

**Bukti Korespondensi Jurnal Internasional Bereputasi terakreditasi  
Q4 (Agriculture and Biological Sciences)**

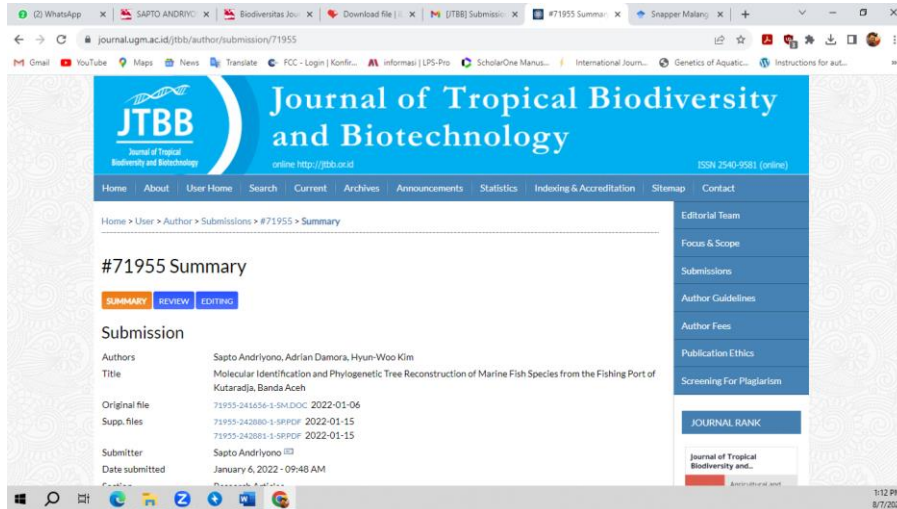
Judul : **Molecular identification and phylogenetic tree reconstruction of marine fish species from the Fishing Port of Kutaradja, Banda Aceh**

Jurnal : **Journal of Tropical Biodiversity and biotechnology**

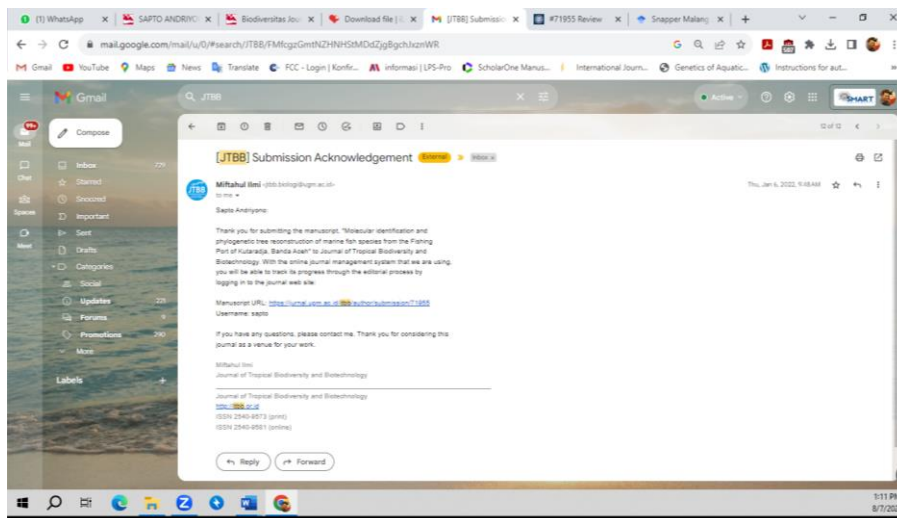
Penulis : **SAPTO ANDRIYONO, ADRIAN DAMORA, HYUN-WOO KIM**

No.	Perihal	Tanggal	Keterangan
1	Bukti Submit dan Artikel yang di submit	06 January 2022	Page 2, Lampiran 1
2	Bukti Review A	16 April 2022	Page 3, Lampiran 2
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4	Bukti Review C	16 April 2022	Page 3, Lampiran 4
5	Bukti Revisi 1	18 Mei 2022	Page 4, Lampiran 5
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7	Bukti Accepted	14 September 2022	Page 5
8	Link Artikel terpublikasi	26 Oktober 2022	Page 5, Lampiran 7

# 1. Bukti Submit dan Artikel yang disubmit 06 January 2022 disystem OJS dan Email Notifikasi



## Email Notifikasi



## 2. Review Roud 1. Reviewer A, B dan C, tanggal 16 April 2022

The screenshot shows the journal submission review page for #71955. The page is titled "#71955 Review" and has tabs for "SUMMARY", "REVIEW", and "EDITING". The "Submission" section lists the authors as Sapto Andriyono, Adrian Damora, and Hyun-Woo Kim. The title is "Molecular Identification and Phylogenetic Tree Reconstruction of Marine Fish Species from the Fishing Port of Kutaradja, Banda Aceh". The section is "Research Articles" and the editor is Furzani Pa'ee. The "Peer Review" section shows "Round 1" with a review version of 71955-241662-1-RVDOC 2022-01-06. It lists the dates for initiation (2022-01-15), last modification (2022-04-24), and the upload dates for Reviewer A (2022-02-14), Reviewer C (2022-01-19), and Reviewer B (2022-02-01). On the right side, there is a "Focus & Scope" menu, a "JOURNAL RANK" section showing a Q4 ranking and an SJR 2022 score of 0.19, and a "TEMPLATE" button.

## Email Notifikasi 3 Reviewer

The screenshot shows an email notification from the journal editor, Liya Audinah, to Sapto Andriyono. The email is titled "[JTBB] Editor Decision" and is dated Sat, Apr 16, 2022, 1:58 PM. The content of the email is as follows:

Dear Sapto Andriyono,

Thank you for submitting your work, titled "Molecular identification and phylogenetic tree reconstruction of marine fish species from the Fishing Port of Kutaradja, Banda Aceh", to Journal of Tropical Biodiversity and Biotechnology. After reviewing your submission, we will consider publishing your manuscript.

However, before we can proceed to publish the manuscript, we invite you to respond to the reviewers' comments and revise your manuscript carefully. Please highlight the changes you make by using the track changes mode in MS Word or by using bold or coloured text. We enclosed the reviewer comments for you to learn.

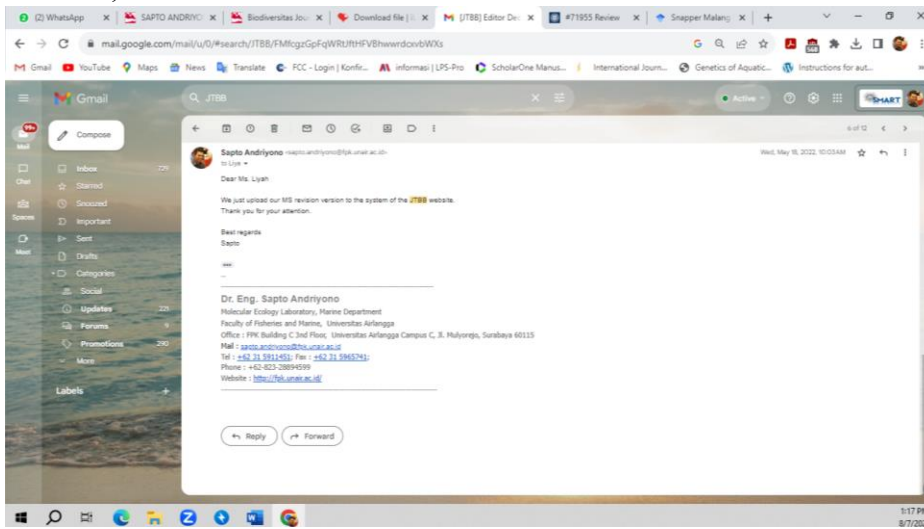
Please send us answers to the reviewers' comments in a separated file.

We expect to receive your revision within two (2) week. If you fail to turn your revision in within the designated time, we may have to decline your manuscript without notification.

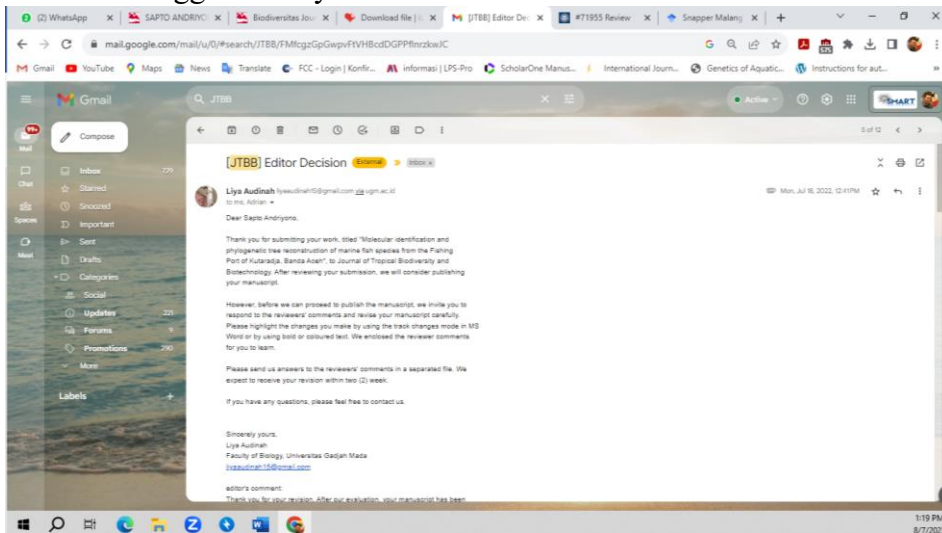
If you have any questions, please feel free to contact us.

Sincerely yours,  
Liya Audinah  
Faculty of Biology, Universitas Gadjah Mada  
liya.audinah15@gmail.com

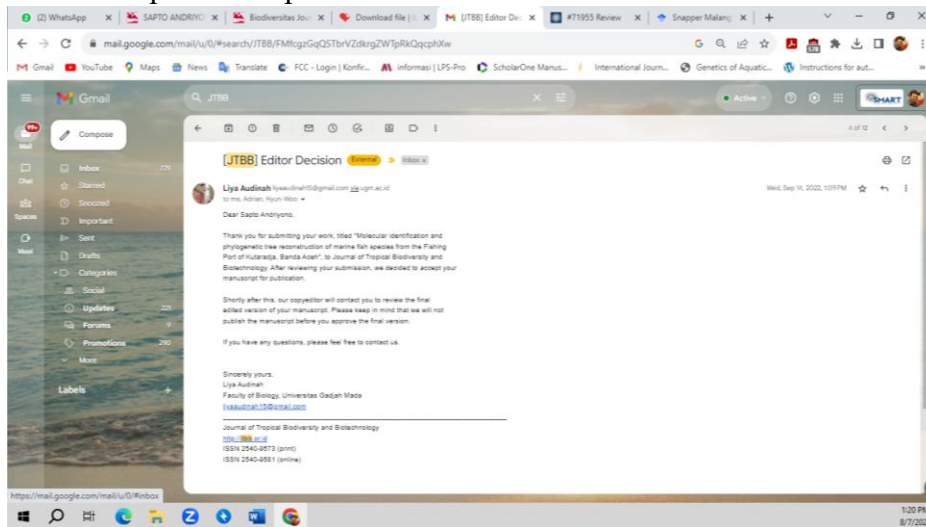
### 3. Revisi 1, 18 Mei 2022



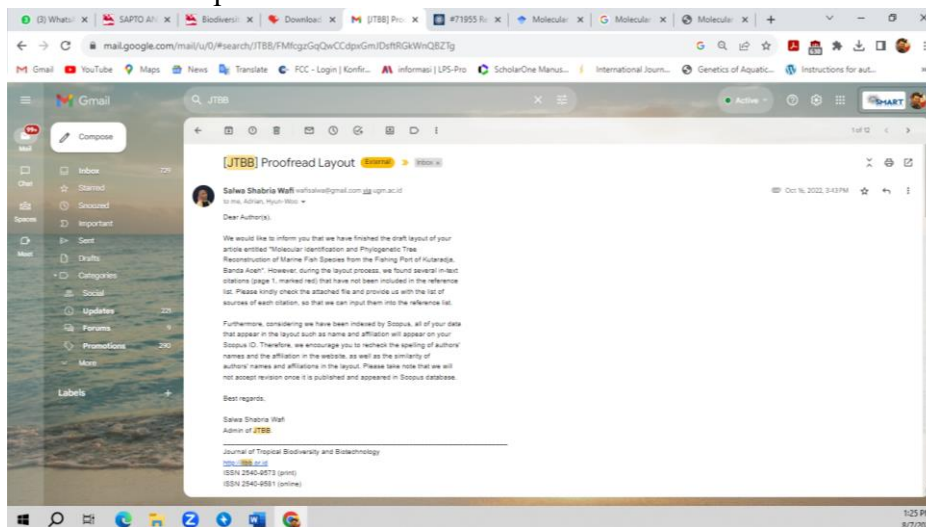
### 4. Revisi 2. Tanggal 18 July 2022



## 5. Bukti Accepted 14 September 2022



## 6. Link Artikel Terpublished 26 Oktober 2022



Your article link: <https://jurnal.ugm.ac.id/jtbb/article/view/71955>



26 landing in Aceh, is the Fishing Port of Kutaradja. Marine fisheries production at this fishing  
27 port increased from 8,922 tons in 2013 to 12,305 tons in 2017 (Yusuf 2003, Yeni and Naufal  
28 2017, Mardhatillah, Damora et al. 2019). The fish landing suffered massive damage due to  
29 the tsunami that struck Aceh Province and was rebuilt in 2004 (Zulmaidah, Zain et al. 2015).  
30 The rebuilding of the Kutaradja fishing port has revived the economy and fisheries activities  
31 in the Banda Aceh region.

32 Regarding the fishing grounds for fishers at this fishing port, all the fishing zone includes the  
33 Indian Ocean, Andaman Sea, and Malacca Strait. Two of the three regions are included in the  
34 Fisheries Management Area (FMA) 571 and 572. The level of utilization of pelagic and  
35 demersal fish resources in these two FMA is included in the overexploited category (Suman,  
36 Irianto et al. 2017, Salmarika and Wisudo 2019).

37 Previous research on the types of fish landed by many traditional fishers of the Kutaradja  
38 fishing port is still being done conventionally. From the inventory carried out at the Kutaradja  
39 fishing port, 11 species have been identified (Munawwarah, Sufi et al. 2016). However,  
40 another report on the types of marine fish species in Banda Aceh (Simeuleu Island) identified  
41 around 77 marine fish species included in 54 genus, 26 families, and seven orders (Batubara,  
42 Muchlisin et al. 2017). The reef-associated fishes inventory at Ulee Lheue, Banda Aceh, also  
43 mentioned that there were 87 species of reef fishes from 28 families in this location (Fadli,  
44 Muchlisin et al. 2019). In different areas, Lhoknga and Lhok Mata Ie Beaches, the eight  
45 orders, 11 families, 19 genera, and 25 species were recorded from 51 fish samples (Nur,  
46 Batubara et al. 2019). The morphological approach is the most widely used method in many  
47 regions in Indonesia, including in Banda Aceh. This research identifies molecular marine fish  
48 in the Cytochrome C Oxidase subunit I (COI) region of the mitochondrial gene to complete  
49 the morphological identification that was also carried out. This COI Region is the region that  
50 some gene markers have agreed on in molecular identification globally. Research on

51 barcoding in several aquatic biota has been carried out such as marine fish in Australia  
52 (Ward, Zemlak et al. 2005), marine fish in India (Lakra, Verma et al. 2011), marine fish in  
53 Turkey (Keskin and Atar 2013), marine fish in China (Wang, Guo et al. 2012, Zhang and  
54 Hanner 2012), and marine fish in Taiwan (Chang, Shao et al. 2017, Bingpeng, Heshan et al.  
55 2018). Whereas research on molecular identification of fish in Aceh has carried out on some  
56 species such as grouper fish (Kamal, Hakim et al. 2019), and *Scomber* spp (Edwarsyah, Nasir  
57 et al. 2019). This research on the identification of marine fish landed at the fishing port of  
58 Kutaradja is the first study to carry out molecular identification.

59 The purpose of this research is to identify species of marine fish to species level by using a  
60 molecular approach to minimize identification errors. Besides, the research carried out  
61 identification of Aceh's haplotype in the Scombridae, Serranidae, and Carangidae groups,  
62 which are pelagic fish resources that have significant economic value.

63

## 64 **2. Materials and methods**

### 65 **2.1 Sampling site**

66 A total of 47 fish samples were collected from the Lampulo traditional fish market  
67 close to the Lampulo fish landing on 19 July 2019. Morphologically, identification, and  
68 species confirmation have been carried out with molecular identification carried out in this  
69 study. No specific permit was required for this study, and a digital camera has taken the  
70 individual photograph. All samples have collected from the local traditional fish market were  
71 dead upon purchasing.

### 72 **2.2 DNA extraction and PCR**

73 Each specimen has been collected based on the morphological characters and after  
74 collection directly preserved in 90% ethanol for further experimental purposes. Genomic  
75 DNA extracted using an Accuprep® Genomic DNA Extraction Kit (Bioneer) according to



76 the manufacturer's guidelines. The anal fin, around 1 cm tissues, was dissected and mix with  
77 6X lysis buffer, which was further homogenized by the TissueLyser II (Qiagen).  
78 Quantification of purified genomic DNA performed by nanoDrop (Thermofisher Scientific  
79 D1000), aliquoted and stored at the -70°C for further analysis.

