

## Research Article

# Molecular Identification and Phylogenetic Tree Reconstruction of Marine Fish Species from the Fishing Port of Kutaradja, Banda Aceh

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### ABSTRACT

The enormous potential of marine resources possessed by Banda Aceh Province is expected to be utilised optimally. Accuracy in marine fish resource identification is a critical requirement to support their utilisation and preservation in Banda Aceh Province. In this study, a molecular identification approach was carried out in addition to conducting a morphological identification, commonly used by several scientists. The results were 47 COI sequences generated representing 33 genera, 19 families, and five orders. From the resulting COI partial sequences, there is one potential haplotype from the Scombridae family (*Auxis thazard*), two potential haplotypes from the Carangidae family (*Elagatis bipinnulata* and *Decapterus macarellus*), and two potential haplotypes from the Serranidae family (*Variola albimarginata* and *Cephalopholis sonnerati*). This study is essential for fisheries biological studies and other fisheries studies to support the sustainable utilisation of marine fisheries potential in Banda Aceh.

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### INTRODUCTION

Aceh is the westernmost province of the Indo-Malaya Archipelago (IMA), an area known as a hot spot of tropical marine biodiversity (Briggs 2005; Hoeksema 2007; Bellwood & Meyer 2009; Veron et al. 2009; Gaither et al. 2011). This province has a high fisheries potential, with a water area reaching 295,370 km<sup>2</sup> and a coastline length of 2,666.3 km (Fikri 2013). One of the centres of fishing activity and the most significant fish landing site in Aceh is the Fishing Port of Kutaradja. Marine fisheries production at this fishing port increased from 8,922 tons in 2013 to 12,305 tons in 2017 (Yusuf 2003; Yeni & Naufal 2017; Mardhatillah et al. 2019) The fish landing site suffered massive damage due to the tsunami that struck Aceh Province and rebuilt in 2004 (Zulmaidah et al. 2015). The rebuilding of the Kutaradja fishing port has revived the economy and fisheries activities in the Banda Aceh region.

Regarding the fishing grounds for fishers at this fishing port, all the fishing zones include the Indian Ocean, Andaman Sea, and Malacca Strait. Two of the three fishing zones are included within two out of the 11 Indone-

sian Fisheries Management Areas (FMA), namely FMA 571 and 572. Since 2009, Indonesia has determined the management of territorial waters into several areas according to Law no. 31 of 2004 in conjunction with Law No. 41 of 2009 (Suman et al. 2017), which called Indonesian Fisheries Management Areas (FMA). The management area in western Sumatra includes FMA 571 in the Malacca Strait (Damanik et al. 2016) and FMA 572 in the Indian Ocean waters west of Sumatra. In the framework of fisheries management policies in Indonesia, the 11 FMAs stretch from the Malacca Strait in the west of Indonesia to the Arafura Sea in the east of Indonesia (Damanik et al. 2016). The level of utilization of pelagic and demersal fish resources in the two FMAs is categorized in the overexploited category (Suman et al. 2017; Salmarika & Wisudo 2019).

Previous research on the types of fish landed by the many traditional fishers of the Kutaradja Fishing Port were conducted based on fish morphology and anatomy. From the inventory carried out at the Kutaradja Fishing Port, 11 species were identified (Munawwarah et al. 2016). However, another report on the types of marine fish species in Simeuleu Island identified around 77 marine fish species which are members of 54 genera, 26 families, and seven orders (Batubara et al. 2017). The reef-associated fish inventory at Ulee Lheue, Banda Aceh, also mentioned that there were 87 species of reef fishes from 28 families in this location (Fadli et al. 2019). In different areas (i.e. Lhoknga and Lhok Mata Ie Beaches) 25 fish species which are members of eight orders, 11 families, and 19 genera were recorded from 51 fish samples (Nur et al. 2019); 71 species were identified in Pusong Bay, Lhokseumawe belonging to 54 genus, 37 families and 15 orders (Damora et al. 2020); 50 species were identified in the Weh Island, Sabang belonging to 24 families and eight orders (Zulfahmi et al. 2022). The morphological approach is the most widely used method in many regions in Indonesia, including in Banda Aceh.

This research identified marine fish at the molecular level in the Cytochrome C Oxidase subunit I (COI) region of the mitochondrial gene to complete the morphological identification that was also carried out. This COI Region is the region that some gene markers have agreed on globally for molecular identification. Research on barcoding of several aquatic biota has been carried out such as for marine fish in Australia (Ward et al. 2005), marine fish in India (Lakra et al. 2011), marine fish in Turkey (Keskin & Atar 2013), marine fish in China (Wang et al. 2012; Zhang & Hanner 2012), and marine fish in Taiwan (Chang et al. 2017; Bingpeng et al. 2018). Whereas research on fish molecular identification in Aceh has been carried out on some species such as groupers (Kamal et al. 2019), and *Scomber* spp. (Edwarsyah et al. 2019). This research is the first study to carry out molecular identification on the marine fish landed at the Kutaradja fishing port.

The purpose of this research is to identify marine fish to species level by using a molecular approach. This approach has higher accuracy of identification until species level. In addition, the research aims to identify Aceh's

potential haplotype for the Scombridae, Serranidae, and Carangidae groups, which are pelagic fish resources with significant economic important. DNA Barcoding will strengthen genetic information availability and it can be used for other studies such as breeding, fishery management, as well as conservation (Afriyie et al. 2019). One of the studies which is essential is haplotype analysis. Haplotype analysis can only be conducted based on genetic information, especially the DNA sequences from the number of unique species in a particular region.

## **MATERIALS AND METHODS**

### **Sampling site**

A total of 47 fish samples were collected from the Kutaradja Fishing Port on 19 July 2019 (5°35'09"N -95°19'06"E) (Nasution et al. 2019). Morphological identification and species confirmation have been carried out together with the molecular identification carried out in this study. Morphological identification by guideline of FAO Species Identification Guide for Fishery Purposes (Carpenter and Niem 2001). No specific permit was required for this study, and a digital camera was used to take individual photographs. All samples collected from the fish market were already dead upon purchasing. All specimens have been deposited to the Fisheries Laboratory, Faculty of Fisheries and Marine, Universitas Airlangga. All specimens keep in ethanol 90% with samples code AC no 01-47.

### **DNA extraction and PCR condition**

Each specimen was collected based on the morphological characters and following collection were directly preserved in 90% ethanol for further experimental purposes. Genomic DNA were extracted using an Accuprep® Genomic DNA Extraction Kit (Bioneer) following the manufacturer's guidelines. Around 1 cm tissue was dissected from the anal fin and mixed with 6X lysis buffer, which was further homogenized using the TissueLyser II (Qiagen). Quantification of purified genomic DNA was performed using NanoDrop (Thermofisher Scientific D1000), aliquoted and stored at the -70°C for further analysis.

