus *Rastrelliger*. DNA Barcoding analysis shows that the species is *Rastrelliger kanagurta* with 99.25% identification similarity. This study successfully identified a sample from Muara Angke by using a DNA code and therefore would be beneficial for managing food controlling and food safety concerns.

Acknowledgements

We want to thank the Biodiversity and Marine Biosystematics Laboratory (BIODIVS-IPB) and the team that helped provide the facilities. Other thanks to Ummu Qonitah (Oniqin) for her contribution to redrawing fish images.

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