### 80 **2.3 PCR condition and Data Analysis**

81 One set universal fish primer targeting cytochrome c oxidase I (COI) region, BCL-BCH  
82 (Baldwin, Mounts et al. 2009, Handy, Deeds et al. 2011), used to obtain the partial sequences  
83 of each gene. The PCR mixture (20µL) included 11.2 µL ultra-pure water, 1 µL primer  
84 forward and reverse (0.5 µM), 0.2 µL Ex Taq DNA polymerase (TaKaRa, Japan), 2 µL 10X  
85 ExTag Buffer, 2 µL dNTPs (1 µM, TaKaRa, Japan), and 2 µL genomic DNA as template.  
86 The PCR condition carried out under the following setting: 95°C for 5 min in initial  
87 denaturation, followed by denaturation at 95°C for 30 s in 40 cycles, 50°C for 30 s in  
88 annealing, and 72°C for 45 s in extension step, and a final extension at 72°C for 5 min. The  
89 PCR products purified with the AccuPrep®Gel purification kit (Bioneer, Korea).

### 90 **2.4 Phylogenetic analysis**

91 All sequences were aligned and submitted to GenBank (Table 1). The pairwise evolutionary  
92 distance among the family determined by the Kimura 2-Parameter method. The Neighbor-  
93 joining (NJ) tree constructed, and 1000 bootstrap analysis was carried by Mega 7 (Kumar,  
94 Stecher et al. 2016).

95

## 96 **3. Results and Discussion**

### 97 **3.1 Species Identification**

98 A total of 47 COI sequences generated representing 33 genera, 19 families, and five  
99 orders. Common names, taxonomic designation, habitat, IUCN list, as well as the GenBank  
100 accession number for all specimens in Table 1. The sequencing of the COI gene produced

101 more than 600 nucleotide base pairs per taxon. The un-ambiguity and simplicity observed  
102 among all the sequences and no stop codons, deletions, and insertions observed in all the  
103 sequences. Here, we cluster into two groups are Perciformes and another order.

104

### 105 **3.2 Perciformes**

106 The nucleotide frequencies of COI sequences are 29.65% (T/U), 23.95% (A), 28.80% (C),  
107 and 17.6% (G). The average of transitional pair ( $si=5.07$ ) and was lower than the average of  
108 transversional pair ( $sv=14.86$ ) with an overall transition/transversion ratio bias is 1.57. The  
109 phylogenetic tree was constructed the COI sequences for the Perciformes and shown the  
110 average K2P distance within taxonomic levels measured for COI sequences is 0.226 (Figure  
111 1).

112

### 113 **3.3 Clupeiformes and Others**

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

120

### 121 **3.4 The haplotype of Scombridae, Serranidae, and Carangidae from Aceh**

122 In this study, the sample from Aceh had several unique haplotypes compared to the  
123 same species from the GenBank database. By aligning the sequence generated with the  
124 reference sequence, some different nucleotides produce genetic variations (Table 2). The  
125 phylogenetics reconstruction of those sequences shown that several haplotypes found in this

126 study (Figure 3). The identified haplotype in the Carangid group was found in the *Decapterus*  
127 *macarellus* species (MN257556) which had similarities with sequences from China and  
128 Malaysia, and had a genetic distance with an Indian sequence is 0.002. Also, *Elagatis*  
129 *bipinnulata* (MN257553) is closer to the similarity of the sequence owned by the same type  
130 of fish (KF461174) from Alabama, USA. While the genetic distance of *Elagatis bipinnulata*  
131 with the same species is 0.003 (Philippines) and 0.02 (India and China). In the Carangid  
132 group, *Caranx sexfasciatus* (MN257546) and *Megalaspis cordyla* (MN257528 and  
133 MN257538) species were not found polymorphic in the sequences obtained.

134 In the Scombridae family group, haplotypes found in *Auxis thazard* fish (MN257554)  
135 which differed from Chinese, Indian, and Spanish haplotypes with a genetic distance of  
136 0.002. While in the Serranidae family, haplotypes found in *Variola albimarginata* fish  
137 (MN257516) and *Cephalopholis sonnerati* (MN257517). This *Variola albimarginata* species  
138 (MN257516) has similarities with sequences from India but is different from Chinese  
139 haplotypes with a genetic distance of 0.007. While species of *Cephalopholis sonnerati*  
140 (MN257517) differ only from Chinese haplotypes, this species merged in one clade with  
141 samples of the Philippines, Australia, and Indonesia with genetic distance 0.00-0.002. In  
142 *Epinephelus arelatus* species, there are no haplotypes and sequences obtained from samples  
143 from China and Saudi Arabia.

144

## 145 **Discussion**

146 Research on molecular identification is now extensive in the field of fisheries and  
147 marine sciences. In this study, molecular identification used to completing the morphological  
148 identification and, at the same time, determine the position of the species identified in the  
149 phylogenetic tree created. Conventional identification that has been done at this time still  
150 finds obstacles with the difficulty of getting taxonomists in determining species, in addition

151 to the long enough time in the identification process, errors in identification also still occur in  
152 some cases. By doing a combination of identification, it is expected to be more valid in  
153 getting the results of fish species obtained.

154 In this study, several marine fish landed at the Kutaradja port became an essential  
155 fishery commodity in Banda Aceh. After the 2004 tsunami disaster in this province, several  
156 activities capable of mobilizing economic activities continue to be carried out, including  
157 capture fisheries activities in this Lampulo fish port (Zulmaidah, Zain et al. 2015). Previous  
158 studies have also reported the identification of marine fish species from Lampulo. There is  
159 still inaccurate information regarding marine fish identification in some reports. Besides, an  
160 identification that is only on morphological-based characteristics that are not done by  
161 taxonomists, then the results in identification may be incorrect on species justification. In an  
162 earlier report, the species *Sardinella sirin* (Serranidae) was reported to exist in this Lampulo  
163 port (Munawwarah, Sufi et al. 2016). Still, an inaccurate in determining taxonomy made the  
164 identification results unreliable. The genus *Sardinella* spp is a group of fish in the family  
165 Clupeidae, order Clupeiformes ([www.fishbase.org](http://www.fishbase.org)), not include in Serranidae.

166 In this report, family Perciformes are identified as a group that dominates the caught  
167 by fishermen in Banda Aceh, who landed at the Kutaradja fishing port. These fish are  
168 consumption fish that are essential export commodities with high economic value such as  
169 Skipjack tuna (57%) followed by yellowfin tuna (23%) (Lubis, Syaifuddin et al. 2016). The  
170 results of identification, the Scombridae family, is a group of pelagic fish that is quite  
171 commonly found. The types identified in this report include *Thunnus albacares*, *Auxis thazar*,  
172 and *Katsuwonus pelamis*. Besides, the genus Lutjanidae (snapper) found three species,  
173 namely *Lutjanus bengalensis*, *Lutjanus lutjanus*, and *Lethrinus rubrioperculatus*. Other  
174 groups that are targeted by fishermen are reef fish that have significant economic value, such  
175 as Grouper and Carangid. The groupers identified in this study include *Epinephelus*

176 *areolatus*, *Variola albimarginata*, and *Cephalopholis sonnerati*, whereas the Carangids group  
177 includes *Parastromateus niger*, *Megalaspis cordyla*, *Caranx sexfasciatus*, and *Decapterus*  
178 *macarellus* (Table 1).

179 In another group on the Clupeiformes order, two families found in the Lampulo, namely  
180 Clupeidae (*Sardinella jussieu*) and Engraulidae (*Stelephorus commersonii* and *Thryssa*  
181 *baelama*). In connection with the types of fish caught by fishermen, it is shown that capture  
182 fisheries in Banda Aceh use purse seine, which finds a group of pelagic fish in large  
183 quantities. Previous studies have explained that the fishermen in Banda Aceh mostly use  
184 purse seine (Wiryawan, Wiyono et al. 2016, Hariati 2017). The purse-seine is also generally  
185 fishing gear to catch fish of *Euthynnus affinis*, *Auxis thazard*, and *Auxis rochei* (Salmarika  
186 and Wisudo 2019).

187 The small number of fish collected in this study are fish that are associated with coral reefs  
188 such as grouper fish groups that make coral reef areas as a nursery ground, feeding ground,  
189 and spawning ground. The diversity of reef fish around Banda Aceh experiences a natural  
190 gradient, which shows an increase in the area far from the mainland of the island of Sumatra.  
191 Variety in the region of small islands around Banda Aceh still shows in the good conditions  
192 when compared to the status of coral reefs in the mainland, Sumatra (Edrus, Wijaya et al.  
193 2016). The species of *Epinephelus areolatus*, *Variola albimarginata*, and *Cephalopholis*  
194 *sonnerati* are a group of fish that become coral reefs as their habitat. However, several  
195 pelagic fish around the shallow seas of Banda Aceh remains the primary target. The skipjack  
196 tuna *Rastrelinger kanagurta* (Hariati, Faizah et al. 2015, Hariati and Fauzi 2017), yellowfin  
197 tuna *Thunnus albacares* (Neliyana, Wiyono et al. 2014), Mackerel scad *Decapterus*  
198 *macrosoma*, dan anchovy *Stolephorus* spp) (Kurnia, Purnawan et al. 2016) were also  
199 obtained in this study.

200 In this report, several Acehnese fish sequences also have similarities in some previous  
201 studies, and some are unique to other sequences. Species *Auxis Thazard*, identified from the  
202 port of Lampulo, may have been collected from the area around the sea waters of Western of  
203 Banda Aceh Province with a catch distance of about 50-190 nautical miles (Salmarika and  
204 Wisudo 2019). Although it is still in the Indian Ocean region, there may be specialization in  
205 this species so that the Aceh haplotype separated from the same species in the resulting  
206 phylogenetic tree analysis.

207 In this study, a phylogenetic tree analysis of 3 prominent marine fish families, namely  
208 Scombridae, Serranidae, and Carangidae, was carried out. The results of the investigation  
209 found that the Scombridae *Auxis thazard* (Aceh) which separates from the same clade species  
210 even though it only has a genetic distance of only 0.002. This haplotype appears likely to  
211 occur due to differences in species populations analyzed from India, China (Xu, Van Damme  
212 et al. 2019), and Spain (Catanese, Catanese et al. 2008). While other haplotypes found in reef  
213 fish are *Variola albimarginata* and *Cephalopholis sonnerati*, the *Variola albimarginata* from  
214 Aceh may be a population with the results of a study conducted in India that allows the  
215 sharing of habitats in the Indian Ocean in the Western part of Sumatra Island. Previous  
216 studies on molecular identification of *Variola albimarginata* species have carried out in the  
217 Andaman Islands and Nicobar Island (Basheer, Vineesh et al. 2017). This area is Indian sea  
218 waters, which have the potential to have reef fish, which are almost the same as the species in  
219 Aceh. While *Cephalopholis sonnerati* fish species also have similarities with populations from  
220 Australia and the Philippines, but slightly different from populations from China (Zhuang, Qu  
221 et al. 2013). The study of *Cephalopholis sonnerati* shows the possibility of differences in the  
222 structure of coral fish populations in the South China Sea with the Indian Ocean, especially in  
223 Aceh waters. Although integrated with Indian Ocean waters, no similarities with Indian  
224 populations found in the *Cephalopholis sonnerati* species only in previous studies conducted

225 in the Philippines (Alcantara and Yambot 2016), and Australia (Ward, Zemlak et al. 2005).  
226 The speciation process that occurs in coral reef ecosystems occurs with an allopathic pattern  
227 that makes geographic isolation the leading cause for the emergence of different species.  
228 However, the presence of pelagic larvae in reef fish species also becomes a big question even  
229 though it is believed that the allopatric pattern is a speciation pattern on the main coral reefs  
230 (Rocha and Bowen 2008).

231

#### 232 **4. Conclusions**

233         The From this study, the identification of marine fish landed at the Kutaradja fishing  
234 port in Aceh confirmed 47 specimens (33 genera) of marine fish. Almost all fish species  
235 were becoming fishery commodities and became the main target of the Province of Banda  
236 Aceh's exports, including Tuna fish (*Thunnus albacares*) and Cakalang fish (*Katsuwonus*  
237 *pelamis*). In this study, the Scombridae group found one Aceh haplotype in the *Auxis thazard*  
238 species (MN257554). In contrast, in the Carangids group, it was known that the *Elagatis*  
239 *bipinnulata* species (MN257553) had similar sequences with the same species from Alabama  
240 USA (KF461174). Another species in the Carangidae order, namely *Decapterus macarellus*  
241 from Aceh, has similarities with the sequence from China (MH638794) and Malaysia  
242 (KY570732). In this type of reef fish, this study found *Variola albimarginata* species (India  
243 KM226315), which had similarities with Aceh samples. In contrast, *Cephalopholis sonnerati*  
244 (MN257517) had similarities with the same sequence species from the Philippines  
245 (KU668631) and Australia (DQ107928). More in-depth research on the haplotypes of the  
246 marine fish mentioned above is very much needed to maintain genetic biodiversity in the  
247 waters of Banda Aceh, which is a precious asset for Indonesia.

248

#### 249 **Author contribution**

250 SA. designed the research and supervised all the process including laboratory analysis  
251 and wrote manuscript, AD. collected and analyzed the data and wrote drfat the manuscript.

252

### 253 **Acknowledgments**

254 This work was supported by an educational grant from the LPDP BUDI-LN batch I  
255 2016 and Molecular Physiology Laboratory, Department of Marine Biology, Pukyong  
256 National University, Korea. This paper as the initiation of the first research collaboration  
257 between Universitas Airlangga and Syiah Kuala University.