One set of universal fish primer targeting cytochrome c oxidase I (COI) region, BCL-BCH (Baldwin et al. 2009, Handy et al. 2011), was used to obtain the partial sequences of each gene. The PCR mixture (20µL) included 11.2 µL ultra-pure water, 1 µL primer forward and reverse (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 template. The PCR condition was carried out under the following setting: 95°C for 5 min in initial denaturation, followed by denaturation at 95°C for 30 s in 40 cycles, 50°C for 30 s in annealing, and 72°C for 45 s in extension step, and a final extension at 72°C for 5 min. The PCR products were purified with the AccuPrep®Gel purification kit (Bioneer, Korea). The experiment was conducted at Molecular Physiology Laboratory, Department of Marine Biology,

School of Fisheries Science, Pukyong National University, Busan Korea. All PCR products were sent to Macrogen (Seoul, Korea) for sequencing.

### Sequence alignment and data analyses

All sequences were aligned and submitted to GenBank (Table 1). All raw files after sequencing were trimmed and the sequences quality were checked using Chromash® (downloaded from <http://technelysium.com.au/wp/chromas/>) to read the ab1 file format. Then, the reverse sequence was aligned with Clustal-omega using online system through <https://www.ebi.ac.uk/Tools/msa/clustalo/>, but reverse complement ([https://www.bioinformatics.org/sms/rev\\_comp.html](https://www.bioinformatics.org/sms/rev_comp.html)) was also performed on reverse sequences to make them have the same direction with the forward sequences. The BLASTN which is provided on NCBI system was applied for sequences identification (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>). After all sequences have been identified (species name) using BLASTN, the phylogenetic tree was then constructed. The pairwise evolutionary distance among the families was determined by the Kimura 2-Parameter method. The Neighbour-joining (NJ) tree constructed, and 1000 bootstrap analysis was carried out by MEGA7 (Kumar et al. 2016). Besides, nucleotides composition and genetic distance were also generated by MEGA7, including sequences alignment and transition/transversion bias after phylogenetic trees reconstruction was conducted.

## RESULTS AND DISCUSSION

### Results

#### Species Identification

In this study, a pair of universal COI primers BCL-BCH succeeded in obtaining DNA target sequences of more than 600 bp (Baldwin et al. 2009, Handy et al. 2011), it is effectiveness and efficiency to be used as a standard for molecular identification at species level. This strengthens previous research which has also succeeded in using these primers in molecular identification down to the species level (Pringgenis & Susilowati 2016; Serdiati et al. 2020). Here, we report the identification of marine fish from the Lampulo fish market, Aceh which is one of the center for fisheries in the province. A total of 47 COI sequences were generated representing 37 species, 33 genera, 19 families, and five orders with % identity ranging between 99-100% when compared to the GenBank dataset on BLASTN online system. Common names, taxonomic designation, habitat, IUCN list, as well as the GenBank accession number for all specimens are listed in Table 1. The sequencing of the COI gene produced more than 600 nucleotide base pairs per taxon. The un-ambiguity and simplicity were observed among all the sequences and no stop codons, deletions, and insertions were observed in all the sequences. Here, we cluster them into two groups in phylogenetic reconstruction, namely “Perciformes” and “other order”.

Table 1. The marine fish species list was identified by COI region from Lampulo fish market, Banda Aceh, Indonesia

ID No. (AC).	Species Name	Family	GenBank Acc No.	Order	Common name	Habitat	IUCN list
1	<i>Myripristis berndti</i>	Holocentridae	MN257521	Beryciformes	Blotcheye soldierfish	Indo-Pacific and Eastern Pacific	LC
2	<i>Myripristis berndti</i>	Holocentridae	MN257522	Beryciformes	Blotcheye soldierfish	Indo-Pacific and Eastern Pacific	LC
3	<i>Sardinella jassieu</i>	Clupeidae	MN257539	Clupeiformes	Mauritian sardinella	Western Indian Ocean	DD
4	<i>Sardinella jassieu</i>	Clupeidae	MN257540	Clupeiformes	Mauritian sardinella	Western Indian Ocean	DD
5	<i>Stolephorus commersonii</i>	Engraulidae	MN257541	Clupeiformes	Commerson's anchovy	Indo-West Pacific	LC
6	<i>Stolephorus commersonii</i>	Engraulidae	MN257542	Clupeiformes	Commerson's anchovy	Indo-West Pacific	LC
7	<i>Thryssa baelama</i>	Engraulidae	MN257543	Clupeiformes	Baelama anchovy	Indo-Pacific	LC
8	<i>Thryssa baelama</i>	Engraulidae	MN257544	Clupeiformes	Baelama anchovy	Indo-Pacific	LC
9	<i>Scolopsis xenochroa</i>	Nemipteridae	MN257509	Perciformes	Oblique-barred monocle bream	Indo-West Pacific	NE
10	<i>Lutjanus bengalensis</i>	Lutjanidae	MN257511	Perciformes	Bengal snapper	Indo-West Pacific:	NE
11	<i>Upeneus sulphureus</i>	Mullidae	MN257512	Perciformes	Sulphur goatfish	Indo-West Pacific	LC
12	<i>Pristipomoides filamentosus</i>	Lutjanidae	MN257513	Perciformes	Crimson jobfish	Indo-Pacific	LC
13	<i>Parascoropsis eriomma</i>	Nemipteridae	MN257514	Perciformes	Rosy dwarf monocle bream	Indo-West Pacific	NE
14	<i>Epinephelus areolatus</i>	Serranidae	MN257515	Perciformes	Arcolate grouper	Indo-Pacific	LC
15	<i>Variola albimarginata</i>	Serranidae	MN257516	Perciformes	White-edged lyretail	Indo-Pacific	LC
16	<i>Cephalopholis sonnerati</i>	Serranidae	MN257517	Perciformes	Tomato hind	Indo-Pacific	LC
17	<i>Parastromateus niger</i>	Carangidae	MN257518	Perciformes	Black pomfret	Indo-West Pacific	LC
18	<i>Paripeneus macronemus</i>	Mullidae	MN257519	Perciformes	Long-barbel goatfish	Indo-West Pacific	LC
19	<i>Paripeneus macronemus</i>	Mullidae	MN257520	Perciformes	Long-barbel goatfish	Indo-West Pacific	LC
20	<i>Priacanthus tayenus</i>	Priacanthidae	MN257523	Perciformes	Purple-spotted bigeye	Indo-Pacific	LC
21	<i>Lebrinus rubrioperculatus</i>	Lethrinidae	MN257524	Perciformes	Spotcheek emperor	Indo-Pacific	LC
22	<i>Megalaspis cordyla</i>	Carangidae	MN257528	Perciformes	Torpedo scad	Indo-West Pacific	LC
23	<i>Pomadasyx argyreus</i>	Haemulidae	MN257529	Perciformes	Bluecheek silver grunt	Indo-West Pacific	NE
24	<i>Terapon jarbua</i>	Terapontidae	MN257530	Perciformes	Jarbua terapon	Indo-Pacific	LC
25	<i>Equulites leuciscus</i>	Leiognathidae	MN257531	Perciformes	Whipfin ponyfish	Indo-West Pacific	LC
26	<i>Gaëza minuta</i>	Leiognathidae	MN257532	Perciformes	Toothpony	Indo-Pacific	LC
27	<i>Leiognathus striatus</i>	Leiognathidae	MN257533	Perciformes	Toothpony	Western Indian Ocean	NE
28	<i>Photopectoralis bindus</i>	Leiognathidae	MN257534	Perciformes	Orangefin ponyfish	Indo-West Pacific	NE
29	<i>Gerres filamentosus</i>	Gerreidae	MN257535	Perciformes	Whipfin silver-biddy	Indo-Pacific	LC
30	<i>Equulites leuciscus</i>	Leiognathidae	MN257536	Perciformes	Whipfin ponyfish	Indo-West Pacific	LC
31	<i>Gaëza minuta</i>	Leiognathidae	MN257537	Perciformes	Toothpony	Indo-Pacific	LC
32	<i>Megalaspis cordyla</i>	Carangidae	MN257538	Perciformes	Torpedo scad	Indo-West Pacific	LC
33	<i>Lutjanus lutjanus</i>	Lutjanidae	MN257545	Perciformes	Bigeye snapper	Indo-West Pacific	LC
34	<i>Caranx sexfasciatus</i>	Carangidae	MN257546	Perciformes	Bigeye trevally	Indo-Pacific	LC
35	<i>Siganus sutor</i>	Siganidae	MN257547	Perciformes	Shoemaker spinefoot	Indian Ocean	LC
36	<i>Siganus sutor</i>	Siganidae	MN257548	Perciformes	Shoemaker spinefoot	Indian Ocean	LC