258

### 259 **Conflict of Interest**

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

262

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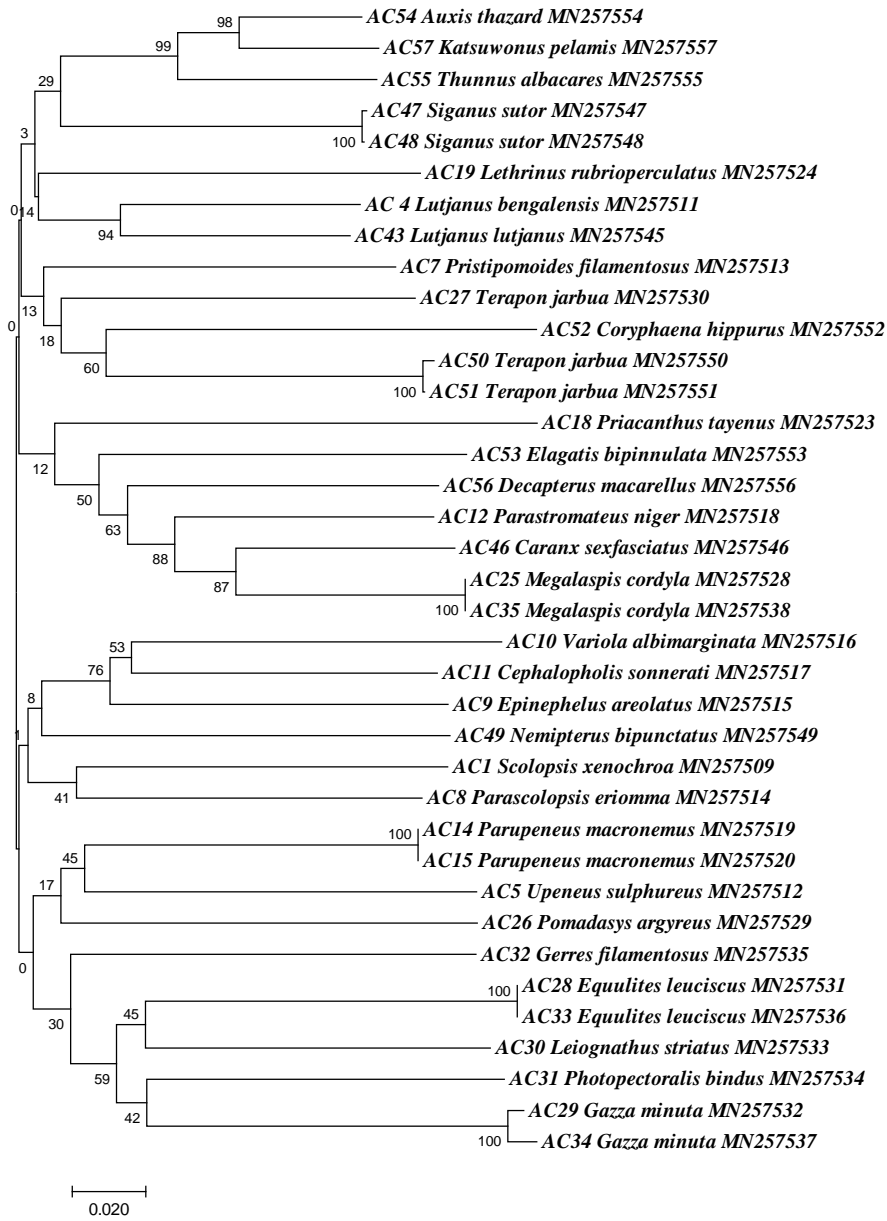
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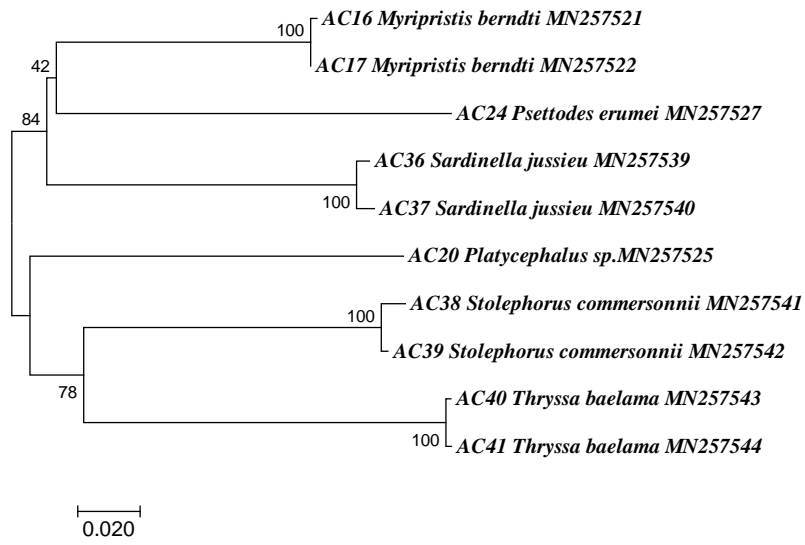
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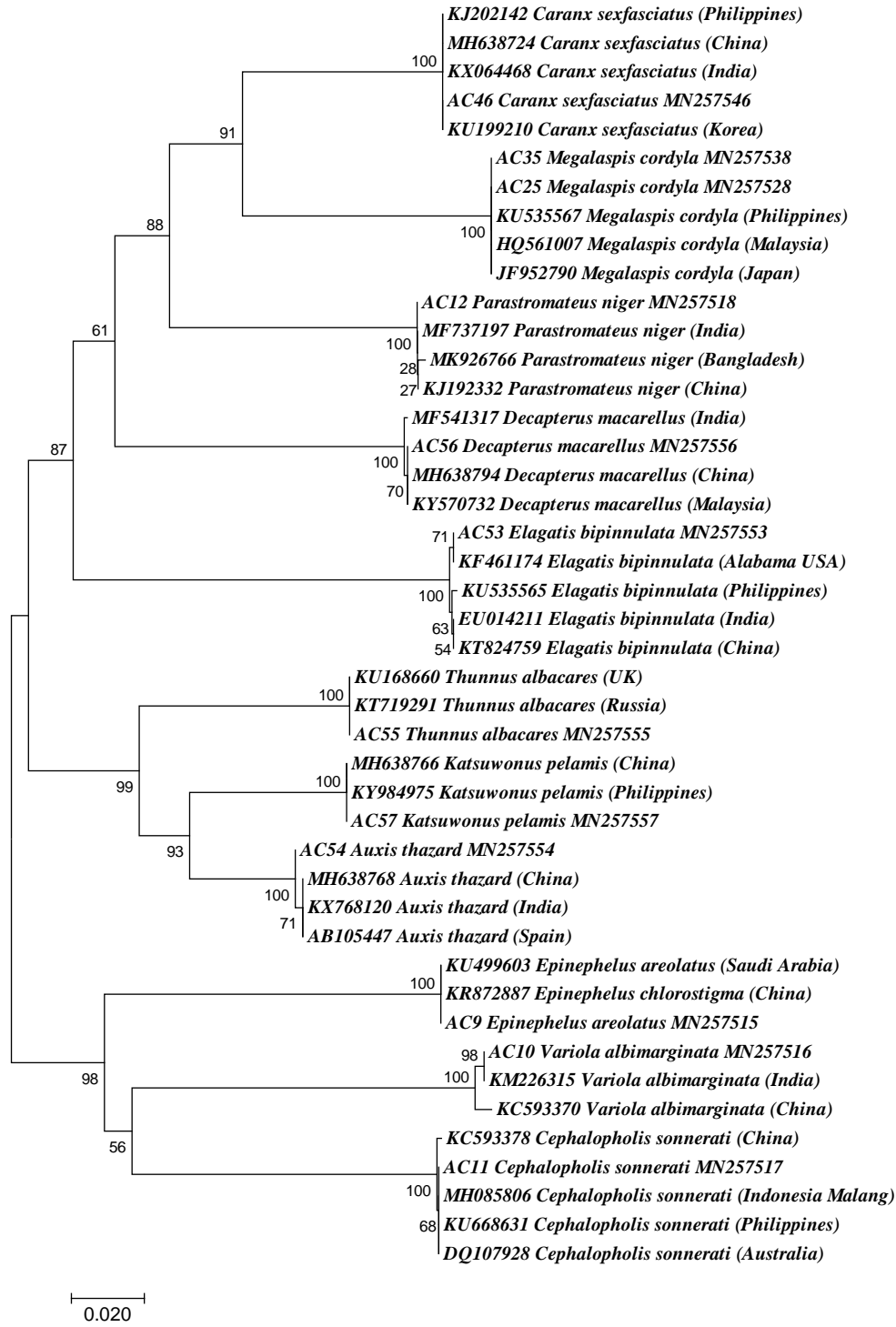


384 **Figure 1.** Phylogenetic tree of Perciformes order by Neighbor-Joining tree algorithm using

385 Mega7



389 **Figure 2.** Phylogenetic tree of small number others order in this study including the  
390 Clupeiformes, Beryciformes, Pleuronectiformes, and Scorpaeniformes by Neighbor-Joining  
391 tree algorithm using Mega7



392

393 **Figure 3.** Phylogenetic reconstruction of three families (Carangidae, Scombridae, and

394 Serranidae) by Neighbor-Joining algorithm using Mega7



395 **Table 1.** The marine fish species list was identified by COI region from Lampulo marine fish

396 landing station, Banda Aceh, Indonesia

No.	ID (AC).	Species Name	Family	GenBank Acc No.	Order	Common name	Habitat	IUCN list
1	16	<i>Myripristis berndti</i>	Holocentridae	MN257521	Beryciformes	Blotcheye soldierfish	Indo-Pacific and Eastern Pacific	LC
2	17	<i>Myripristis berndti</i>	Holocentridae	MN257522	Beryciformes	Blotcheye soldierfish	Indo-Pacific and Eastern Pacific	LC
3	36	<i>Sardinella jussieu</i>	Clupeidae	MN257539	Clupeiformes	Mauritian sardinella	Western Indian Ocean	DD
4	37	<i>Sardinella jussieu</i>	Clupeidae	MN257540	Clupeiformes	Mauritian sardinella	Western Indian Ocean	DD
5	38	<i>Stolephorus commersonii</i>	Engraulidae	MN257541	Clupeiformes	Commerson's anchovy	Indo-West Pacific	LC
6	39	<i>Stolephorus commersonii</i>	Engraulidae	MN257542	Clupeiformes	Commerson's anchovy	Indo-West Pacific	LC
7	40	<i>Thryssa baelama</i>	Engraulidae	MN257543	Clupeiformes	Baelama anchovy	Indo-Pacific	LC
8	41	<i>Thryssa baelama</i>	Engraulidae	MN257544	Clupeiformes	Baelama anchovy	Indo-Pacific	LC
9	1	<i>Scolopsis xenochroa</i>	Nemipteridae	MN257509	Perciformes	Oblique-barred monocle bream	Indo-West Pacific	NE
10	4	<i>Lutjanus bengalensis</i>	Lutjanidae	MN257511	Perciformes	Bengal snapper	Indo-West Pacific:	NE
11	5	<i>Upeneus sulphureus</i>	Mullidae	MN257512	Perciformes	Sulphur goatfish	Indo-West Pacific	LC
12	7	<i>Pristipomoides filamentosus</i>	Lutjanidae	MN257513	Perciformes	Crimson jobfish	Indo-Pacific	LC
13	8	<i>Parascolopsis eriomma</i>	Nemipteridae	MN257514	Perciformes	Rosy dwarf monocle bream	Indo-West Pacific	NE
14	9	<i>Epinephelus areolatus</i>	Serranidae	MN257515	Perciformes	Areolate grouper	Indo-Pacific	LC
15	10	<i>Variola albimarginata</i>	Serranidae	MN257516	Perciformes	White-edged lyretail	Indo-Pacific	LC
16	11	<i>Cephalopholis sonnerati</i>	Serranidae	MN257517	Perciformes	Tomato hind	Indo-Pacific	LC
17	12	<i>Parastromateus niger</i>	Carangidae	MN257518	Perciformes	Black pomfret	Indo-West Pacific	LC
18	14	<i>Parupeneus macronemus</i>	Mullidae	MN257519	Perciformes	Long-barbel goatfish	Indo-West Pacific	LC
19	15	<i>Parupeneus macronemus</i>	Mullidae	MN257520	Perciformes	Long-barbel goatfish	Indo-West Pacific	LC
20	18	<i>Priacanthus tayenus</i>	Priacanthidae	MN257523	Perciformes	Purple-spotted bigeye	Indo-West Pacific	LC
21	19	<i>Lethrinus rubrioperculatus</i>	Lethrinidae	MN257524	Perciformes	Spotcheek emperor	Indo-Pacific	LC
22	25	<i>Megalaspis cordyla</i>	Carangidae	MN257528	Perciformes	Torpedo scad	Indo-West Pacific	LC
23	26	<i>Pomadasyus argyreus</i>	Haemulidae	MN257529	Perciformes	Bluecheek silver	Indo-West Pacific	NE

						grunt		
24	27	<i>Terapon jarbua</i>	Terapontidae	MN257530	Perciformes	Jarbua terapon	Indo-Pacific	LC
25	28	<i>Equulites leuciscus</i>	Leiognathidae	MN257531	Perciformes	Whipfin ponyfish	Indo-West Pacific	LC
26	29	<i>Gazza minuta</i>	Leiognathidae	MN257532	Perciformes	Toothpony	Indo-Pacific	LC
27	30	<i>Leiognathus striatus</i>	Leiognathidae	MN257533	Perciformes	Toothpony	Western Indian Ocean	NE
28	31	<i>Photopectoralis bindus</i>	Leiognathidae	MN257534	Perciformes	Orangefin ponyfish	Indo-West Pacific	NE
29	32	<i>Gerres filamentosus</i>	Gerreidae	MN257535	Perciformes	Whipfin silver-biddy	Indo-Pacific	LC
30	33	<i>Equulites leuciscus</i>	Leiognathidae	MN257536	Perciformes	Whipfin ponyfish	Indo-West Pacific	LC
31	34	<i>Gazza minuta</i>	Leiognathidae	MN257537	Perciformes	Toothpony	Indo-Pacific	LC
32	35	<i>Megalaspis cordyla</i>	Carangidae	MN257538	Perciformes	Torpedo scad	Indo-West Pacific	LC
33	43	<i>Lutjanus lutjanus</i>	Lutjanidae	MN257545	Perciformes	Bigeye snapper	Indo-West Pacific	LC
34	46	<i>Caranx sexfasciatus</i>	Carangidae	MN257546	Perciformes	Bigeye trevally	Indo-Pacific	LC
35	47	<i>Siganus sutor</i>	Siganidae	MN257547	Perciformes	Shoemaker spinefoot	Indian Ocean	LC
36	48	<i>Siganus sutor</i>	Siganidae	MN257548	Perciformes	Shoemaker spinefoot	Indian Ocean	LC
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>Katsuwonus pelamis</i>	Scombridae	MN257557	Perciformes	Skipjack tuna	Cosmopolitan in tropical and warm-temperate waters	LC
46	24	<i>Psettodes erumei</i>	Psettodidae	MN257527	Pleuronectiformes	Indian halibut	Indo-West Pacific	NE
47	20	<i>Platycephalus sp.</i>	Platycephalidae	MN257525	Scorpaeniformes	Bartail flathead	Indo-West Pacific	DD

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

399 **Table 2.** Alignment result of several marine fish species from Aceh showing nucleotides  
 400 different from the references (GenBank database)

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	-	-	-

401

402

403

1     **Molecular identification and phylogenetic tree reconstruction of marine fish species**  
2                     **from the Fishing Port of Kutaradja, Banda Aceh**

3  
4     **Abstract**

5     The enormous potential of marine resources possessed by Banda Aceh Province is expected  
6     to be utilized optimally. Accurate in the identification of marine fish resources is a critical  
7     requirement to support their utilization and preservation in Banda Aceh Province. In this  
8     study, a molecular identification approach carried out in addition to conducting a  
9     morphological identification, which several scientists commonly used. The results obtained  
10    were 47 COI sequences were generated representing 33 genera, 19 families, and five orders.  
11    From the resulting COI partial sequence, there is one haplotype from the Scombridae family  
12    (*Auxis thazard*), two haplotypes from the Carangidae family (*Elagatis bipinnulata* and  
13    *Decapterus macarellus*), and two haplotypes from the Serranidae family (*Variola*  
14    *albimarginata* and *Cephalopholis sonnerati*). This study is essential in fisheries biology  
15    studies and other fisheries studies to support the sustainable utilization of marine fisheries  
16    potential in Banda Aceh.

17  
18    Keywords: molecular, DNA barcoding, haplotype analysis, Aceh, marine fish

19  
20    **1. Introduction**

21    Aceh is the westernmost province of the Indo-Malaya Archipelago (IMA) known as the hot  
22    spot of tropical marine biodiversity (Gaither et al. 2011; Veron et al. 2009; Bellwood and  
23    Meyer 2009; Hoeksema 2007; Briggs 2005). This province has considerable fishery potential  
24    with waters reaching 295,370 km<sup>2</sup> and with a coastline length reaching 2,666.3 km<sup>2</sup>  
25    (Mukhtar, 2017). One of the centers of fishing activities, as well as the most significant fish

**Commented [Reviewer1]:** Please correct grammatical errors throughout the manuscript

26 landing in Aceh, is the Fishing Port of Kutaradja. Marine fisheries production at this fishing  
27 port increased from 8,922 tons in 2013 to 12,305 tons in 2017 (Yusuf 2003, Yeni and Naufal  
28 2017, Mardhatillah, Damora et al. 2019). The fish landing suffered massive damage due to  
29 the tsunami that struck Aceh Province and was rebuilt in 2004 (Zulmaidah, Zain et al. 2015).  
30 The rebuilding of the Kutaradja fishing port has revived the economy and fisheries activities  
31 in the Banda Aceh region.

32 Regarding the fishing grounds for fishers at this fishing port, all the fishing zone includes the  
33 Indian Ocean, Andaman Sea, and Malacca Strait. Two of the three regions are included in the  
34 Fisheries Management Area (FMA) 571 and 572. The level of utilization of pelagic and  
35 demersal fish resources in these two FMA is included in the overexploited category (Suman,  
36 Irianto et al. 2017, Salmarika and Wisudo 2019).

37 Previous research on the types of fish landed by many traditional fishers of the Kutaradja  
38 fishing port is still being done conventionally. From the inventory carried out at the Kutaradja  
39 fishing port, 11 species have been identified (Munawwarah, Sufi et al. 2016). However,  
40 another report on the types of marine fish species in Banda Aceh (Simeuleu Island) identified  
41 around 77 marine fish species included in 54 genus, 26 families, and seven orders (Batubara,  
42 Muchlisin et al. 2017). The reef-associated fishes inventory at Ulee Lheue, Banda Aceh, also  
43 mentioned that there were 87 species of reef fishes from 28 families in this location (Fadli,  
44 Muchlisin et al. 2019). In different areas, Lhoknga and Lhok Mata Ie Beaches, the eight  
45 orders, 11 families, 19 genera, and 25 species were recorded from 51 fish samples (Nur,  
46 Batubara et al. 2019). The morphological approach is the most widely used method in many  
47 regions in Indonesia, including in Banda Aceh. This research identifies molecular marine fish  
48 in the Cytochrome C Oxidase subunit I (COI) region of the mitochondrial gene to complete  
49 the morphological identification that was also carried out. This COI Region is the region that  
50 some gene markers have agreed on in molecular identification globally. Research on

51 barcoding in several aquatic biota has been carried out such as marine fish in Australia  
52 (Ward, Zemlak et al. 2005), marine fish in India (Lakra, Verma et al. 2011), marine fish in  
53 Turkey (Kesk n and Atar 2013), marine fish in China (Wang, Guo et al. 2012, Zhang and  
54 Hanner 2012), and marine fish in Taiwan (Chang, Shao et al. 2017, Bingpeng, Heshan et al.  
55 2018). Whereas research on molecular identification of fish in Aceh has carried out on some  
56 species such as grouper fish (Kamal, Hakim et al. 2019), and *Scomber* spp (Edwarsyah, Nasir  
57 et al. 2019). This research on the identification of marine fish landed at the fishing port of  
58 Kutaradja is the first study to carry out molecular identification.

59 The purpose of this research is to identify species of marine fish to species level by using a  
60 molecular approach to minimize identification errors. Besides, the research carried out  
61 identification of Aceh's haplotype in the Scombridae, Serranidae, and Carangidae groups,  
62 which are pelagic fish resources that have significant economic value.

63

## 64 **2. Materials and methods**

### 65 **2.1 Sampling site**

66 A total of 47 fish samples were collected from the Lampulo traditional fish market  
67 close to the Lampulo fish landing on 19 July 2019. Morphologically, identification, and  
68 species confirmation have been carried out with molecular identification carried out in this  
69 study. No specific permit was required for this study, and a digital camera has taken the  
70 individual photograph. All samples have collected from the local traditional fish market were  
71 dead upon purchasing.

### 72 **2.2 DNA extraction and PCR**

73 Each specimen has been collected based on the morphological characters and after  
74 collection directly preserved in 90% ethanol for further experimental purposes. Genomic  
75 DNA extracted using an Accuprep® Genomic DNA Extraction Kit (Bioneer) according to

**Commented [Reviewer2]:** So this market only sells local fishes? No imported fishes?

76 the manufacturer's guidelines. The anal fin, around 1 cm tissues, was dissected and mix with  
77 6X lysis buffer, which was further homogenized by the TissueLyser II (Qiagen).  
78 Quantification of purified genomic DNA performed by nanoDrop (Thermofisher Scientific  
79 D1000), aliquoted and stored at the -70°C for further analysis.

### 80 **2.3 PCR condition and Data Analysis**

81 One set universal fish primer targeting cytochrome c oxidase I (COI) region, BCL-BCH  
82 (Baldwin, Mounts et al. 2009, Handy, Deeds et al. 2011), used to obtain the partial sequences  
83 of each gene. The PCR mixture (20µL) included 11.2 µL ultra-pure water, 1 µL primer  
84 forward and reverse (0.5 µM), 0.2 µL Ex Taq DNA polymerase (TaKaRa, Japan), 2 µL 10X  
85 ExTag Buffer, 2 µL dNTPs (1 µM, TaKaRa, Japan), and 2 µL genomic DNA as template.  
86 The PCR condition carried out under the following setting: 95°C for 5 min in initial  
87 denaturation, followed by denaturation at 95°C for 30 s in 40 cycles, 50°C for 30 s in  
88 annealing, and 72°C for 45 s in extension step, and a final extension at 72°C for 5 min. The  
89 PCR products purified with the AccuPrep®Gel purification kit (Bioneer, Korea).

### 90 **2.4 Phylogenetic analysis**

91 All sequences were aligned and submitted to GenBank (Table 1). The pairwise evolutionary  
92 distance among the family determined by the Kimura 2-Parameter method. The Neighbor-  
93 joining (NJ) tree constructed, and 1000 bootstrap analysis was carried by Mega 7 (Kumar,  
94 Stecher et al. 2016).

95

## 96 **3. Results and Discussion**

### 97 **3.1 Species Identification**

98 A total of 47 COI sequences generated representing 33 genera, 19 families, and five  
99 orders. Common names, taxonomic designation, habitat, IUCN list, as well as the GenBank  
100 accession number for all specimens in Table 1. The sequencing of the COI gene produced

101 more than 600 nucleotide base pairs per taxon. The un-ambiguity and simplicity observed  
102 among all the sequences and no stop codons, deletions, and insertions observed in all the  
103 sequences. Here, we cluster into two groups are Perciformes and another order.

104

### 105 **3.2 Perciformes**

106 The nucleotide frequencies of COI sequences are 29.65% (T/U), 23.95% (A), 28.80% (C),  
107 and 17.6% (G). The average of transitional pair ( $si=5.07$ ) and was lower than the average of  
108 transversional pair ( $sv=14.86$ ) with an overall transition/transversion ratio bias is 1.57. The  
109 phylogenetic tree was constructed the COI sequences for the Perciformes and shown the  
110 average K2P distance within taxonomic levels measured for COI sequences is 0.226 (Figure  
111 1).

112

### 113 **3.3 Clupeiformes and Others**

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

120

### 121 **3.4 The haplotype of Scombridae, Serranidae, and Carangidae from Aceh**

122 In this study, the sample from Aceh had several unique haplotypes compared to the  
123 same species from the GenBank database. By aligning the sequence generated with the  
124 reference sequence, some different nucleotides produce genetic variations (Table 2). The  
125 phylogenetics reconstruction of those sequences shown that several haplotypes found in this



126 study (Figure 3). The identified haplotype in the Carangid group was found in the *Decapterus*  
127 *macarellus* species (MN257556) which had similarities with sequences from China and  
128 Malaysia, and had a genetic distance with an Indian sequence is 0.002. Also, *Elagatis*  
129 *bipinnulata* (MN257553) is closer to the similarity of the sequence owned by the same type  
130 of fish (KF461174) from Alabama, USA. While the genetic distance of *Elagatis bipinnulata*  
131 with the same species is 0.003 (Philippines) and 0.02 (India and China). In the Carangid  
132 group, *Caranx sexfasciatus* (MN257546) and *Megalaspis cordyla* (MN257528 and  
133 MN257538) species were not found polymorphic in the sequences obtained.

134 In the Scombridae family group, haplotypes found in *Auxis thazard* fish (MN257554)  
135 which differed from Chinese, Indian, and Spanish haplotypes with a genetic distance of  
136 0.002. While in the Serranidae family, haplotypes found in *Variola albimarginata* fish  
137 (MN257516) and *Cephalopholis sonnerati* (MN257517). This *Variola albimarginata* species  
138 (MN257516) has similarities with sequences from India but is different from Chinese  
139 haplotypes with a genetic distance of 0.007. While species of *Cephalopholis sonnerati*  
140 (MN257517) differ only from Chinese haplotypes, this species merged in one clade with  
141 samples of the Philippines, Australia, and Indonesia with genetic distance 0.00-0.002. In  
142 *Epinephelus arelatus* species, there are no haplotypes and sequences obtained from samples  
143 from China and Saudi Arabia.

144

## 145 **Discussion**

146 Research on molecular identification is now extensive in the field of fisheries and  
147 marine sciences. In this study, molecular identification used to completing the morphological  
148 identification and, at the same time, determine the position of the species identified in the  
149 phylogenetic tree created. Conventional identification that has been done at this time still  
150 finds obstacles with the difficulty of getting taxonomists in determining species, in addition

151 to the long enough time in the identification process, errors in identification also still occur in  
152 some cases. By doing a combination of identification, it is expected to be more valid in  
153 getting the results of fish species obtained.

154 In this study, several marine fish landed at the Kutaradja port became an essential  
155 fishery commodity in Banda Aceh. After the 2004 tsunami disaster in this province, several  
156 activities capable of mobilizing economic activities continue to be carried out, including  
157 capture fisheries activities in this Lampulo fish port (Zulmaidah, Zain et al. 2015). Previous  
158 studies have also reported the identification of marine fish species from Lampulo. There is  
159 still inaccurate information regarding marine fish identification in some reports. Besides, an  
160 identification that is only on morphological-based characteristics that are not done by  
161 taxonomists, then the results in identification may be incorrect on species justification. In an  
162 earlier report, the species *Sardinella sirin* (Serranidae) was reported to exist in this Lampulo  
163 port (Munawwarah, Sufi et al. 2016). Still, an inaccurate in determining taxonomy made the  
164 identification results unreliable. The genus *Sardinella* spp is a group of fish in the family  
165 Clupeidae, order Clupeiformes ([www.fishbase.org](http://www.fishbase.org)), not include in Serranidae.

166 In this report, family Perciformes are identified as a group that dominates the caught  
167 by fishermen in Banda Aceh, who landed at the Kutaradja fishing port. These fish are  
168 consumption fish that are essential export commodities with high economic value such as  
169 Skipjack tuna (57%) followed by yellowfin tuna (23%) (Lubis, Syaifuddin et al. 2016). The  
170 results of identification, the Scombridae family, is a group of pelagic fish that is quite  
171 commonly found. The types identified in this report include *Thunnus albacares*, *Auxis thazar*,  
172 and *Katsuwonus pelamis*. Besides, the genus Lutjanidae (snapper) found three species,  
173 namely *Lutjanus bengalensis*, *Lutjanus lutjanus*, and *Lethrinus rubrioperculatus*. Other  
174 groups that are targeted by fishermen are reef fish that have significant economic value, such  
175 as Grouper and Carangid. The groupers identified in this study include *Epinephelus*

176 *areolatus*, *Variola albimarginata*, and *Cephalopholis sonnerati*, whereas the Carangids group  
177 includes *Parastromateus niger*, *Megalaspis cordyla*, *Caranx sexfasciatus*, and *Decapterus*  
178 *macarellus* (Table 1).

179 In another group on the Clupeiformes order, two families found in the Lampulo, namely  
180 Clupeidae (*Sardinella jussieu*) and Engraulidae (*Stelephorus commersonii* and *Thryssa*  
181 *baelama*). In connection with the types of fish caught by fishermen, it is shown that capture  
182 fisheries in Banda Aceh use purse seine, which finds a group of pelagic fish in large  
183 quantities. Previous studies have explained that the fishermen in Banda Aceh mostly use  
184 purse seine (Wiryawan, Wiyono et al. 2016, Hariati 2017). The purse-seine is also generally  
185 fishing gear to catch fish of *Euthynnus affinis*, *Auxis thazard*, and *Auxis rochei* (Salmarika  
186 and Wisudo 2019).

187 The small number of fish collected in this study are fish that are associated with coral reefs  
188 such as grouper fish groups that make coral reef areas as a nursery ground, feeding ground,  
189 and spawning ground. The diversity of reef fish around Banda Aceh experiences a natural  
190 gradient, which shows an increase in the area far from the mainland of the island of Sumatra.  
191 Variety in the region of small islands around Banda Aceh still shows in the good conditions  
192 when compared to the status of coral reefs in the mainland, Sumatra (Edrus, Wijaya et al.  
193 2016). The species of *Epinephelus areolatus*, *Variola albimarginata*, and *Cephalopholis*  
194 *sonnerati* are a group of fish that become coral reefs as their habitat. However, several  
195 pelagic fish around the shallow seas of Banda Aceh remains the primary target. The skipjack  
196 tuna *Rastrelinger kanagurta* (Hariati, Faizah et al. 2015, Hariati and Fauzi 2017), yellowfin  
197 tuna *Thunnus albacares* (Neliyana, Wiyono et al. 2014), Mackerel scad *Decapterus*  
198 *macrosoma*, dan anchovy *Stolephorus* spp) (Kurnia, Purnawan et al. 2016) were also  
199 obtained in this study.

200 In this report, several Acehnese fish sequences also have similarities in some previous  
201 studies, and some are unique to other sequences. Species *Auxis Thazard*, identified from the  
202 port of Lampulo, may have been collected from the area around the sea waters of Western of  
203 Banda Aceh Province with a catch distance of about 50-190 nautical miles (Salmarika and  
204 Wisudo 2019). Although it is still in the Indian Ocean region, there may be specialization in  
205 this species so that the Aceh haplotype separated from the same species in the resulting  
206 phylogenetic tree analysis.

207 In this study, a phylogenetic tree analysis of 3 prominent marine fish families, namely  
208 Scombridae, Serranidae, and Carangidae, was carried out. The results of the investigation  
209 found that the Scombridae *Auxis thazard* (Aceh) which separates from the same clade species  
210 even though it only has a genetic distance of only 0.002. This haplotype appears likely to  
211 occur due to differences in species populations analyzed from India, China (Xu, Van Damme  
212 et al. 2019), and Spain (Catanese, Catanese et al. 2008). While other haplotypes found in reef  
213 fish are *Variola albimarginata* and *Cephalopholis sonnerati*, the *Variola albimarginata* from  
214 Aceh may be a population with the results of a study conducted in India that allows the  
215 sharing of habitats in the Indian Ocean in the Western part of Sumatra Island. Previous  
216 studies on molecular identification of *Variola albimarginata* species have carried out in the  
217 Andaman Islands and Nicobar Island (Basheer, Vineesh et al. 2017). This area is Indian sea  
218 waters, which have the potential to have reef fish, which are almost the same as the species in  
219 Aceh. While *Cephalopholis sonnerati* fish species also have similarities with populations from  
220 Australia and the Philippines, but slightly different from populations from China (Zhuang, Qu  
221 et al. 2013). The study of *Cephalopholis sonnerati* shows the possibility of differences in the  
222 structure of coral fish populations in the South China Sea with the Indian Ocean, especially in  
223 Aceh waters. Although integrated with Indian Ocean waters, no similarities with Indian  
224 populations found in the *Cephalopholis sonnerati* species only in previous studies conducted

225 in the Philippines (Alcantara and Yambot 2016), and Australia (Ward, Zemlak et al. 2005).  
226 The speciation process that occurs in coral reef ecosystems occurs with an allopathic pattern  
227 that makes geographic isolation the leading cause for the emergence of different species.  
228 However, the presence of pelagic larvae in reef fish species also becomes a big question even  
229 though it is believed that the allopatric pattern is a speciation pattern on the main coral reefs  
230 (Rocha and Bowen 2008).

231

#### 232 **4. Conclusions**

233 The From this study, the identification of marine fish landed at the Kutaradja fishing  
234 port in Aceh confirmed 47 specimens (33 genera) of marine fish. Almost all fish species  
235 were becoming fishery commodities and became the main target of the Province of Banda  
236 Aceh's exports, including Tuna fish (*Thunnus albacares*) and Cakalang fish (*Katsuwonus*  
237 *pelamis*). In this study, the Scombridae group found one Aceh haplotype in the *Auxis thazard*  
238 species (MN257554). In contrast, in the Carangids group, it was known that the *Elagatis*  
239 *bipinnulata* species (MN257553) had similar sequences with the same species from Alabama  
240 USA (KF461174). Another species in the Carangidae order, namely *Decapterus macarellus*  
241 from Aceh, has similarities with the sequence from China (MH638794) and Malaysia  
242 (KY570732). In this type of reef fish, this study found *Variola albimarginata* species (India  
243 KM226315), which had similarities with Aceh samples. In contrast, *Cephalopholis somnerati*  
244 (MN257517) had similarities with the same sequence species from the Philippines  
245 (KU668631) and Australia (DQ107928). More in-depth research on the haplotypes of the  
246 marine fish mentioned above is very much needed to maintain genetic biodiversity in the  
247 waters of Banda Aceh, which is a precious asset for Indonesia.

248

#### 249 **Author contribution**

250 SA. designed the research and supervised all the process including laboratory analysis  
251 and wrote manuscript, AD. collected and analyzed the data and wrote drfat the manuscript.

252

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258

### 259 **Conflict of Interest**

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

262

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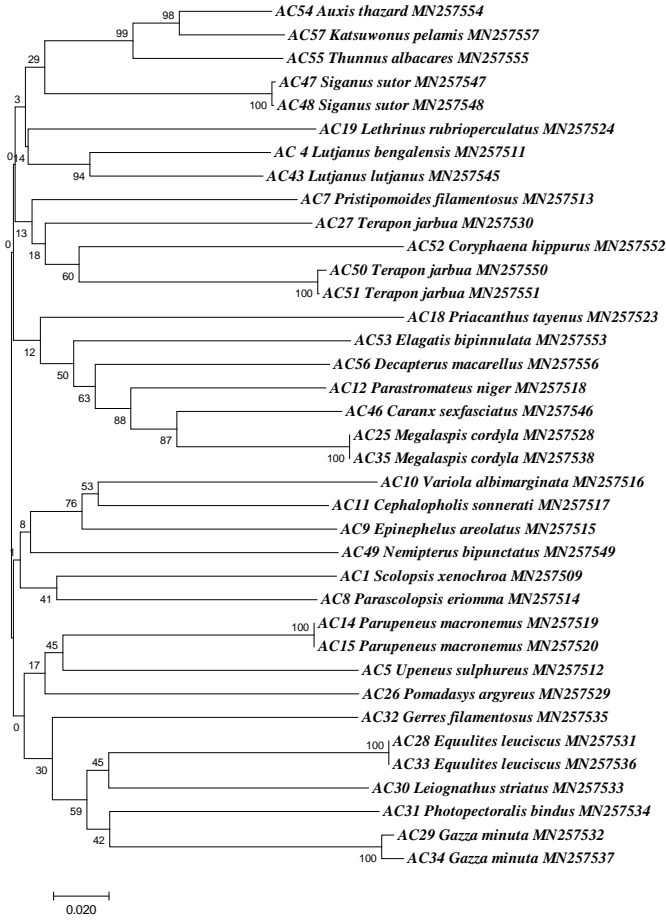
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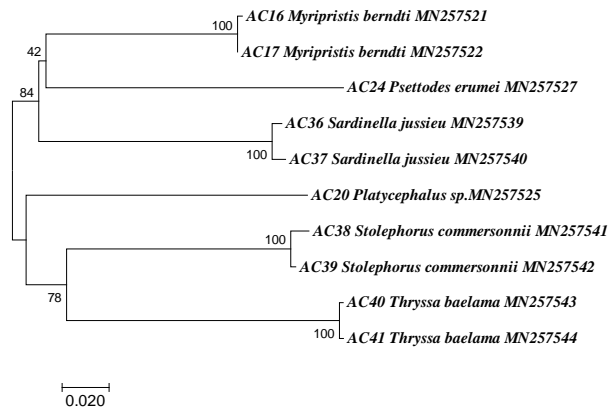
384 **Figure 1.** Phylogenetic tree of Perciformes order by Neighbor-Joining tree algorithm using