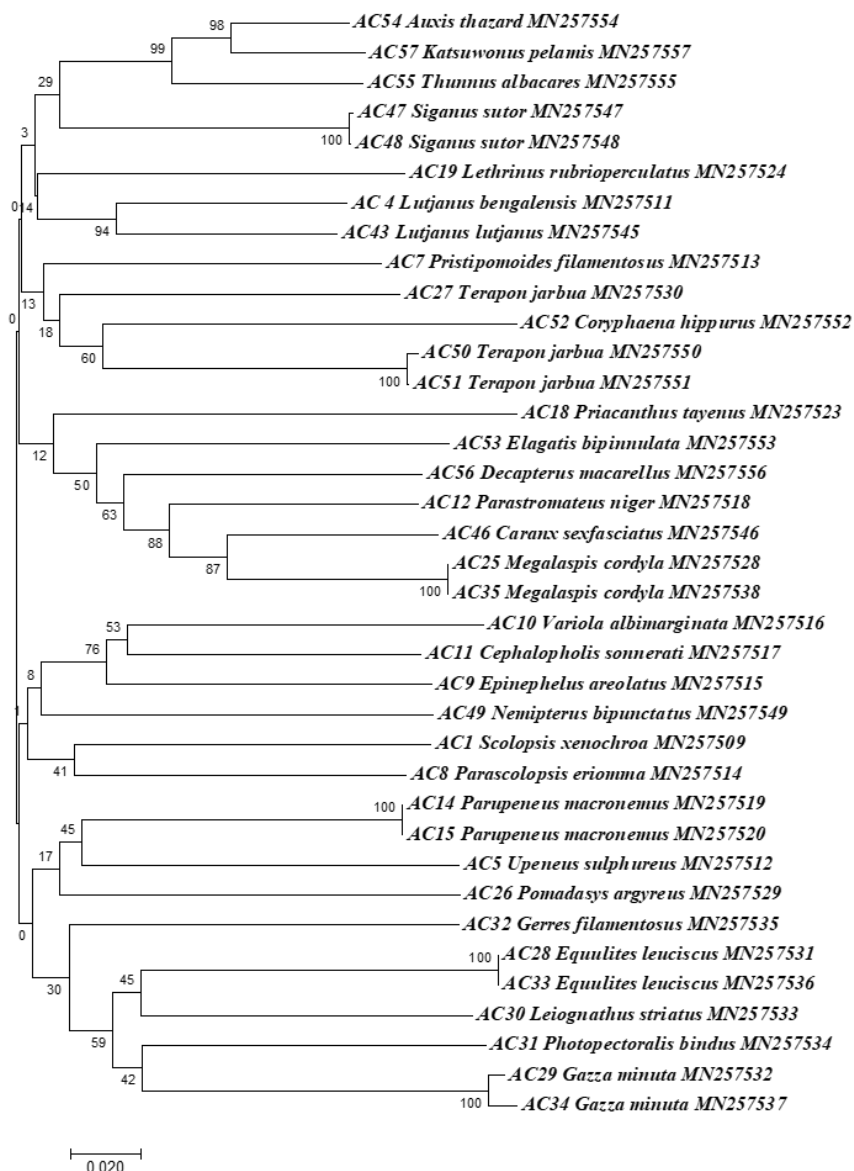
Table 1. Contd.

No.	ID (AC).	Species Name	Family	GenBank Acc No.	Order	Common name	Habitat	IUCN list
37	49	<i>Nemipterus bipunctatus</i>	Nemipteridae	MN257549	Perciformes	Delagoa threadfin bream	Indian Ocean	NE
38	50	<i>Terapon jarbua</i>	Terapontidae	MN257550	Perciformes	Jarbua terapon	Indo-Pacific	LC
39	51	<i>Terapon jarbua</i>	Terapontidae	MN257551	Perciformes	Jarbua terapon	Indo-Pacific	LC
40	52	<i>Coryphaena hippurus</i>	Coryphaenidae	MN257552	Perciformes	Common dolphinfish	Atlantic, Indian and Pacific	LC
41	53	<i>Auxis thazard</i>	Scombridae	MN257553	Perciformes	Frigate tuna	Atlantic, Indian and Pacific (Western Central)	LC
42	54	<i>Auxis thazard</i>	Scombridae	MN257554	Perciformes	Frigate tuna	Atlantic, Indian and Pacific (Western Central)	LC
43	55	<i>Thunnus albacares</i>	Scombridae	MN257555	Perciformes	Yellowfin tuna	Worldwide in tropical and subtropical seas	NT
44	56	<i>Decapterus macarellus</i>	Carangidae	MN257556	Perciformes	Mackerel scad	Circumglobal	LC
45	57	<i>Katsunonus pelamis</i>	Scombridae	MN257557	Perciformes	Skipjack tuna	Cosmopolitan in tropical and warm-temperate waters	LC
46	24	<i>Psettodes erumei</i>	Psettridae	MN257527	Pleuronectiformes	Indian halibut	Indo-West Pacific	NE
47	20	<i>Platycephalus sp.</i>	Platycephalidae	MN257525	Scorpaeniformes	Bartail flathead	Indo-West Pacific	DD

Least Concern (LC); Not Evaluated (NE); Data deficient (DD); Near Threatened (NT)

### Perciformes

From the total of 37 samples, we successfully identified 31 species from 14 families under Perciformes (30 genera). The nucleotide frequencies of COI sequences are 29.65% (T/U), 23.95% (A), 28.80% (C), and 17.6% (G). The average of transitional pair (si=5.07) was lower than the average of transver-tional pair (sv=14.86) with an overall transition/transversion ratio bias of 1.57. The phylogenetic tree was constructed from the COI sequences for the Perciformes and shows that the average K2P distance within taxonomic lev-els measured for COI sequences is 0.226 (Figure 1).