385 Mega7

386

**Commented [Reviewer3]:** I suggest label each cluster on the tree e.g. Genus *Segatus* and others

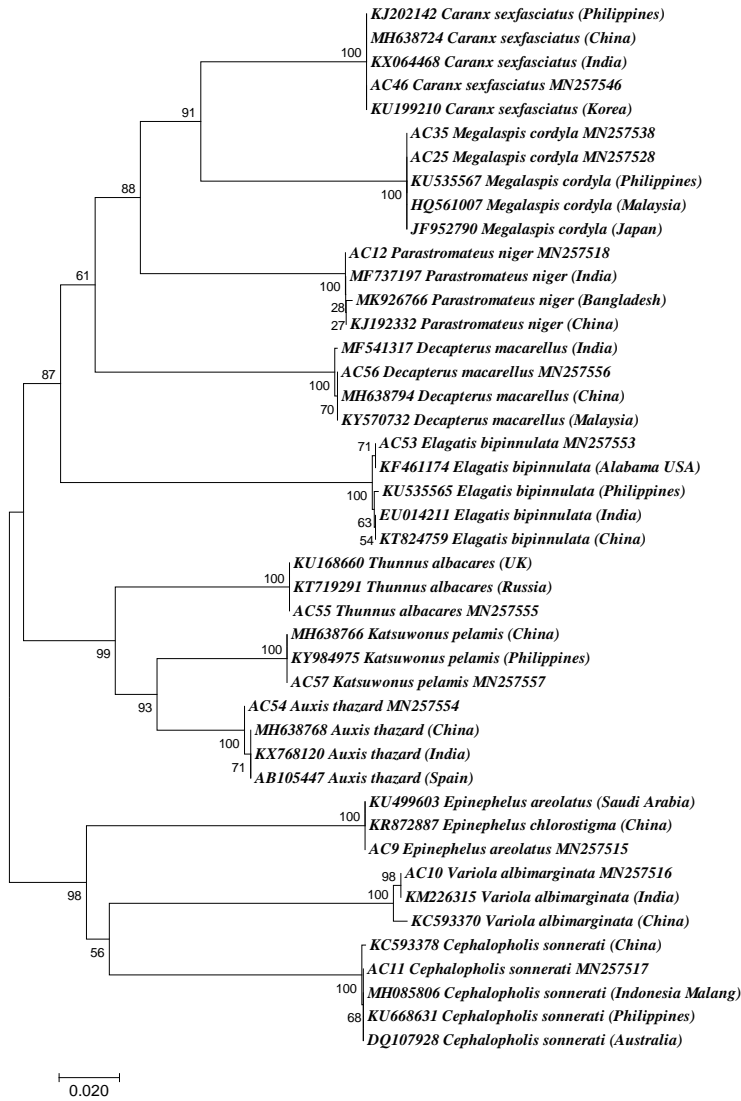
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389 **Figure 2.** Phylogenetic tree of small number others order in this study including the  
390 Clupeiformes, Beryciformes, Pleuronectiformes, and Scorpaeniformes by Neighbor-Joining  
391 tree algorithm using Mega7

**Commented [Reviewer4]:** I suggest label each cluster on the tree e.g. Genus *Stolephorus* and others



392

393 **Figure 3.** Phylogenetic reconstruction of three families (Carangidae, Scombridae, and

394 Serranidae) by Neighbor-Joining algorithm using [Mega7](#)

**Commented [Reviewer5]:** I suggest label each cluster on the tree e.g. Genus *Cephalopholis* and others

395 **Table 1.** The marine fish species list was identified by COI region from Lampulo marine fish  
 396 landing station, Banda Aceh, Indonesia

No.	ID (AC).	Species Name	Family	GenBank Acc No.	Order	Common name	Habitat	IUCN list
1	16	<i>Myripristis berndti</i>	Holocentridae	MN257521	Beryciformes	Blotcheye soldierfish	Indo-Pacific and Eastern Pacific	LC
2	17	<i>Myripristis berndti</i>	Holocentridae	MN257522	Beryciformes	Blotcheye soldierfish	Indo-Pacific and Eastern Pacific	LC
3	36	<i>Sardinella jussieu</i>	Clupeidae	MN257539	Clupeiformes	Mauritian sardinella	Western Indian Ocean	DD
4	37	<i>Sardinella jussieu</i>	Clupeidae	MN257540	Clupeiformes	Mauritian sardinella	Western Indian Ocean	DD
5	38	<i>Stolephorus commersonii</i>	Engraulidae	MN257541	Clupeiformes	Commerson's anchovy	Indo-West Pacific	LC
6	39	<i>Stolephorus commersonii</i>	Engraulidae	MN257542	Clupeiformes	Commerson's anchovy	Indo-West Pacific	LC
7	40	<i>Thryssa baelama</i>	Engraulidae	MN257543	Clupeiformes	Baelama anchovy	Indo-Pacific	LC
8	41	<i>Thryssa baelama</i>	Engraulidae	MN257544	Clupeiformes	Baelama anchovy	Indo-Pacific	LC
9	1	<i>Scolopsis xenochroa</i>	Nemipteridae	MN257509	Perciformes	Oblique-barred monocle bream	Indo-West Pacific	NE
10	4	<i>Lutjanus bengalensis</i>	Lutjanidae	MN257511	Perciformes	Bengal snapper	Indo-West Pacific:	NE
11	5	<i>Upeneus sulphureus</i>	Mullidae	MN257512	Perciformes	Sulphur goatfish	Indo-West Pacific	LC
12	7	<i>Pristipomoides filamentosus</i>	Lutjanidae	MN257513	Perciformes	Crimson jobfish	Indo-Pacific	LC
13	8	<i>Parascolopsis eriomma</i>	Nemipteridae	MN257514	Perciformes	Rosy dwarf monocle bream	Indo-West Pacific	NE
14	9	<i>Epinephelus areolatus</i>	Serranidae	MN257515	Perciformes	Areolate grouper	Indo-Pacific	LC
15	10	<i>Variola albimarginata</i>	Serranidae	MN257516	Perciformes	White-edged lyretail	Indo-Pacific	LC
16	11	<i>Cephalopholis sonnerati</i>	Serranidae	MN257517	Perciformes	Tomato hind	Indo-Pacific	LC
17	12	<i>Parastromateus niger</i>	Carangidae	MN257518	Perciformes	Black pomfret	Indo-West Pacific	LC
18	14	<i>Parupeneus macronemus</i>	Mullidae	MN257519	Perciformes	Long-barbel goatfish	Indo-West Pacific	LC
19	15	<i>Parupeneus macronemus</i>	Mullidae	MN257520	Perciformes	Long-barbel goatfish	Indo-West Pacific	LC
20	18	<i>Priacanthus tayenus</i>	Priacanthidae	MN257523	Perciformes	Purple-spotted bigeye	Indo-West Pacific	LC
21	19	<i>Lethrinus rubrioperculatus</i>	Lethrinidae	MN257524	Perciformes	Spotcheek emperor	Indo-Pacific	LC
22	25	<i>Megalaspis cordyla</i>	Carangidae	MN257528	Perciformes	Torpedo scad	Indo-West Pacific	LC
23	26	<i>Pomadasys argyreus</i>	Haemulidae	MN257529	Perciformes	Bluecheek silver	Indo-West Pacific	NE

						grunt		
24	27	<i>Terapon jarbua</i>	Terapontidae	MN257530	Perciformes	Jarbua terapon	Indo-Pacific	LC
25	28	<i>Equulites leuciscus</i>	Leiognathidae	MN257531	Perciformes	Whipfin ponyfish	Indo-West Pacific	LC
26	29	<i>Gazza minuta</i>	Leiognathidae	MN257532	Perciformes	Toothpony	Indo-Pacific	LC
27	30	<i>Leiognathus striatus</i>	Leiognathidae	MN257533	Perciformes	Toothpony	Western Indian Ocean	NE
28	31	<i>Photopectoralis bindus</i>	Leiognathidae	MN257534	Perciformes	Orangefin ponyfish	Indo-West Pacific	NE
29	32	<i>Gerres filamentosus</i>	Gerreidae	MN257535	Perciformes	Whipfin silver-biddy	Indo-Pacific	LC
30	33	<i>Equulites leuciscus</i>	Leiognathidae	MN257536	Perciformes	Whipfin ponyfish	Indo-West Pacific	LC
31	34	<i>Gazza minuta</i>	Leiognathidae	MN257537	Perciformes	Toothpony	Indo-Pacific	LC
32	35	<i>Megalaspis cordyla</i>	Carangidae	MN257538	Perciformes	Torpedo scad	Indo-West Pacific	LC
33	43	<i>Lutjanus lutjanus</i>	Lutjanidae	MN257545	Perciformes	Bigeye snapper	Indo-West Pacific	LC
34	46	<i>Caranx sexfasciatus</i>	Carangidae	MN257546	Perciformes	Bigeye trevally	Indo-Pacific	LC
35	47	<i>Siganus sutor</i>	Siganidae	MN257547	Perciformes	Shoemaker spinefoot	Indian Ocean	LC
36	48	<i>Siganus sutor</i>	Siganidae	MN257548	Perciformes	Shoemaker spinefoot	Indian Ocean	LC
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>	Scombriidae	MN257553	Perciformes	Frigate tuna	Atlantic, Indian and Pacific (Western Central)	LC
42	54	<i>Auxis thazard</i>	Scombriidae	MN257554	Perciformes	Frigate tuna	Atlantic, Indian and Pacific (Western Central)	LC
43	55	<i>Thunnus albacares</i>	Scombriidae	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>Katsuwonus pelamis</i>	Scombriidae	MN257557	Perciformes	Skipjack tuna	Cosmopolitan in tropical and warm-temperate waters	LC
46	24	<i>Psettodes erumei</i>	Psettodidae	MN257527	Pleuronectiformes	Indian halibut	Indo-West Pacific	NE
47	20	<i>Platycephalus sp.</i>	Platycephalidae	MN257525	Scorpaeniformes	Bartail flathead	Indo-West Pacific	DD

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



399 **Table 2.** Alignment result of several marine fish species from Aceh showing nucleotides  
 400 different from the references (GenBank database)

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	-	-	-

401

402

403

The manuscript 71955-241662-1-RV reports molecular identification through DNA barcoding principle in one of the fishing ports in Aceh, the westernmost region of Indonesia. I consider the MS can be indexed in JTBB. However, I found some flaws in the MS and the authors need to require appropriate revision to improve the MS. Additionally, I found many grammatical mistakes throughout the MS which undermine the presentation of the results and findings of the article. Please consider re-checking the grammar before submitting the revised one. I list my comments below and hope the authors find them useful:

1. Introduction, L 33-35: Some readers may understand regarding FMA, but many others don't. I suggest explaining briefly about FMA. Which part of fishing zone in Aceh waters belongs to FMA 571 and which one under FMA 572?
2. Introduction, L 38: "...still being done conventionally". What does the authors mean by done conventionally? Is it done by morphological-based analyses?
3. Introduction, paragraph 3, L 47-58: these sentences can be separated from paragraph 3 to create paragraph 4. I also recommend adding some sentences on the promise of DNA barcoding for species identification
4. 2.1 Sampling site, L 66: Lampulo traditional fish market or Kutaradja fishing port? Which one is correct? The authors should avoid making this kind of mistake
5. 2.1 Sampling site: did the authors record the geographic coordinate of the sampling site? If yes I recommend to mention it in this section
6. 2.1 Sampling site: what references (books, articles and other type literatures) were used for morphological identification and taxonomic nomenclature? The authors should explain methods and references used for morphological observation in this section
7. 2.1 Sampling site: Are the specimens used in this study were stored in a specimen depository with voucher number? If yes please mention it. Deposition of specimens with voucher numbers are important e.g., other researcher are able to verify the identified specimens or it can provide credibility of the article
8. PCR condition at the section 2.3 can be moved to section 2.2. Then the section 2.2 will be -> 2.2 DNA extraction and PCR condition, or it can be -> 2.2 DNA extraction and PCR amplification. In this section the authors can insert additional information which are lacking e.g., what is the next step after purification of PCR product? Sequencing process? Where did the sequencing process take place? at the same lab or being sent to the outsource company?
9. Meanwhile, I suggest section 2.3 can be renamed into -> 2.3 Sequence alignment and data analyses, put information in section 2.4 in this section. Then section 2.4 Phylogenetic analyses can be deleted. In section 2.3, the authors can insert additional information such as explain method for editing sequences (alignment of forward and revers primers, trimming sequences etc.). The authors can also expand explanation on how to create the dataset: It seems for Perciformes, all of the sequences for phylogenetic reconstruction came from this study. As for Clupeiformes, Beryciformes, Pleuronectiformes, and Scorpaeniformes, all of the

sequences to build phylogenetic tree were from this study whereas many sequences for Carangidae, Scombridae, and Serranidae were retrieved from the GenBank database. What makes the strategy different?

10. Section 2.4: Did the authors calculated within and between species (or between genera) genetic divergence? If yes, please mention it in this section
11. Results and Discussion, section 3.1: I recommend inserting general information in the beginning of the paragraph e.g., that the pair of primers could amplify the target region for wide range of marine fish species, thus, showed its effectiveness and efficiency to be used as a standard for molecular identification at species level.
12. Results and Discussion, section 3.1: Forty-seven sequences belong to how many species?
13. Results and Discussion: are species in the phylogenetic tree clustered together in a monophyletic clade? Did results from phylogenetic analyses suggest misplace of phylogenetic position of a particular species? It is important to state this information in the MS
14. I found in L 96 -> 3.1 Results and discussion. Then in L 145 appears Discussion section. Which one is correct?
15. Results and Discussion: the authors provided IUCN red list categories in the Table 1. However, no further discussion developed in the MS. In my opinion, discussion on the basis of IUCN red list is important and this point match towards the storyline develops by the authors in the introduction section.
16. Consider circumvent placing simple reference in the MS (for example L 195-199). Citation of references should be made to provide important and crucial information for readers.
17. Conclusion: the authors may address comprehensive conclusion in this section e.g., how findings from this study will help stakeholders to monitor and to conserve marine fish diversity in this region.
18. Figure 3: how readers can instantly see the difference between sequences obtain from this study with those retrieved from the GenBank? Consider revise presentation of the figure
19. References: Many references cited in the MS are in Bahasa Indonesia, the authors can cite them only if it is important and relevant.
20. Replace Mega 7 -> MEGA7 or MEGA 7

**General comments:**

The manuscript 71955-241662-RV reports the importance of molecular method to identify species using a molecular approach (DNA barcoding with COI gene marker). The result of this study is quite straightforward. I found that the manuscript is interesting and important, especially on how the tool (DNA barcoding) shows its effectiveness to identify species to discriminate closely related taxa when morphological identification can be difficult to resolve the species ambiguity, and also determine haplotypes of the three genus (Scrombridae, Serranidae, and Carangidae). Before the manuscript can be accepted, I provide my comments below and hope the authors find it useful to improve the quality of the manuscript:

1. Introduction
  - a. The introduction is good, but it would be better using the current papers (last 7 years, > 2015).
  - b. Please look at the rules how to write the citation in this journal
2. Material and methods
  - a. Line 78, it should be NanoDrop
  - b. Please check how to cite the references (look at the template of this journal)
  - c. In the section 2.4. which program that you use to get consensus sequence of each sample (ex. Bioedit?, MEGA, DNASTAR? or others)?. This is due to COI is coding gene and you must consider about the stop codon
  - d. Which program that you use to identify each sample (ex. BLAST? Identification Engine from BOLD? Or others). Please explain it in this section
  - e. Which program you use for alignment? Please explain it in this section
  - f. Which program you use to determine haplotypes? Please explain it in this section
3. Results
  - a. How to identity each sample? You used BLAST or identification engine from BOLD. How many range identity percentage you got from the analysis
  - b. In section 3.2. How many genus, species and famili in order Perciformes you identified
  - c. In section 3.3. How many genus, species and famili in order Clupeiformes and other orders you identified
  - d. Figure 3 do not show the clear haplotypes that you report. It would be better you use NETWORK program to show about haplotypes including unique haplotypes even sharing haplotypes between population of each species.
  - e. In the Figure 1, 2, and 3, the phylogenetic tree was reconstructed using sequences from this study, and from the GenBank. Are these sequences resulted from a published article? If yes, I recommend the authors to cite the article.

4. Discussion
  - a. Please check how to cite the references (look at the template of this journal)
  - b. It would be better the author explain the reasons why unique haplotype of species identified occur in the area of this study
5. Conclusions
  - a. Make it shorter and no need explanation such as in line 238.
  - b. Line 238-245 should be put in Results and not in conclusion
6. References  
Please follow the rules of the JTBB

# Molecular identification and phylogenetic tree reconstruction of marine fish species from the Kutaradja fish market, Banda Aceh

## Abstract

The enormous potential of marine resources possessed by Banda Aceh Province is expected to be utilised optimally. Accuracy in identifying marine fish resources 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, which is commonly used by several scientists. The results obtained 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 biology studies and other fisheries studies to support the sustainable utilisation of marine fisheries potential in Banda Aceh.