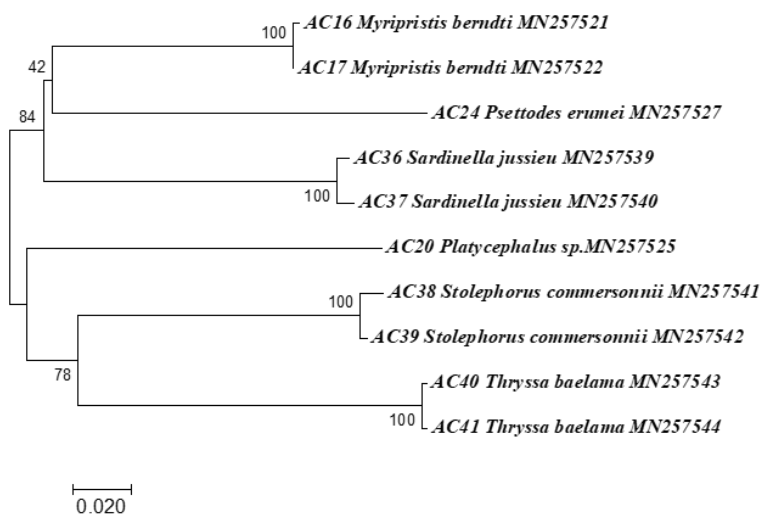
**Figure 1.** Phylogenetic tree of several Perciformes order by Neighbor-Joining tree algorithm using Mega7

### Clupeiformes and Others

In addition to Perciformes, Clupeiformes (3 genera) were also identified from 6 samples which were distributed in three species and three families. For the rest of the samples, one species was from Scorpaeniformes (*Platycephalus* sp.), one species from Pleuronectiformes (*Psettodes erumei*), and one species from



Beryciformes (*Myripristis berndti*). The nucleotide frequencies of the COI sequences were 28.17% (T/U), 23.04% (A), 30.11% (C), and 18.68% (G). The average of transitional pair (si=1.43) was lower than the average of transversional pair (sv=22.13) with an average transition/transversion bias of 8.71. The phylogenetic tree was constructed using the COI sequences for the small number order, including the Clupeiformes, Beryciformes, Pleuronectiformes, and Scorpaeniformes (Figure 2). The average K2P distance within taxonomic levels measured for COI sequences is 0.214.



**Figure 2.** Phylogenetic tree of Clupeiformes including Beryciformes, Pleuronectiformes, and Scorpaeniformes by Neighbor-Joining tree algorithm using MEGA7.

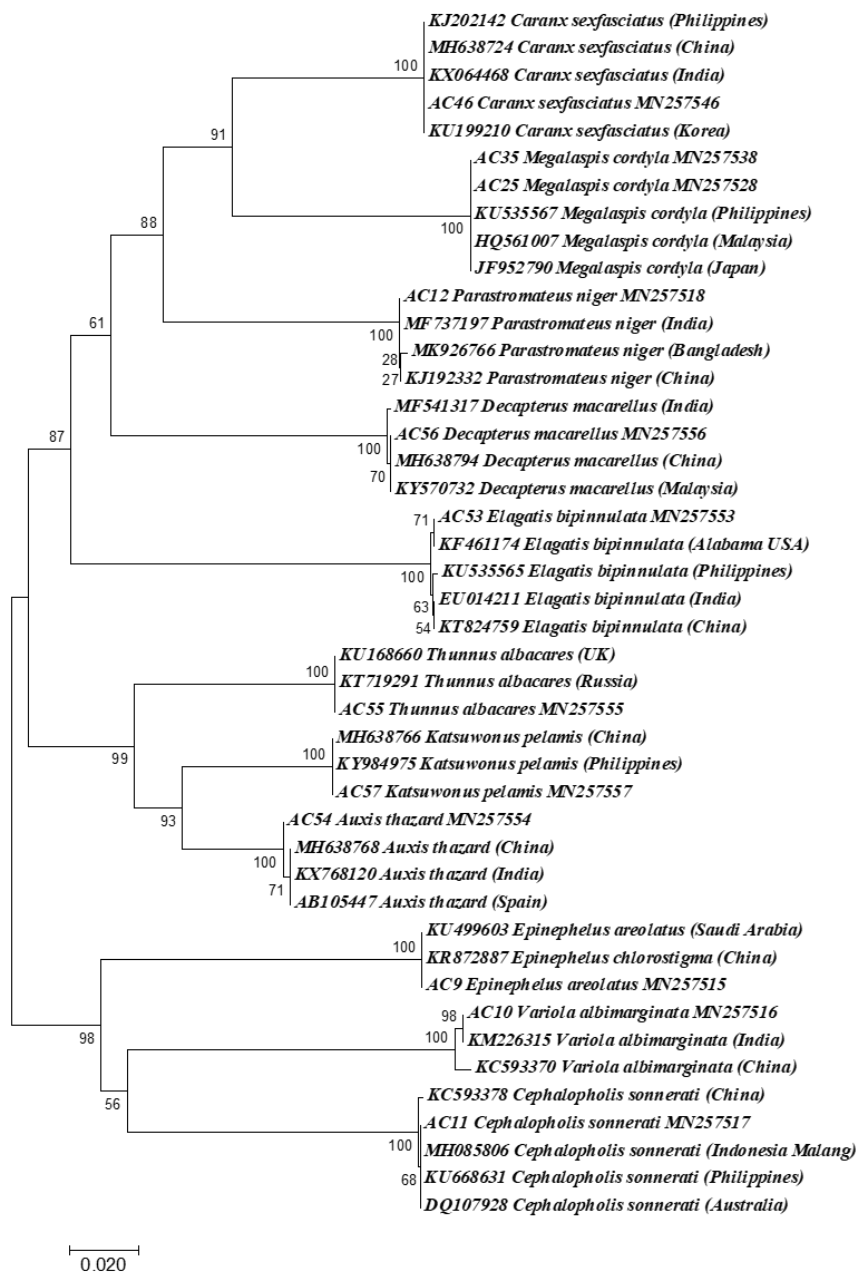
### The haplotype of Scombridae, Serranidae, and Carangidae from Aceh

In this study, the sample from Aceh had several unique potential haplotypes when compared to the same species from the GenBank database. By aligning the sequence generated with the reference sequence, some different nucleotides produced genetic variations (Table 2). The phylogenetics tree reconstruction of those sequences show that several potential haplotypes were found in this study (Figure 3). The identified haplotype in the Carangid group was found in the *Decapterus macarellus* species (MN257556) which had similarities with sequences from China and Malaysia, having a genetic distance with an Indian sequence of 0.002. Also, *Elagatis bipinnulata* (MN257553) is closer to the similarity of the sequence owned by the same type of fish (KF461174) from Alabama, USA. While the genetic distance of *Elagatis bipinnulata* with the same species is 0.003 (Philippines) and 0.02 (India and China). In the Carangid group, *Caranx sexfasciatus* (MN257546) and *Megalaspis cordyla* (MN257528 and MN257538) species were not found to be polymorphic in the sequences obtained.