Keywords: molecular, DNA barcoding, haplotype analysis, Aceh, marine fish

## 1. Introduction

Aceh is the westernmost province of the Indo-Malaya Archipelago (IMA), an area known as a hot spot of tropical marine biodiversity (Gaither et al. 2011; Veron et al. 2009; Bellwood and Meyer 2009; Hoeksema 2007; Briggs 2005). This province has a high level of fisheries potential, with water area reaching 295,370 km<sup>2</sup> and a coastline length corresponding to 6.3 km<sup>2</sup> (Mukhtar, 2017). One of the centres of fishing activity and the most significant fish

26 landing site in Aceh is the Fishing Port of Kutaradja. Marine fisheries production at this  
27 fishing port increased from 8,922 tons in 2013 to 12,305 tons in 2017 (Mardhatillah et al.  
28 2019, Yeni and Naufal 2017, Yusuf 2003). The fish landing site suffered massive damage  
29 due to the tsunami that struck Aceh Province and was rebuilt in 2004 (Zulmaidah et al. 2015).  
30 The rebuilding of the Kutaradja fishing port has revived the economy and fisheries activities  
31 in the Banda Aceh region.

32 Regarding the fishing grounds for fishers at this fishing port, all the fishing zones include the  
33 Indian Ocean, Andaman Sea, and Malacca Strait. Two of the three fishing zones are included  
34 within two out of the 11 Indonesian Fisheries Management Areas (FMA), namely FMA 571  
35 and 572. In the framework of fisheries management policies in Indonesia, the 11 FMAs  
36 stretch from the Malacca Strait in the west of Indonesia to the Arafura Sea in the east of  
37 Indonesia (Damanik et al. 2016). The level of utilization of pelagic and demersal fish  
38 resources in the two FMAs is categorized in the overexploited category (Salmarika and  
39 Wisudo 2019, Suman et al. 2017).

40 Previous research on the types of fish landed by the many traditional fishers of the Kutaradja  
41 fishing port were conducted based on fish morphology and anatomy. From the inventory  
42 carried out at the Kutaradja fishing port, 11 species were identified (Munawwarah et al. 2016).  
43 However, another report on the types of marine fish species in Simeuleu Island identified  
44 around 77 marine fish species which are members of 54 genus, 26 families, and seven orders  
45 (Batubara et al. 2017). The reef-associated fish inventory at Ulee Lheue, Banda Aceh, also  
46 mentioned that there were 87 species of reef fishes from 28 families in this location (Fadli et  
47 al. 2019). In different areas (i.e. Lhoknga and Lhok Mata Ie Beaches) 25 fish species which are  
48 members of eight orders, 11 families, and 19 genera were recorded from 51 fish samples (Nur  
49 et al. 2019); 71 species were identified in Pusong Bay, Lhokseumawe belonging to 54 genus,  
50 37 families and 15 orders (Damora et al. 2020); 50 species were identified in Weh Island,

51 Sabang belonging to 24 families and eight orders (Zulfahmi et al. 2022). The morphological  
52 approach is the most widely used method in many regions in Indonesia, including in Banda  
53 Aceh. This research identifies marine fish at the molecular level in the Cytochrome C Oxidase  
54 subunit I (COI) region of the mitochondrial gene to complete the morphological identification  
55 that was also carried out. This COI Region is the region that some gene markers have agreed  
56 on globally for molecular identification. Research on barcoding of several aquatic biota has  
57 been carried out such as for marine fish in Australia (Ward et al. 2005), marine fish in India  
58 (Lakra et al. 2011), marine fish in Turkey (Keskİn and Atar 2013), marine fish in China (Wang  
59 et al. 2012, Zhang and Hanner 2012), and marine fish in Taiwan (Bingpeng et al. 2018, Chang  
60 et al. 2017). Whereas research on fish molecular identification in Aceh has been carried out on  
61 some species such as groupers (Kamal et al. 2019), and *Scomber* spp. (Edwarsyah et al. 2019).  
62 This research is the first study to carry out molecular identification on the marine fish landed  
63 at the fishing port of Kutaradja.

64 The purpose of this research is to identify marine fish down to species level by using a  
65 molecular approach to increase the accuracy of species level identification. In addition, the  
66 research aims to identify Aceh's potential haplotype for the Scombridae, Serranidae, and  
67 Carangidae groups, which are pelagic fish resources with significant economic value. DNA  
68 Barcoding will strengthen genetic information availability and it can be used for other studies  
69 such as breeding, fishery management, as well as conservation (Afriyie et al. 2019). One of  
70 the studies which is essential is haplotype analysis. Haplotype analysis can only be conducted  
71 based on genetic information, especially the DNA sequences from the number of unique  
72 species in a particular region.

## 73 **2. Materials and methods**

### 74 **2.1 Sampling site**



75 A total of 47 fish samples were collected from the Kutaradja fish market close to the  
76 Kutaradja fish landing on 19 July 2019 (5°35'09"N -95°19'06"E) (Nasution et al. 2019).  
77 Morphological identification and species confirmation have been carried out together with  
78 the molecular identification carried out in this study. No specific permit was required for this  
79 study, and a digital camera was used to take individual photographs. All samples collected  
80 from the fish market were already dead upon purchasing. All specimens have been deposited  
81 to the Fisheries Laboratory, Faculty of Fisheries and Marine, Universitas Airlangga.

## 82 **2.2 DNA extraction**

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

## 90 **2.3 PCR amplification and sequencing**

91 One set of universal fish primer targeting cytochrome c oxidase I (COI) region, BCL-BCH  
92 (Baldwin et al. 2009, Handy et al. 2011), was used to obtain the partial sequences of each  
93 gene. The PCR mixture (20µL) included 11.2 µL ultra-pure water, 1 µL primer forward and  
94 reverse (0.5 µM), 0.2 µL Ex Taq DNA polymerase (TaKaRa, Japan), 2 µL 10X ExTag  
95 Buffer, 2 µL dNTPs (1 µM, TaKaRa, Japan), and 2 µL genomic DNA as template. The PCR  
96 condition was carried out under the following setting: 95°C for 5 min in initial denaturation,  
97 followed by denaturation at 95°C for 30 s in 40 cycles, 50°C for 30 s in annealing, and 72°C  
98 for 45 s in extension step, and a final extension at 72°C for 5 min. The PCR products were

99 purified with the AccuPrep®Gel purification kit (Bioneer, Korea). All PCR products were  
100 sent to Macrogen (Seoul, Korea) for sequencing.

## 101 **2.4 Sequence alignment and data analyses**

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

117

## 118 **3. Results**

### 119 **3.1 Species Identification**

120 In this study, a pair of universal COI primers succeeded in obtaining DNA target  
121 sequences of more than 600 bp. This strengthens previous research which has also succeeded  
122 in using these primers in molecular identification down to the species level (Pringgennis and  
123 Susilowati 2016, Serdiati et al. 2020). Here, we report the identification of marine fish from

124 the Kutaradja fish market, Aceh which is one of the center for fisheries in the province. A  
125 total of 47 COI sequences were generated representing 37 species, 33 genera, 19 families,  
126 and five orders with % identity ranging between 99-100% when compared to the GenBank  
127 dataset on BLASTN online system. Common names, taxonomic designation, habitat, IUCN  
128 list, as well as the GenBank accession number for all specimens are listed in Table 1. The  
129 sequencing of the COI gene produced more than 600 nucleotide base pairs per taxon. The un-  
130 ambiguity and simplicity was observed among all the sequences and no stop codons,  
131 deletions, and insertions were observed in all the sequences. Here, we cluster them into two  
132 groups in phylogenetic reconstruction, namely “Perciformes” and “other order”.

133

### 134 **3.2 Perciformes**

135 From the total of 37 samples, we successfully identified 31 species from 14 families under  
136 Perciformes. The nucleotide frequencies of COI sequences are 29.65% (T/U), 23.95% (A),  
137 28.80% (C), and 17.6% (G). The average of transitional pair ( $si=5.07$ ) was lower than the  
138 average of transvertional pair ( $sv=14.86$ ) with an overall transition/transversion ratio bias of  
139 1.57. The phylogenetic tree was constructed from the COI sequences for the Perciformes and  
140 shows that the average K2P distance within taxonomic levels measured for COI sequences is  
141 0.226 (Figure 1).

142

### 143 **3.3 Clupeiformes and Others**

144 In addition to Perciformes, Clupeiformes were also identified from 6 samples which were  
145 distributed in three species and three families. For the rest of the samples, one species was  
146 from Scorpaeniformes (*Platycephalus* sp.), one species from Pleuronectiformes (*Psettodes*  
147 *erumei*), and one species from Beryciformes (*Myripristis berndti*). The nucleotide  
148 frequencies of the COI sequences were 28.17% (T/U), 23.04% (A), 30.11% (C), and 18.68%

149 (G). The average of transitional pair (si=1.43) was lower than the average of transversional  
150 pair (sv=22.13) with an average transition/transversion bias of 8.71. The phylogenetic tree  
151 was constructed using the COI sequences for the small number order, including the  
152 Clupeiformes, Beryciformes, Pleuronectiformes, and Scorpaeniformes (Figure 2). The  
153 average K2P distance within taxonomic levels measured for COI sequences is 0.214.

154

### 155 **3.4 The haplotype of Scombridae, Serranidae, and Carangidae from Aceh**

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

169 In the Scombridae family group, potential haplotypes were found in *Auxis thazard*  
170 fish (MN257554) which differed from Chinese, Indian, and Spanish haplotypes with a  
171 genetic distance of 0.002. While in the Serranidae family, haplotypes were found in *Variola*  
172 *albimarginata* fish (MN257516) and *Cephalopholis sonnerati* (MN257517). This *Variola*  
173 *albimarginata* species (MN257516) has similarities with sequences from India but is

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

179

## 180 **Discussion**

181         Research on molecular identification is now extensive in the field of fisheries and  
182 marine sciences. In this study, molecular identification is used to complete the morphological  
183 identification and, at the same time, determine the position of the species identified in the  
184 phylogenetic tree created. Conventional identification that has been done at this time still face  
185 obstacles with the difficulty of getting taxonomists in the process of determining species, in  
186 addition to the long time period required for the identification process, errors in identification  
187 also still occur in some cases. By doing a combination identification approach, the results is  
188 expected to be more valid in identifying the fish species obtained.

189         In this study, several marine fish that were landed at the Kutaradja Fishing Port are  
190 part of the essential fishery commodity in Banda Aceh. After the 2004 tsunami disaster in this  
191 province, several activities that are able to mobilize economic activities continue to be carried  
192 out, including capture fisheries activities in the Kutaradja Fishing Port (Zulmaidah et al.  
193 2015). Previous studies have also reported the identification of marine fish species from  
194 Kutaradja Fishing Port (Lampulo). There are still inaccurate information regarding marine  
195 fish identification in some reports. Some identification were also only done based on  
196 morphological-based characteristics and were not done by taxonomists, the results of which  
197 may be incorrect for species justification. In an earlier report, the species *Sardinella sirin*  
198 (*Serranidae*) was reported to exist in this Kutaradja (Lampulo) Fishing Port (Munawwarah et

199 al. 2016). Still, an inaccurate determination of taxonomy made the identification results  
200 unreliable. The genus *Sardinella* spp. is a group of fish in the family Clupeidae, order  
201 Clupeiformes ([www.fishbase.org](http://www.fishbase.org)), and is not included in Serranidae.

202 In this report, the family Perciformes is identified as a group that dominates the fish  
203 composition caught by fishermen in Banda Aceh, who landed their catch at the Kutaradja  
204 Fishing Port. These are fish used for human consumption that are essential export  
205 commodities with high economic value such as skipjack tuna (57%) followed by yellowfin  
206 tuna (23%) (Lubis et al. 2016). Based on the identification results, the Scombridae family is a  
207 group of pelagic fish that is quite commonly found. The types identified in this report include  
208 *Thunnus albacares*, *Auxis thazard*, and *Katsuwonus pelamis*. In addition, three species from  
209 the genus Lutjanidae (snapper) were also found, namely *Lutjanus bengalensis*, *Lutjanus*  
210 *lutjanus*, and *Lethrinus rubrioperculatus*. Other groups that are targeted by fishermen are reef  
211 fish that have significant economic value, such as groupers and carangids. The groupers  
212 identified in this study include *Epinephelus areolatus*, *Variola albimarginata*, and  
213 *Cephalopholis sonnerati*, whereas the carangids group includes *Parastromateus niger*,  
214 *Megalaspis cordyla*, *Caranx sexfasciatus*, and *Decapterus macarellus* (Table 1).

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

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

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

242 In this study, a phylogenetic tree analysis of three prominent marine fish families, namely  
243 Scombridae, Serranidae, and Carangidae, was carried out. The results of the investigation  
244 found that the Scombridae *Auxis thazard* (Aceh) became separated from the same clade  
245 species even though it only has a genetic distance of only 0.002. This haplotype appears  
246 likely to occur due to differences compared to species populations analyzed from India,  
247 China (Xu et al. 2019), and Spain (Catanese et al. 2008). While for other haplotypes found

248 from the reef fish *Variola albimarginata* and *Cephalopholis sonnerati*, the *Variola*  
249 *albimarginata* from Aceh may be from a population previously described from the results of  
250 a study conducted in India that allows the sharing of habitats in the Indian Ocean in the  
251 Western part of Sumatra Island. Previous studies on molecular identification of *Variola*  
252 *albimarginata* species have been carried out in the Andaman Islands and Nicobar Island  
253 (Basheer et al. 2017). This area is part of Indian sea territory, which may potentially have reef  
254 fish that are of almost the same as the species in Aceh. While *Cephalopholis sonnerati* fish  
255 species also have similarities with populations from Australia and the Philippines, however  
256 they are slightly different to populations from China (Zhuang et al. 2013). The study of  
257 *Cephalopholis sonnerati* shows the possibility of differences in the structure of coral fish  
258 populations in the South China Sea with the Indian Ocean, especially in Aceh waters.  
259 Although integrated with Indian Ocean waters, no similarities with Indian populations were  
260 found in the *Cephalopholis sonnerati* sample species, similarities were only found with  
261 previous studies conducted in the Philippines (Alcantara and Yambot 2016), and Australia  
262 (Ward et al. 2005). The speciation process that occurs in coral reef ecosystems occurs with an  
263 allopathic pattern that makes geographic isolation the leading cause for the emergence of  
264 different species. However, the presence of pelagic larvae in reef fish species also becomes a  
265 big question even though it is believed that the allopatric pattern is the main speciation  
266 pattern occurring in coral reefs (Rocha and Bowen 2008).

267

#### 268 **4. Conclusions**

269 From this study, the identification of marine fish landed at the Kutaradja fishing port  
270 in Aceh confirmed 47 specimens (33 genera) of marine fish. Almost all fish species  
271 were important fishery commodities and became the main target of the Province of Banda  
272 Aceh's exports, including the yellowfin tuna (*Thunnus albacares*) and the skipjack tuna



273 (*Katsuwonus pelamis*). Perciformes is dominant among the fisheries resources landed at the  
274 Fishing Port of Kutaradja. Several coral reef fish were also identified such as Serranidae,  
275 Lethrinidae and Lutjanidae which are also popular as consumption fish. More in-depth  
276 research on haplotype analysis using suitable application is very much needed to maintain a  
277 record of the genetic biodiversity present in the waters of Banda Aceh, Indonesia.