In the Scombridae family group, potential haplotypes were found in *Auxis thazard* fish (MN257554) which differed from Chinese, Indian, and Spanish haplotypes with a genetic distance of 0.002. While in the Serranidae family, haplotypes were found in *Variola albimarginata* fish (MN257516) and *Cephalopholis sonnerati* (MN257517). This *Variola albimarginata* species



(MN257516) has similarities with sequences from India but is different from Chinese haplotypes with a genetic distance of 0.007. While species of *Cephalopholis sonnerati* (MN257517) differ only from Chinese haplotypes, this species merged in one clade with samples from the Philippines, Australia, and Indonesia with genetic distance 0.00-0.002. In *Epinephelus arelatus* species, there are no potential haplotypes and sequences obtained from samples originating from China and Saudi Arabia.



**Figure 3.** Phylogenetic tree reconstruction of three families (Carangidae, Scombridae, and Serranidae) by Neighbor-Joining algorithm using MEGA7. All sequence on this figure have code with AC, then another sequence has been downloaded from GenBank databased as the reference.

### Discussion

Research on molecular identification is now extensive in the field of fisheries and marine sciences. In this study, molecular identification is used to com-

**Table 2.** Alignment result of several marine fish species from Aceh showing nucleotides different from the references (GenBank database) based on Clustal Omega online system.

No.	Species name	GenBank Acc Number	Origin	Sequence number							
				123	171	213	249	258	328	408	471
1	<i>Elagatis bipinnulata</i>	MN257553	Aceh 53	-	-	A	-	-	T	-	-
		KU535565	Philippines	-	-	G	-	-	C	-	-
		KF461174	USA	-	-	A	-	-	T	-	-
		EU014211	India	-	-	A	-	-	C	-	-
		KT824759	China	-	-	A	-	-	C	-	-
2	<i>Decapterus macarellus</i>	MN257556	Aceh 6	-	C	-	-	-	-	-	-
		MH638794	China	-	C	-	-	-	-	-	-
		KY570732	Malaysia	-	C	-	-	-	-	-	-
		MF541317	India	-	T	-	-	-	-	-	-
3	<i>Auxis thazard</i>	MN257554	Aceh 54	-	-	-	-	-	-	-	-
		MH638768	China	-	-	-	-	-	-	-	-
		KX768120	India	-	-	-	-	-	-	-	-
		AB105447	Spain	-	-	-	-	-	-	-	-
4	<i>Variola albimarginata</i>	MN257516	Aceh 10	C	-	-	G	-	-	G	C
		KM226315	India	C	-	-	G	-	-	G	C
		KC593370	China	T	-	-	A	-	-	A	T
5	<i>Cephalopholis sonnerati</i>	MN257517	Aceh 11	-	-	-	-	A	-	-	-
		MH085806	Indonesia	-	-	-	-	A	-	-	-
		KU668631	Philippines	-	-	-	-	A	-	-	-
		DQ107928	Australia	-	-	-	-	A	-	-	-
		KC593378	China	-	-	-	-	G	-	-	-

plete the morphological identification and, at the same time, determine the position of the species identified in the phylogenetic tree created. Conventional identification that has been done at this time still faces obstacles with the difficulty of getting taxonomists in the process of determining species, in addition to the long time period required for the identification process, errors in identification also still occur in some cases. By doing a combination identification approach, the results are expected to be more valid in identifying the fish species obtained.

In this study, several marine fish that were landed at the Kutaradja Fishing Port are part of the essential fishery commodity in Banda Aceh. After the 2004 tsunami disaster in this province, several activities that are able to mobilize economic activities continue to be carried out, including capture fisheries activities in the Kutaradja Fishing Port (Zulmaidah et al. 2015). Previous studies have also reported the identification of marine fish species from Kutaradja Fishing Port at Lampulo. There are still inaccurate information regarding marine fish identification in some reports. Some identifications were also only done based on morphological-based characteristics and were not done by taxonomists, the results of which may be incorrect for species justification. In an earlier report, the species *Sardinella sirin* (Serranidae) was reported to exist in the Kutaradja Fishing Port (Munawwarah et al. 2016). Still, an inaccurate determination of taxonomy made the identification results unreliable. The genus *Sardinella* spp. is a group of fish in the family Clupeidae, order Clupeiformes (www.fishbase.org), and is not included in Serranidae.

In this report, the family Perciformes is identified as a group that dominates the fish composition caught by fishermen in Banda Aceh, who landed their catch at the Kutaradja Fishing Port. These are fish used for human consumption that are essential export commodities with high economic value

such as skipjack tuna (57%) followed by yellowfin tuna (23%) (Lubis et al. 2016). Based on the identification results, the Scombridae family is a group of pelagic fish that is quite commonly found. The types identified in this report include *Thunnus albacares*, *Auxis thazard*, and *Katsuwonus pelamis*. In addition, three species from the genus Lutjanidae (snapper) were also found, namely *Lutjanus bengalensis*, *Lutjanus lutjanus*, and *Lethrinus rubrioperculatus*. Other groups that are targeted by fishermen are reef fish that have significant economic value, such as groupers and carangids. The groupers identified in this study include *Epinephelus areolatus*, *Variola albimarginata*, and *Cephalopholis sonnerati*, whereas the carangids group includes *Parastrumateus niger*, *Megalaspis cordyla*, *Caranx sexfasciatus*, and *Decapterus macarellus* (Table 1).

In another group from the Clupeiformes order, two families were found in Lampulo fish market, namely Clupeidae (*Sardinella jussieu*) and Engraulidae (*Stolephorus commersonnii* and *Thryssa baelama*). In connection with the types of fish caught by fishermen, it is shown that captured fisheries in Banda Aceh use purse seine, which collects a group of pelagic fish in large quantities. Previous studies have explained that the fishermen in Banda Aceh mostly use purse seine (Wiryawan et al. 2016; Hariati 2017). The purse-seine is also a fishing gear generally used to catch *Thunnus tonggol*, *Euthynnus affinis*, *Auxis thazard*, and *Auxis rochei* (Salmarika & Wisudo 2019).