278

#### 279 **Author contribution**

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

283

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289

#### 290 **Conflict of Interest**

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

293

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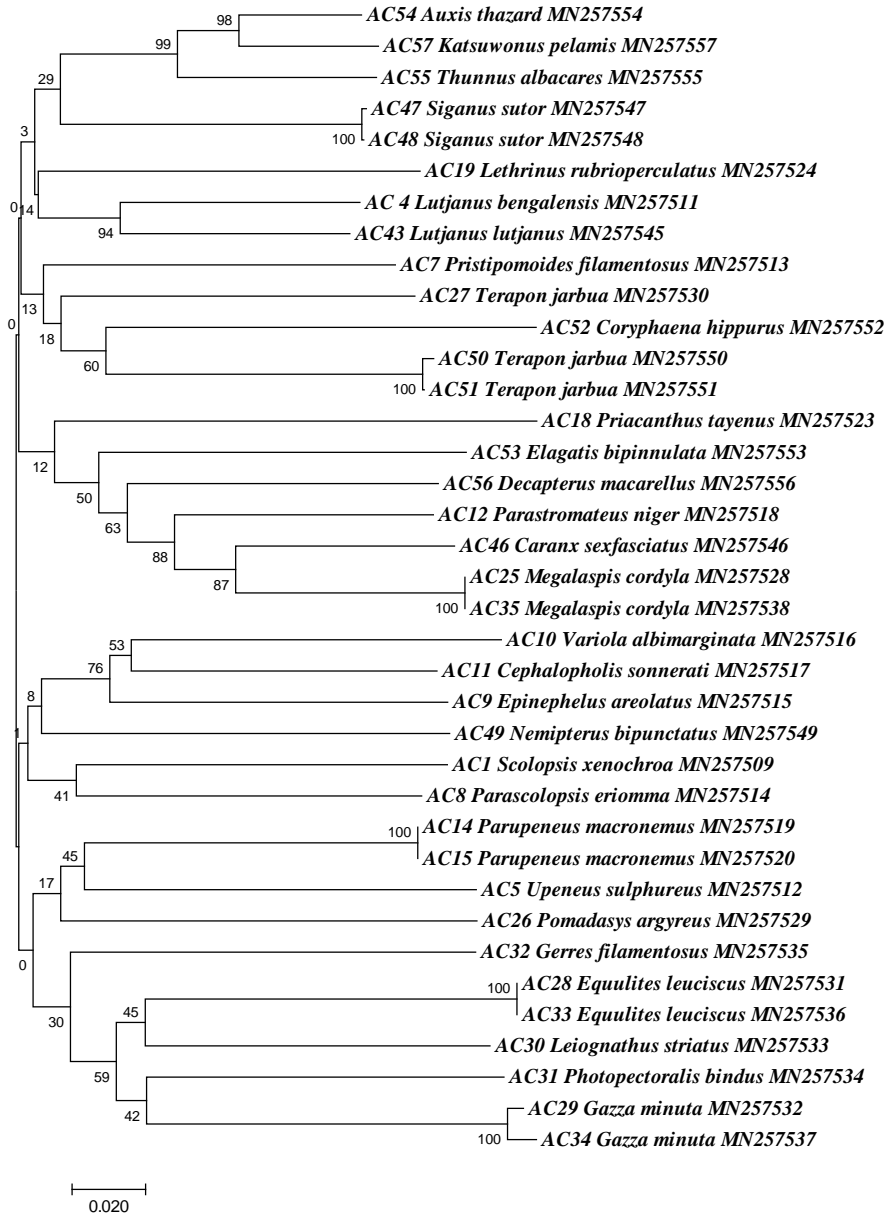
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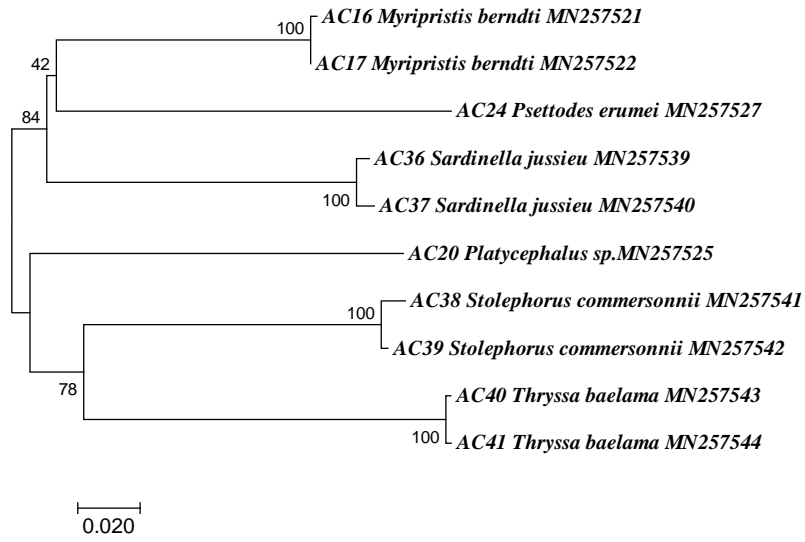
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445 **Figure 1.** Phylogenetic tree of several Perciformes order by Neighbor-Joining tree algorithm

446 using Mega7

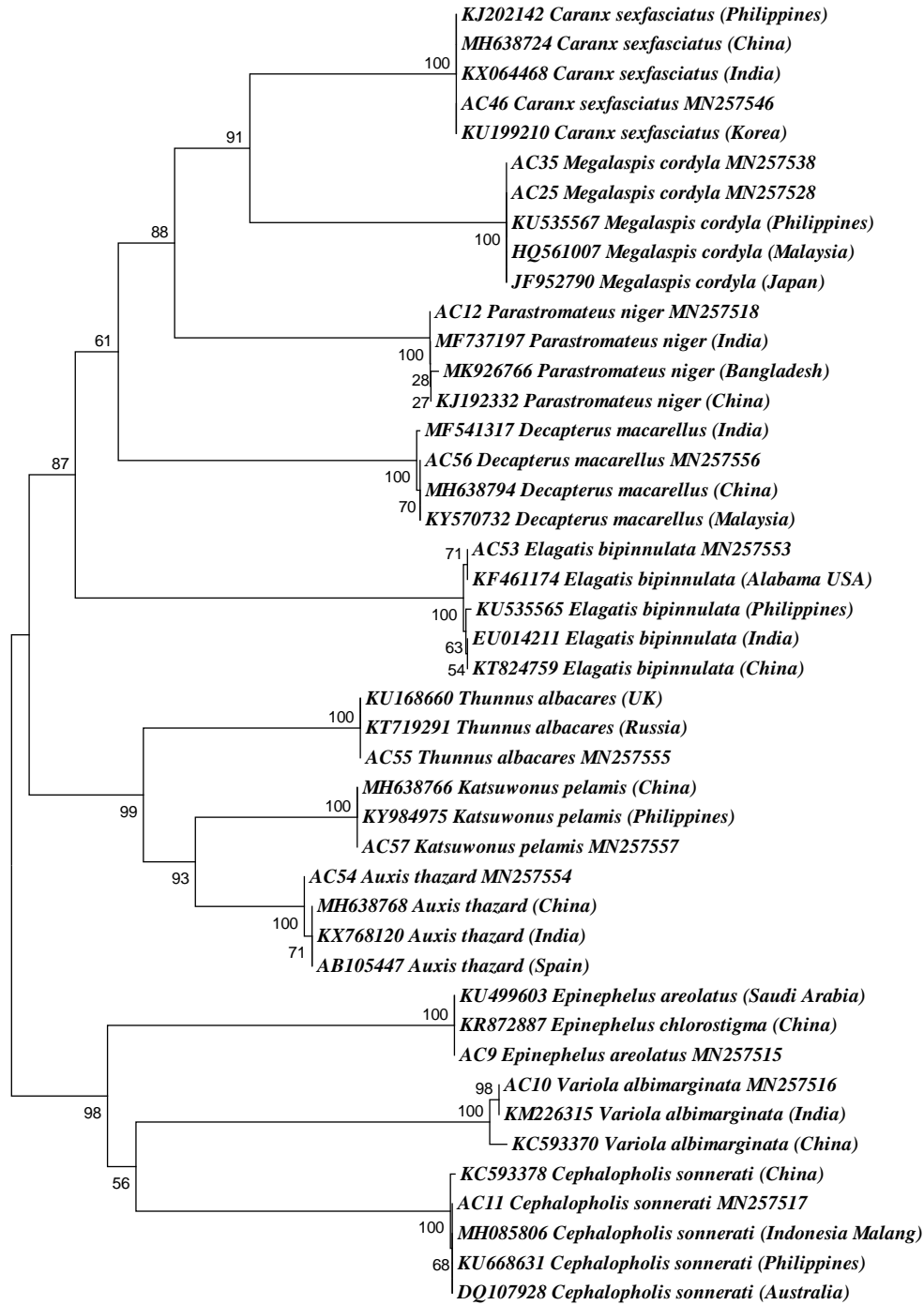
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449 **Figure 2.** Phylogenetic tree of Clupeiformes including Beryciformes, Pleuronectiformes, and  
 450 Scorpaeniformes by Neighbor-Joining tree algorithm using Mega7





451

0.020

452 **Figure 3.** Phylogenetic tree reconstruction of three families (Carangidae, Scombridae, and  
 453 Serranidae) by Neighbor-Joining algorithm using Mega7

454 **Table 1.** The marine fish species list was identified by COI region from Lampulo marine fish  
 455 landing station, Banda Aceh, Indonesia

No.	ID (AC).	Species Name	Family	GenBank Acc No.	Order	Common name	Habitat	IUCN list
1	16	<i>Myripristis berndti</i>	Holocentridae	MN257521	Beryciformes	Blotcheye soldierfish	Indo-Pacific and Eastern Pacific	LC
2	17	<i>Myripristis berndti</i>	Holocentridae	MN257522	Beryciformes	Blotcheye soldierfish	Indo-Pacific and Eastern Pacific	LC
3	36	<i>Sardinella jussieu</i>	Clupeidae	MN257539	Clupeiformes	Mauritian sardinella	Western Indian Ocean	DD
4	37	<i>Sardinella jussieu</i>	Clupeidae	MN257540	Clupeiformes	Mauritian sardinella	Western Indian Ocean	DD
5	38	<i>Stolephorus commersonnii</i>	Engraulidae	MN257541	Clupeiformes	Commerson's anchovy	Indo-West Pacific	LC
6	39	<i>Stolephorus commersonnii</i>	Engraulidae	MN257542	Clupeiformes	Commerson's anchovy	Indo-West Pacific	LC
7	40	<i>Thryssa baelama</i>	Engraulidae	MN257543	Clupeiformes	Baelama anchovy	Indo-Pacific	LC
8	41	<i>Thryssa baelama</i>	Engraulidae	MN257544	Clupeiformes	Baelama anchovy	Indo-Pacific	LC
9	1	<i>Scolopsis xenochroa</i>	Nemipteridae	MN257509	Perciformes	Oblique-barred monocle bream	Indo-West Pacific	NE
10	4	<i>Lutjanus bengalensis</i>	Lutjanidae	MN257511	Perciformes	Bengal snapper	Indo-West Pacific:	NE
11	5	<i>Upeneus sulphureus</i>	Mullidae	MN257512	Perciformes	Sulphur goatfish	Indo-West Pacific	LC
12	7	<i>Pristipomoides filamentosus</i>	Lutjanidae	MN257513	Perciformes	Crimson jobfish	Indo-Pacific	LC
13	8	<i>Parascolopsis eriomma</i>	Nemipteridae	MN257514	Perciformes	Rosy dwarf monocle bream	Indo-West Pacific	NE
14	9	<i>Epinephelus areolatus</i>	Serranidae	MN257515	Perciformes	Areolate grouper	Indo-Pacific	LC
15	10	<i>Variola albimarginata</i>	Serranidae	MN257516	Perciformes	White-edged lyretail	Indo-Pacific	LC
16	11	<i>Cephalopholis sonnerati</i>	Serranidae	MN257517	Perciformes	Tomato hind	Indo-Pacific	LC
17	12	<i>Parastromateus niger</i>	Carangidae	MN257518	Perciformes	Black pomfret	Indo-West Pacific	LC
18	14	<i>Parupeneus macronemus</i>	Mullidae	MN257519	Perciformes	Long-barbel goatfish	Indo-West Pacific	LC
19	15	<i>Parupeneus macronemus</i>	Mullidae	MN257520	Perciformes	Long-barbel goatfish	Indo-West Pacific	LC
20	18	<i>Priacanthus tayenus</i>	Priacanthidae	MN257523	Perciformes	Purple-spotted bigeye	Indo-West Pacific	LC
21	19	<i>Lethrinus rubrioperculatus</i>	Lethrinidae	MN257524	Perciformes	Spotcheek emperor	Indo-Pacific	LC
22	25	<i>Megalaspis cordyla</i>	Carangidae	MN257528	Perciformes	Torpedo scad	Indo-West Pacific	LC
23	26	<i>Pomadasys argyreus</i>	Haemulidae	MN257529	Perciformes	Bluecheek silver grunt	Indo-West Pacific	NE

24	27	<i>Terapon jarbua</i>	Terapontidae	MN257530	Perciformes	Jarbua terapon	Indo-Pacific	LC
25	28	<i>Equulites leuciscus</i>	Leiognathidae	MN257531	Perciformes	Whipfin ponyfish	Indo-West Pacific	LC
26	29	<i>Gazza minuta</i>	Leiognathidae	MN257532	Perciformes	Toothpony	Indo-Pacific	LC
27	30	<i>Leiognathus striatus</i>	Leiognathidae	MN257533	Perciformes	Toothpony	Western Indian Ocean	NE
28	31	<i>Photopectoralis bindus</i>	Leiognathidae	MN257534	Perciformes	Orangefin ponyfish	Indo-West Pacific	NE
29	32	<i>Gerres filamentosus</i>	Gerreidae	MN257535	Perciformes	Whipfin silver-biddy	Indo-Pacific	LC
30	33	<i>Equulites leuciscus</i>	Leiognathidae	MN257536	Perciformes	Whipfin ponyfish	Indo-West Pacific	LC
31	34	<i>Gazza minuta</i>	Leiognathidae	MN257537	Perciformes	Toothpony	Indo-Pacific	LC
32	35	<i>Megalaspis cordyla</i>	Carangidae	MN257538	Perciformes	Torpedo scad	Indo-West Pacific	LC
33	43	<i>Lutjanus lutjanus</i>	Lutjanidae	MN257545	Perciformes	Bigeye snapper	Indo-West Pacific	LC
34	46	<i>Caranx sexfasciatus</i>	Carangidae	MN257546	Perciformes	Bigeye trevally	Indo-Pacific	LC
35	47	<i>Siganus sutor</i>	Siganidae	MN257547	Perciformes	Shoemaker spinefoot	Indian Ocean	LC
36	48	<i>Siganus sutor</i>	Siganidae	MN257548	Perciformes	Shoemaker spinefoot	Indian Ocean	LC
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>Katsuwonus pelamis</i>	Scombridae	MN257557	Perciformes	Skipjack tuna	Cosmopolitan in tropical and warm-temperate waters	LC
46	24	<i>Psettodes erumei</i>	Psettodidae	MN257527	Pleuronectiformes	Indian halibut	Indo-West Pacific	NE
47	20	<i>Platycephalus sp.</i>	Platycephalidae	MN257525	Scorpaeniformes	Bartail flathead	Indo-West Pacific	DD

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

457

458 **Table 2.** Alignment result of several marine fish species from Aceh showing nucleotides  
 459 different from the references (GenBank database) based on Cluastal 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	-	-	-

1 **Molecular identification and phylogenetic tree reconstruction of marine fish species**  
 2 **from the Fishing Port of Kutaradja, Banda Aceh**

3 **For our recheck (if there is an update data), please rewrite for the author and affiliation**

4 **Abstract**

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

17  
 18 Keywords: molecular, DNA barcoding, haplotype analysis, Aceh, marine fish

19  
 20 **1. Introduction**

21 Aceh is the westernmost province of the Indo-Malaya Archipelago (IMA), an area known as  
 22 a hot spot of tropical marine biodiversity (Gaither et al. 2011, Veron et al. 2009, Bellwood  
 23 and Meyer 2009; Hoeksema 2007, Briggs 2005). This province has a high fisheries potential,  
 24 with a water area reaching 295,370 km<sup>2</sup> and a coastline length of 2,666.3 km (Mukhtar 2017).  
 25 One of the centres of fishing activity and the most significant fish landing site in Aceh is the

**Commented [U1]:** Mohon bisa ditambahkan untuk Authirnya Hyun-Woo Kim<sup>3</sup>

Afiliasi:  
 3. Department of Marine Biology, Pukyong National University, Busan 48513, Korea

**Commented [a2]:** Please correct grammatical errors throughout the manuscript

Accurate in the identification → Accuracy in identifying

**Commented [a3]:** Please follow our template

26 Fishing Port of Kutaradja. Marine fisheries production at this fishing port increased from  
27 8,922 tons in 2013 to 12,305 tons in 2017 (Mardhatillah et al. 2019, Yeni and Naufal 2017,  
28 Yusuf 2003). The fish landing site suffered massive damage due to the tsunami that struck  
29 Aceh Province and was rebuilt in 2004 (Zulmaidah et al. 2015). The rebuilding of the  
30 Kutaradja fishing port has revived the economy and fisheries activities in the Banda Aceh  
31 region.

32 Regarding the fishing grounds for fishers at this fishing port, all the fishing zones include the  
33 Indian Ocean, Andaman Sea, and Malacca Strait. Two of the three fishing zones are included  
34 within two out of the 11 Indonesian Fisheries Management Areas (FMA), namely FMA 571  
35 and 572. Since 2009, Indonesia has determined the management of territorial waters into  
36 several areas according to Law no. 31 of 2004 in conjunction with Law No. 41 of 2009  
37 (Suman et al. 2017), which called Indonesian Fisheries Management Areas (FMA). The  
38 management area in western Sumatra includes FMA 571 in the Malacca Strait (Damanik et  
39 al. 2016) and FMA 572 in the Indian Ocean waters west of Sumatra. In the framework of  
40 fisheries management policies in Indonesia, the 11 FMAs stretch from the Malacca Strait in  
41 the west of Indonesia to the Arafura Sea in the east of Indonesia (Damanik et al. 2016). The  
42 level of utilization of pelagic and demersal fish resources in the two FMAs is categorized in  
43 the overexploited category (Salmarika and Wisudo 2019, Suman et al. 2017).

44 Previous research on the types of fish landed by the many traditional fishers of the Kutaradja  
45 Fishing Port were conducted based on fish morphology and anatomy. From the inventory  
46 carried out at the Kutaradja Fishing Port, 11 species were identified (Munawwarah et al.  
47 2016). However, another report on the types of marine fish species in Simeuleu Island  
48 identified around 77 marine fish species which are members of 54 genera, 26 families, and  
49 seven orders (Batubara et al. 2017). The reef-associated fish inventory at Ulee Lheue, Banda  
50 Aceh, also mentioned that there were 87 species of reef fishes from 28 families in this

Commented [U4]: Q1 regarding FMA

Commented [U5]: Q2. still being done conventionally = identification based on morphology and anatomy.

51 location (Fadli et al. 2019). In different areas (i.e. Lhoknga and Lhok Mata Ie Beaches) 25  
52 fish species which are members of eight orders, 11 families, and 19 genera were recorded  
53 from 51 fish samples (Nur et al. 2019); 71 species were identified in Pusong Bay,  
54 Lhokseumawe belonging to 54 genus, 37 families and 15 orders (Damora et al. 2020); 50  
55 species were identified in Weh Island, Sabang belonging to 24 families and eight orders  
56 (Zulfahmi et al. 2022). The morphological approach is the most widely used method in many  
57 regions in Indonesia, including in Banda Aceh.