The small number of fish collected in this study are fish that are associated with coral reefs such as grouper fish groups that use coral reef areas as their nursery ground, feeding ground, and spawning ground. The diversity of reef fish around Banda Aceh experiences a natural gradient, which shows an increase in the area far from the mainland of the island of Sumatra. Variety in the region of small islands around Banda Aceh still shows good conditions when compared to the status of coral reefs on the shores of mainland Sumatra (Edrus et al. 2016). The species of *Epinephelus areolatus*, *Variola albimarginata*, and *Cephalopholis sonnerati* are groups of fish that utilize coral reefs as their habitat. However, several pelagic fish found around the shallow seas of Banda Aceh are still the primary target. The Indian mackerel *Rastrelliger kanagurta* (Hariati et al. 2015; Hariati & Fauzi 2017), yellowfin tuna *Thunnus albacares* (Neliyana et al. 2014), mackerel scad *Decapterus macrosoma*, and the anchovy *Stolephorus* spp. (Kurnia et al. 2016) were also obtained in this study.

In this report, sequences from several Acehnese fish also have similarities with those collected in some previous studies, and some are unique to other sequences. Species *Auxis thazard* that was identified from the Kutaradja Fishing Port at Lampulo, may have been caught from the area around the seas of Western Banda Aceh Province, indicating a catch distance of about 50-190 nautical miles (Salmarika & Wisudo 2019). Although it is still in the Indian Ocean region, there may be specialization in this species so that the Aceh haplotype separated from the same species in the resulting phylogenetic tree analysis.

In this study, a phylogenetic tree analysis of three prominent marine fish families, namely Scombridae, Serranidae, and Carangidae, was carried

out. The results of the investigation found that the Scombridae *Auxis thazard* (Aceh) became separated from the same clade species even though it only has a genetic distance of only 0.002. This haplotype appeared likely to occur due to differences compared to species populations analyzed from India, China (Xu et al. 2019), and Spain (Catanese et al. 2008). While for other haplotypes found from the reef fish *Variola albimarginata* and *Cephalopholis sonnerati*, the *Variola albimarginata* from Aceh might be from a population previously described from the results of a study conducted in India that allows the sharing of habitats in the Indian Ocean in the Western part of Sumatra Island. Previous studies on molecular identification of *Variola albimarginata* species have been carried out in the Andaman Islands and Nicobar Island (Basheer et al. 2017). This area is part of Indian sea territory, which may potentially have reef fish that are of almost the same as the species in Aceh. While *Cephalopholis sonnerati* fish species also have similarities with populations from Australia and the Philippines, however they are slightly different to populations from China (Zhuang et al. 2013). The study of *Cephalopholis sonnerati* shows the possibility of differences in the structure of coral fish populations in the South China Sea with the Indian Ocean, especially in Aceh waters. Although integrated with Indian Ocean waters, no similarity with Indian populations was found in the *Cephalopholis sonnerati* sample species, similarities were only found in previous studies conducted in the Philippines (Alcantara and Yambot 2016), and Australia (Ward et al. 2005). The speciation process that occurs in coral reef ecosystems occurs with an allopathic pattern that makes geographic isolation becomes the leading cause for the emergence of different species. However, the presence of pelagic larvae in reef fish species also becomes a big question even though it is believed that the allopatric pattern is the main speciation pattern occurring in coral reefs (Rocha and Bowen 2008).

Referring to the IUCN data, almost all marine fish in this study are included in the LC category (Table 1). In addition, there are also fish species that are categorized as Not Evaluated (NE), Data deficient (DD) and even Near Threatened (NT). This shows that studies on marine fish species in Indonesia need to be improved so that the conservation status of marine fish is in a well-monitored condition. The type of fish *Thunnus albacares* is getting a lot of attention because it is one of the world's important fishery commodities. Research on biological characteristics (Pecoraro et al. 2017; Mullins et al. 2018), migration (Wang et al. 2018) and various aspects has been carried out. Moreover, this fish also has a fairly high price in the world fish market (Krčmář et al. 2019; Primyastanto et al. 2021).

## CONCLUSION

From this study, the identification of marine fish landed at the Kutaradja fishing port in Aceh confirmed 47 specimens (33 genera) of marine fish. Almost all fish species were considered important as fishery commodities and became the main target of the Province of Banda Aceh's exports, including

the yellowfin tuna (*Thunnus albacares*) and the skipjack tuna (*Katsuwonus pelamis*). Beside Perciformes, Serranidae, Lethrinidae and Lutjanidae was identified as fisheries resources of Banda Aceh. More in-depth research on haplotype analysis using suitable application (bioinformatic software) is very much needed to maintain a record of the genetic biodiversity presence in the waters of Banda Aceh, Indonesia.

### AUTHORS CONTRIBUTION

SA. designed the research and supervised all the process including laboratory analysis and wrote the manuscript, AD. collected and analysed the data and wrote the draft manuscript, HWK. designed research and manuscript finalization

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### CONFLICT OF INTEREST

The authors state that they do not have any conflict of interest. The authors are solely responsible for the article's content and writing.

### REFERENCES

- Afriyie, G. et al., 2019. DNA barcoding of Ghanaian fish species: Status and prospects. *African Journal of Biotechnology*, 18, pp.659-663. doi: 10.5897/AJB2019.16792
- Alcantara, S.G. & Yambot, A.V., 2016. DNA barcoding of commercially important grouper species (Perciformes, Serranidae) in the Philippines. *Mitochondrial DNA Part A*, 27, pp.3837-3845. doi: 10.3109/19401736.2014.958672
- Baldwin, C.C. et al., 2009. Genetic identification and color descriptions of early life-history stages of Belizean Phaeoptyx and Astrapogon (Teleostei: Apogonidae) with comments on identification of adult Phaeoptyx. *Zootaxa*, 2008, pp.1-22. doi: 10.11646/zootaxa.2008.1.1
- Basheer, V. et al., 2017. Mitochondrial signatures for identification of grouper species from Indian waters. *Mitochondrial DNA Part A*, 28, pp.451-457. doi: 10.3109/19401736.2015.1137899
- Batubara, A.S. et al., 2017. Check list of marine fishes from Simeulue Island waters, Aceh Province, Indonesia. *Aceh Journal of Animal Science*, 2, pp.77-84. doi: 10.13170/ajas.2.2.9584.

- Bellwood, D.R. & Meyer, C.P. 2009. Searching for heat in a marine biodiversity hotspot. *Journal of Biogeography*, 36, 569–576. doi: 10.1111/j.1365-2699.2008.02029.x
- Bingpeng, X. et al., 2018. DNA barcoding for identification of fish species in the Taiwan Strait. *PLoS one*, 13, e0198109. doi: 10.1371/journal.pone.0198109
- Briggs, J.C., 2005. The marine East Indies : diversity and speciation. *Biogeography*, 32, pp.1517–1522. doi: 10.1111/j.1365-2699.2005.01266.x
- Carpenter, K.E. & Niem, V.H., 2001. FAO species identification guide for fishery purposes. The living marine resources of the Western Central Pacific. Volume 6. Bony fishes part 4 (Labridae to Latimeriidae), estuarine crocodiles, sea turtles, sea snakes and marine mammals. FAO Library.
- Catanese, G. et al., 2008. Complete mitochondrial DNA sequences of the frigate tuna *Auxis thazard* and the bullet tuna *Auxis rochei*: Full Length Research Paper. *DNA Sequence*, 19, pp.159-166. doi: [10.1080/10425170701207117](https://doi.org/10.1080/10425170701207117)
- Chang, C.H. et al., 2017. DNA barcodes of the native ray-finned fishes in Taiwan. *Molecular ecology resources*, 17, pp.796-805. doi: [10.1111/1755-0998.12601](https://doi.org/10.1111/1755-0998.12601)
- Damanik, M.R.S. et al., 2016. Kajian pendekatan ekosistem dalam pengelolaan perikanan di wilayah pengelolaan perikanan (WPP) 571 Selat Malaka Provinsi Sumatera Utara. *Jurnal Geografi*, 8, pp.165-176. doi:[10.24114/jg.v8i2.5780](https://doi.org/10.24114/jg.v8i2.5780)
- Damora, A. et al., 2020. Diversity of marine fish and their conservation status in Pusong Bay, Lhokseumawe City, Aceh Province, Indonesia. *European Journal of Environmental Sciences*, 10, pp.115-123. doi:[10.14712/23361964.2020.13](https://doi.org/10.14712/23361964.2020.13)
- Edrus, I.N. et al., 2016. Struktur komunitas ikan karang di perairan Pulau Raya, Pulau Rusa, Pulau Rondo dan taman laut Rinoi dan Rubiah, Nanggroe Aceh Darussalam. *Jurnal Penelitian Perikanan Indonesia*, 19, pp.175-186. Doi: 10.15578/jppi.19.4.2013.175-186
- Edwarsyah, E. et al., 2019. The DNA Barcoding of Several Commercial Fish from Simeulue Islands Coast of Aceh Using Cytochrome Oxidase Sub Unit I (COI) Gene Marker. *Budapest International Research in Exact Sciences (BirEx) Journal*, 1, pp.62-70. doi:[10.33258/birex.v1i1.140](https://doi.org/10.33258/birex.v1i1.140)
- Fadli, N. et al., 2019. The composition of coral reefs in Ulee Lheue breakwater, Banda Aceh, Aceh, Indonesia. *IOP Conference Series: Earth and Environmental Science*, 012077. doi:10.1088/1755-1315/348/1/012077
- Fikri, F., 2013. Pelaksanaan Perlindungan Terhadap Sumberdaya Perikanan dalam Laut Teritorial dan Zona Ekonomi Eksklusif Indonesia di Provinsi Aceh. *Kanun Jurnal Ilmu Hukum*, 15(3), 415-435. <http://www.jurnal.unsyiah.ac.id/kanun/article/view/6181>

- Gaither, M. et al., 2011. Phylogeography of the reef fish *Cephalopholis argus* (Epinephelidae) indicates Pleistocene isolation across the indo-pacific barrier with contemporary overlap in the coral triangle. *BMC Evolutionary Biology*, 11, 189. doi:10.1186/1471-2148-11-189
- Handy, S.M. et al., 2011. A single-laboratory validated method for the generation of DNA barcodes for the identification of fish for regulatory compliance. *Journal of AOAC International*, 94, pp.201-210. doi: 10.1093/jaoac/94.1.201
- Hariati, T. et al., 2015. Umur, Pertumbuhan Dan Laju Pemanfaatan Ikan Banyar (*Rastrelliger kanagurta* Cuvier, 1816), di Selat Malaka (Wilayah Pengelolaan Perikanan-571). *Jurnal Penelitian Perikanan Indonesia*, 21, pp.1-8. doi: 10.15578/jppi.21.1.2015.1-8
- Hariati, T., 2017. Status dan perkembangan perikanan pukat cincin di Banda Aceh. *Jurnal Penelitian Perikanan Indonesia*, 17, pp.157-167. doi: 10.15578/jppi.17.3.2011.157-167
- Hariati, T. & Fauzi, M., 2017. Aspek reproduksi ikan banyar, *Rastrelliger kanagurta* (Cuv. 1817) di perairan utara Aceh [Reproductive aspect of indian mackerel *Rastrelliger kanagurta* (Cuv. 1817) of northern Aceh waters]. *Jurnal Iktiologi Indonesia*, 11, pp.47-53. doi: 10.32491/jii.v11i1.155
- Hoeksema, B.W., 2007. *Delineation of the Indo-Malayan centre of maximum marine biodiversity: the Coral Triangle*. In Biogeography, time, and place: distributions, barriers, and islands (pp. 117-178). Springer, Dordrecht. doi: 10.1007/978-1-4020-6374-9\_5
- Kamal, M.M. et al., 2019. Autentikasi spesies ikan kerapu berdasarkan marka gen MT-COI dari perairan Peukan Bada, Aceh. *Jurnal Biologi Tropis*, 19, pp.116-123. doi: [10.29303/jbt.v19i2.1245](https://doi.org/10.29303/jbt.v19i2.1245)
- Keskİn, E. & Atar, H.H., 2013. DNA barcoding commercially important fish species of Turkey. *Molecular ecology resources*, 13, pp.788-797. doi: [10.1111/1755-0998.12120](https://doi.org/10.1111/1755-0998.12120)
- Krĉmář, P. et al., 2019. Identification of tuna species *Thunnus albacares* and *Katsuwonus pelamis* in canned products by real-time PCR method. *Acta Veterinaria Brno*, 88, pp.323-328. doi:10.2754/avb201988030323
- Kumar, S. et al., 2016. MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Molecular biology and evolution*, 33, pp.1870-1874. doi:[10.1093/molbev/msw054](https://doi.org/10.1093/molbev/msw054)
- Kurnia, K. et al., 2016. Pemetaan daerah penangkapan ikan pelagis kecil di Perairan Utara Aceh. *Jurnal Ilmiah Mahasiswa Kelautan Perikanan Unsyiah*, 1.
- Lakra, W. et al., 2011. DNA barcoding Indian marine fishes. *Molecular Ecology Resources*, 11, pp.60-71. [10.1111/j.1755-0998.2010.02894.x](https://doi.org/10.1111/j.1755-0998.2010.02894.x)
- Lubis, A.M. et al., 2016. The Contribution of the Lampulo Fishing Port, for Fishery Sector in Banda Aceh City, Nanggroe Aceh Darussalam Province. *Jurnal Online Mahasiswa Fakultas Perikanan dan Ilmu Kelautan Universitas Riau*, 3, pp.1-13.



- Mardhatillah, I. et al., 2019. Application of Schaefer model on mackerels fishery in Aceh waters, Indonesia. *IOP Conference Series: Earth and Environmental Science*, 012114. doi: 10.1088/1755-1315/348/1/012114
- Mullins, R.B. et al., 2018. Genomic analysis reveals multiple mismatches between biological and management units in yellowfin tuna (*Thunnus albacares*). *ICES Journal of Marine Science*, 75, pp.2145-2152. doi: [10.1093/icesjms/fsy102](https://doi.org/10.1093/icesjms/fsy102)
- Munawwarah, A. et al., 2016. Identifikasi Jenis-Jenis Ikan Yang Terdapat Di Tempat Pelelangan Ikan (TPI) Di Gampong Lampulo Kecamatan Kuta Alam Banda Aceh. *Serambi Saintia: Jurnal Sains dan Aplikasi*, 4. doi: 10.32672/jss.v4i1.115
- Nasution, M.H. et al., 2019. Forecasting The Amount of Tuna/Madidihang (Yellowfin tuna) Landed in PPS Kutaraja Banda Aceh City With The Triple Exponential Smoothing Method. *Samakia: Jurnal Ilmu Perikanan*, 10, pp.08-14. doi: 10.35316/jsapi.v10i1.231
- Neliyana, W.B. et al., 2014. Analisis Kelayakan Usaha Perikanan Pukat Cincin di Pelabuhan Perikanan Pantai (PPP) Lampulo Banda Aceh Propinsi Aceh. *Jurnal Marine Fisheries*, 5, pp.163-169.
- Nur, F.M. et al., 2019. Checklist of coral fishes in Lhoknga and Lhok Mata Ie Beaches, Aceh Besar, Indonesia. *IOP Conference Series: Earth and Environmental Science*, 012104. doi: 10.1088/1755-1315/348/1/012104
- Pecoraro, C. et al., 2017. Putting all the pieces together: integrating current knowledge of the biology, ecology, fisheries status, stock structure and management of yellowfin tuna (*Thunnus albacares*). *Reviews in Fish Biology and Fisheries*, 27, pp.811-841. doi: 10.1007/s11160-016-9460-z
- Primyastanto, M. et al., 2021. Sustainable operational analysis of the cultivation of Indonesian *Thunnus albacares* by bioeconomic approach. *Croatian Journal of Fisheries: Ribarstvo*, 79, pp.61-70. doi: 10.2478/cjf-2021-0007
- Pringgenis, D. & Susilowati, R., 2016. Highly commercial fisheries tawar fish: molecular analysis DNA mitochondrial COI gene sequence and proximate analysis from malacca Strait, Riau. *Jurnal Teknologi*, 78. doi: 10.11113/jt.v78.8149
- Rocha, L. & Bowen, B. 2008. Speciation in coral-reef fishes. *Journal of Fish Biology*, 72, pp.1101-1121. doi: 10.1111/j.1095-8649.2007.01770.x
- Salmarika, S. & Wisudo, S.H., 2019. Status Pengelolaan Sumber Daya Ikan Tongkol di Perairan Samudera Hindia Berbasis Pendaratan Pukat Cincin di Pelabuhan Perikanan Samudera Lampulo, Aceh: Suatu Pendekatan Ekosistem. *Jurnal Penelitian Perikanan Indonesia*, 24, pp.263-272.
- Serdiati, N. et al., 2020. Morphological variations and phylogenetic analysis of *Oryzias nigrimas* Kottelat, 1990 (rice fish) from Lake Poso, Central Sulawesi, Indonesia. *Biodiversitas Journal of Biological Diversity*, 21. doi: 10.13057/biodiv/d210305

- Suman, A. et al., 2017. Potensi dan tingkat pemanfaatan sumber daya ikan di wilayah pengelolaan perikanan Negara Republik Indonesia (WPP NRI) Tahun 2015 serta Opsi Pengelolaannya. *Jurnal Kebijakan Perikanan Indonesia*, 8, pp.97-100. doi: 10.15578/jkpi.8.2.2016.97-100
- Veron, J.E.N. et al., 2009. Delineating the Coral Triangle. *Galaxea. Journal of Cournal Reef Studies*, 11, pp.91-100. doi:10.3755/galaxea.11.91
- Wang, X. et al., 2018. Relationship between water migration and quality changes of yellowfin tuna (*Thunnus albacares*) during storage at 0 C and 4 C by LF-NMR. *Journal of aquatic food product technology*, 27, pp.35-47. doi: 10.1080/10498850.2017.1400630
- Wang, Z-D. et al., 2012. DNA barcoding South China Sea fishes. *Mitochondrial DNA*, 23, pp.405-410. doi: 10.3109/19401736.2012.710204
- Ward, R.D. et al., 2005. DNA barcoding Australia's fish species. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 360, pp.1847-1857. doi: 10.1098/rstb.2005.1716
- Wiryawan, B. et al., 2016. Analisis Kelayakan Usaha Perikanan Pukat Cincin Di Pelabuhan Perikanan Pantai (Ppp) Lampulo Banda Aceh Propinsi Aceh (Analysis Financial Fisheries of Purse Seine in Lampulo Fishing Port Banda Aceh Provinsi Aceh). *Marine Fisheries: Journal of Marine Fisheries Technology and Management*, 5, pp.163-169. doi: 10.29244/jmf.5.2.163-169
- Xu, L. et al., 2019. A molecular approach to the identification of marine fish of the Dongsha Islands (South China Sea). *Fisheries Research*, 213, pp.105-112. doi: 10.1016/j.fishres.2019.01.011
- Yeni, E. & Naufal, A., 2017. Identifikasi Aktivitas Pengembangan Fasilitas Pelabuhan Perikanan Pantai Lampulo Banda Aceh. *Prosiding SEMDI-UNAYA (Seminar Nasional Multi Disiplin Ilmu UNAYA)*, pp.355-363.
- Yusuf, Q., 2003. Empowerment of Panglima Laot in Aceh. *International workshop on Marine Science and Resource*, pp.11-13.
- Zhang, J. & Hanner, R., 2012. Molecular approach to the identification of fish in the South China Sea. *PLoS One*, 7, e30621. doi: 10.1371/journal.pone.0030621
- Zhuang, X. et al., 2013. A comprehensive description and evolutionary analysis of 22 grouper (Perciformes, Epinephelidae) mitochondrial genomes with emphasis on two novel genome organizations. *PLoS One*, 8, e73561. doi: 10.1371/journal.pone.0073561
- Zulfahmi, I. et al., 2022. Commercial marine fish species from Weh Island, Indonesia: Checklist, distribution pattern and conservation status. *Biodiversitas Journal of Biological Diversity*, 23. doi: 10.13057/biodiv/d230432
- Zulmaidah Z, Zain J, Hutauruk RM. 2015. Facilities Utilization in Lampulo Fisheries Port, District of Kuta Alam, Banda Aceh City, Province Nanggroe Aceh Darussalam. *Jurnal Online Mahasiswa*, 2, pp.1-13.