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

69 The purpose of this research is to identify marine fish to species level by using a molecular  
70 approach. This approach has higher accuracy of identification until species level. In addition,  
71 the research aims to identify Aceh's potential haplotype for the Scombridae, Serranidae, and  
72 Carangidae groups, which are pelagic fish resources with significant economic important.  
73 DNA Barcoding will strengthen genetic information availability and it can be used for other  
74 studies such as breeding, fishery management, as well as conservation (Afriyie et al. 2019).  
75 One of the studies which is essential is haplotype analysis. Haplotype analysis can only be

Commented [U6]: Q3. Split paragraph 3 and new paragraph 4

76 conducted based on genetic information, especially the DNA sequences from the number of  
77 unique species in a particular region.

## 78 2. Materials and methods

### 79 2.1 Sampling site

80 A total of 47 fish samples were collected from the Kutaradja Fishing Port on 19 July  
81 2019 (5°35'09"N -95°19'06"E) (Nasution et al. 2019). Morphological identification and  
82 species confirmation have been carried out together with the molecular identification carried  
83 out in this study. Morphological identification by guideline of FAO Species Identification  
84 Guide for Fishery Purposes (Carpenter and Niem 2001). No specific permit was required for  
85 this study, and a digital camera was used to take individual photographs. All samples  
86 collected from the fish market were already dead upon purchasing. All specimens have been  
87 deposited to the Fisheries Laboratory, Faculty of Fisheries and Marine, Universitas  
88 Airlangga. All specimens keep in ethanol 90% with samples code AC no 01-47.

Commented [U7]: Q6 Reference for morphological identification

Commented [U8]: Q7 Sample code

### 89 2.2 DNA extraction and PCR condition

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

Commented [a9]:  
Line 78, it should be NanoDrop

97 One set of universal fish primer targeting cytochrome c oxidase I (COI) region, BCL-  
98 BCH (Baldwin et al. 2009, Handy et al. 2011), was used to obtain the partial sequences of  
99 each gene. The PCR mixture (20µL) included 11.2 µL ultra-pure water, 1 µL primer forward  
100 and reverse (0.5 µM), 0.2 µL Ex Taq DNA polymerase (TaKaRa, Japan), 2 µL 10X ExTag

Commented [U10]: Q8 2.3 was removed and merged with 2.2.



101 Buffer, 2  $\mu$ L dNTPs (1  $\mu$ M, TaKaRa, Japan), and 2  $\mu$ L genomic DNA as template. The PCR  
102 condition was carried out under the following setting: 95°C for 5 min in initial denaturation,  
103 followed by denaturation at 95°C for 30 s in 40 cycles, 50°C for 30 s in annealing, and 72°C  
104 for 45 s in extension step, and a final extension at 72°C for 5 min. The PCR products were  
105 purified with the AccuPrep®Gel purification kit (Bioneer, Korea). The experiment was  
106 conducted at Molecular Physiology Laboratory, Department of Marine Biology, School of  
107 Fisheries Science, Pukyong National University, Busan Korea. All PCR products were sent to  
108 Macrogen (Seoul, Korea) for sequencing.

**Commented [U11]:** Q8 Lab for experiments

#### 109 2.4 Sequence alignment and data analyses

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

**Commented [a12]:** a. In the section 2.4, which program that you use to get consensus sequence of each sample (ex. Bioedit?, MEGA, DNASTAR? or others)?. This is due to COI is coding gene and you must consider about the stop codon  
b. Which program you use for alignment? Please explain it in this section  
c. Which program you use to determine haplotypes? Please explain it in this section

**Commented [U13R12]:** a. We use Chromas to check the quality of Sequences and we don't use all sequences which have a low quality (value less than 20). Low-quality sequence especially in the beginning and the last of sequences.  
b. We use MEGA7  
c. In this report, we don't analyse the haplotype in detail. We determine based on the genetic distance of the sequences which were generated by MEGA7.

**Commented [U14]:** Q10 no we just focus on molecular identification.

125

126 **3. Results**

127 **3.1 Species Identification**

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

144 **3.2 Perciformes**

145 From the total of 37 samples, we successfully identified 31 species from 14 families under  
146 Perciformes (30 genera). The nucleotide frequencies of COI sequences are 29.65% (T/U),  
147 23.95% (A), 28.80% (C), and 17.6% (G). The average of transitional pair (si=5.07) was lower  
148 than the average of transvertional pair (sv=14.86) with an overall transition/transversion ratio  
149 bias of 1.57. The phylogenetic tree was constructed from the COI sequences for the

Commented [U15]: Q14 Results and Discussion has been separated

Commented [U16]: Q11

Commented [U17]: Q12 37 species, 33 genera, 19 families

Commented [a18]: a.How to identify each sample? You used BLAST or identification engine from BOLD. How many range identity percentage you got from the analysis

Commented [U19R18]: We use BLASTN from NCBI not BOLD. We ensure that 99-100% is confirmed species.

Commented [a20]: In section 3.2. How many genus, species and famili in order Perciformes you identified

Commented [U21R20]: 30 genera (genus)

150 Perciformes and shows that the average K2P distance within taxonomic levels measured for  
151 COI sequences is 0.226 (Figure 1).

152

### 153 3.3 Clupeiformes and Others

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

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

164

### 165 3.4 The haplotype of Scombridae, Serranidae, and Carangidae from Aceh

166 In this study, the sample from Aceh had several unique potential haplotypes when  
167 compared to the same species from the GenBank database. By aligning the sequence  
168 generated with the reference sequence, some different nucleotides produced genetic  
169 variations (Table 2). The phylogenetics tree reconstruction of those sequences show that  
170 several potential haplotypes were found in this study (Figure 3). The identified haplotype in  
171 the Carangid group was found in the *Decapterus macarellus* species (MN257556) which had  
172 similarities with sequences from China and Malaysia, having a genetic distance with an  
173 Indian sequence of 0.002. Also, *Elagatis bipinnulata* (MN257553) is closer to the similarity  
174 of the sequence owned by the same type of fish (KF461174) from Alabama, USA. While the

**Commented [a22]:** a.In section 3.3. How many genus, species and famili in order Clupeiformes and other orders you identified

**Commented [U23R22]:** 3 genera (genus)

**Commented [a24]:** b.In the Figure 1, 2, and 3, the phylogenetic tree was reconstructed using sequences from this study, and from the GenBank. Are these sequences resulted from a published article? If yes, I recommend the authors to cite the article.

175 genetic distance of *Elagatis bipinnulata* with the same species is 0.003 (Philippines) and 0.02  
176 (India and China). In the Carangid group, *Caranx sexfasciatus* (MN257546) and *Megalaspis*  
177 *cordyla* (MN257528 and MN257538) species were not found to be polymorphic in the  
178 sequences obtained.

179 In the Scombridae family group, potential haplotypes were found in *Auxis thazard*  
180 fish (MN257554) which differed from Chinese, Indian, and Spanish haplotypes with a  
181 genetic distance of 0.002. While in the Serranidae family, haplotypes were found in *Variola*  
182 *albimarginata* fish (MN257516) and *Cephalopholis sonnerati* (MN257517). This *Variola*  
183 *albimarginata* species (MN257516) has similarities with sequences from India but is  
184 different from Chinese haplotypes with a genetic distance of 0.007. While species of  
185 *Cephalopholis sonnerati* (MN257517) differ only from Chinese haplotypes, this species  
186 merged in one clade with samples from the Philippines, Australia, and Indonesia with genetic  
187 distance 0.00-0.002. In *Epinephelus arelatus* species, there are no potential haplotypes and  
188 sequences obtained from samples originating from China and Saudi Arabia.

189

## 190 **Discussion**

191 Research on molecular identification is now extensive in the field of fisheries and  
192 marine sciences. In this study, molecular identification is used to complete the morphological  
193 identification and, at the same time, determine the position of the species identified in the  
194 phylogenetic tree created. Conventional identification that has been done at this time still face  
195 obstacles with the difficulty of getting taxonomists in the process of determining species, in  
196 addition to the long time period required for the identification process, errors in identification  
197 also still occur in some cases. By doing a combination identification approach, the results is  
198 expected to be more valid in identifying the fish species obtained.

199 In this study, several marine fish that were landed at the Kutaradja Fishing Port are  
200 part of the essential fishery commodity in Banda Aceh. After the 2004 tsunami disaster in this  
201 province, several activities that are able to mobilize economic activities continue to be carried  
202 out, including capture fisheries activities in the Kutaradja Fishing Port (Zulmaidah et al.  
203 2015). Previous studies have also reported the identification of marine fish species from  
204 Kutaradja Fishing Port at Lampulo. There are still inaccurate information regarding marine  
205 fish identification in some reports. Some identifications were also only done based on  
206 morphological-based characteristics and were not done by taxonomists, the results of which  
207 may be incorrect for species justification. In an earlier report, the species *Sardinella sirin*  
208 (Serranidae) was reported to exist in the Kutaradja Fishing Port (Munawwarah et al. 2016).  
209 Still, an inaccurate determination of taxonomy made the identification results unreliable. The  
210 genus *Sardinella* spp. is a group of fish in the family Clupeidae, order Clupeiformes  
211 ([www.fishbase.org](http://www.fishbase.org)), and is not included in Serranidae.

212 In this report, the family Perciformes is identified as a group that dominates the fish  
213 composition caught by fishermen in Banda Aceh, who landed their catch at the Kutaradja  
214 Fishing Port. These are fish used for human consumption that are essential export  
215 commodities with high economic value such as skipjack tuna (57%) followed by yellowfin  
216 tuna (23%) (Lubis et al. 2016). Based on the identification results, the Scombridae family is a  
217 group of pelagic fish that is quite commonly found. The types identified in this report include  
218 *Thunnus albacares*, *Auxis thazard*, and *Katsuwonus pelamis*. In addition, three species from  
219 the genus Lutjanidae (snapper) were also found, namely *Lutjanus bengalensis*, *Lutjanus*  
220 *lutjanus*, and *Lethrinus rubrioperculatus*. Other groups that are targeted by fishermen are reef  
221 fish that have significant economic value, such as groupers and carangids. The groupers  
222 identified in this study include *Epinephelus areolatus*, *Variola albimarginata*, and

223 *Cephalopholis sonnerati*, whereas the carangids group includes *Parastromateus niger*,  
224 *Megalaspis cordyla*, *Caranx sexfasciatus*, and *Decapterus macarellus* (Table 1).

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

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

245 In this report, sequences from several Acehese fish also have similarities with those  
246 collected in some previous studies, and some are unique to other sequences. Species *Auxis*  
247 *thazard* that was identified from the Kutaradja Fishing Port at Lampulo, may have been

248 caught from the area around the seas of Western Banda Aceh Province, indicating a catch  
249 distance of about 50-190 nautical miles (Salmarika and Wisudo 2019). Although it is still in  
250 the Indian Ocean region, there may be specialization in this species so that the Aceh  
251 haplotype separated from the same species in the resulting phylogenetic tree analysis.

252 In this study, a phylogenetic tree analysis of three prominent marine fish families, namely  
253 Scombridae, Serranidae, and Carangidae, was carried out. The results of the investigation  
254 found that the Scombridae *Auxis thazard* (Aceh) became separated from the same clade  
255 species even though it only has a genetic distance of only 0.002. This haplotype appears  
256 likely to occur due to differences compared to species populations analyzed from India,  
257 China (Xu et al. 2019), and Spain (Catanese et al. 2008). While for other haplotypes found  
258 from the reef fish *Variola albimarginata* and *Cephalopholis sonnerati*, the *Variola*  
259 *albimarginata* from Aceh may be from a population previously described from the results of  
260 a study conducted in India that allows the sharing of habitats in the Indian Ocean in the  
261 Western part of Sumatra Island. Previous studies on molecular identification of *Variola*  
262 *albimarginata* species have been carried out in the Andaman Islands and Nicobar Island  
263 (Basheer et al. 2017). This area is part of Indian sea territory, which may potentially have reef  
264 fish that are of almost the same as the species in Aceh. While *Cephalopholis sonnerati* fish  
265 species also have similarities with populations from Australia and the Philippines, however  
266 they are slightly different to populations from China (Zhuang et al. 2013). The study of  
267 *Cephalopholis sonnerati* shows the possibility of differences in the structure of coral fish  
268 populations in the South China Sea with the Indian Ocean, especially in Aceh waters.  
269 Although integrated with Indian Ocean waters, no similarities with Indian populations were  
270 found in the *Cephalopholis sonnerati* sample species, similarities were only found with  
271 previous studies conducted in the Philippines (Alcantara and Yambot 2016), and Australia  
272 (Ward et al. 2005). The speciation process that occurs in coral reef ecosystems occurs with an

273 allopathic pattern that makes geographic isolation the leading cause for the emergence of  
274 different species. However, the presence of pelagic larvae in reef fish species also becomes a  
275 big question even though it is believed that the allopatric pattern is the main speciation  
276 pattern occurring in coral reefs (Rocha and Bowen 2008).

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

286

#### 287 4. Conclusions

288 From this study, the identification of marine fish landed at the Kutaradja fishing port  
289 in Aceh confirmed 47 specimens (33 genera) of marine fish. Almost all fish species were  
290 important fishery commodities and became the main target of the Province of Banda Aceh's  
291 exports, including the yellowfin tuna (*Thunnus albacares*) and the skipjack tuna (*Katsuwonus*  
292 *pelamis*). Beside Perciformes, Serranidae, Lethrinidae and Lutjanidae was identified as  
293 fisheries resources of Banda Aceh. More in-depth research on haplotype analysis using  
294 suitable application (bioinformatic software) is very much needed to maintain a record of the  
295 genetic biodiversity present in the waters of Banda Aceh, Indonesia.

296

#### 297 Author contribution

**Commented [a25]:** a. Make it shorter and no need explanation such as in line 238.  
b. Line 238-245 should be put in Results and not in conclusion



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

301

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304 2016 and Molecular Physiology Laboratory, Department of Marine Biology, Pukyong  
305 National University, Korea. This paper is the initiation of the first research collaboration  
306 between Universitas Airlangga and Syiah Kuala University.

307

## 308 **Conflict of Interest**

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

311

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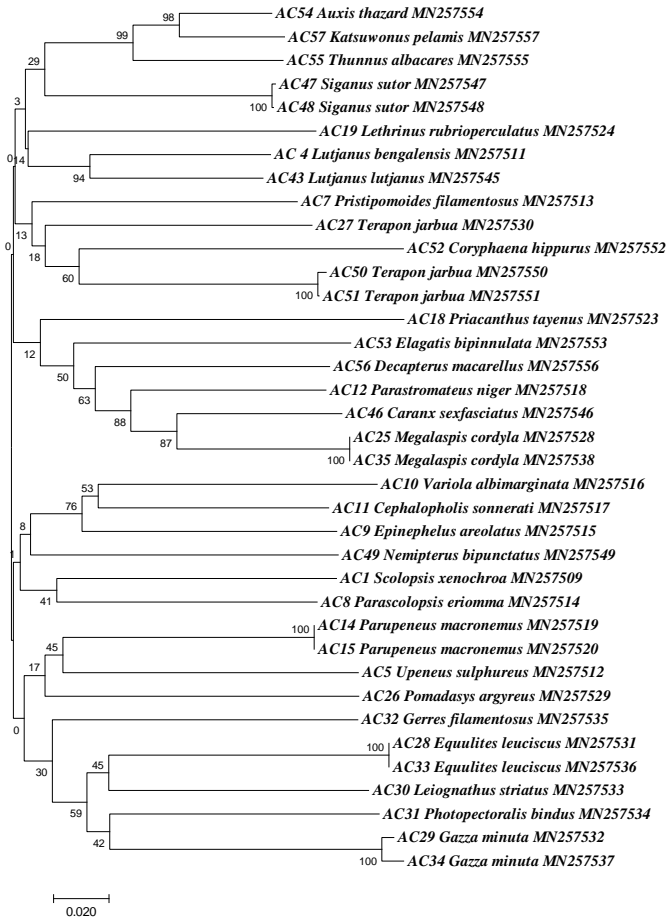
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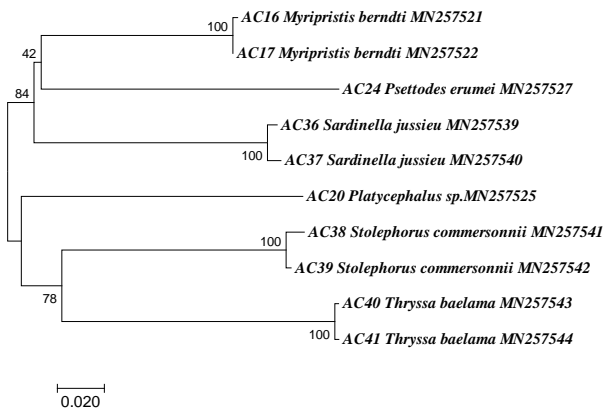


484  
485 **Figure 1.** Phylogenetic tree of several Perciformes order by Neighbor-Joining tree algorithm

486 using [Mega7](#)

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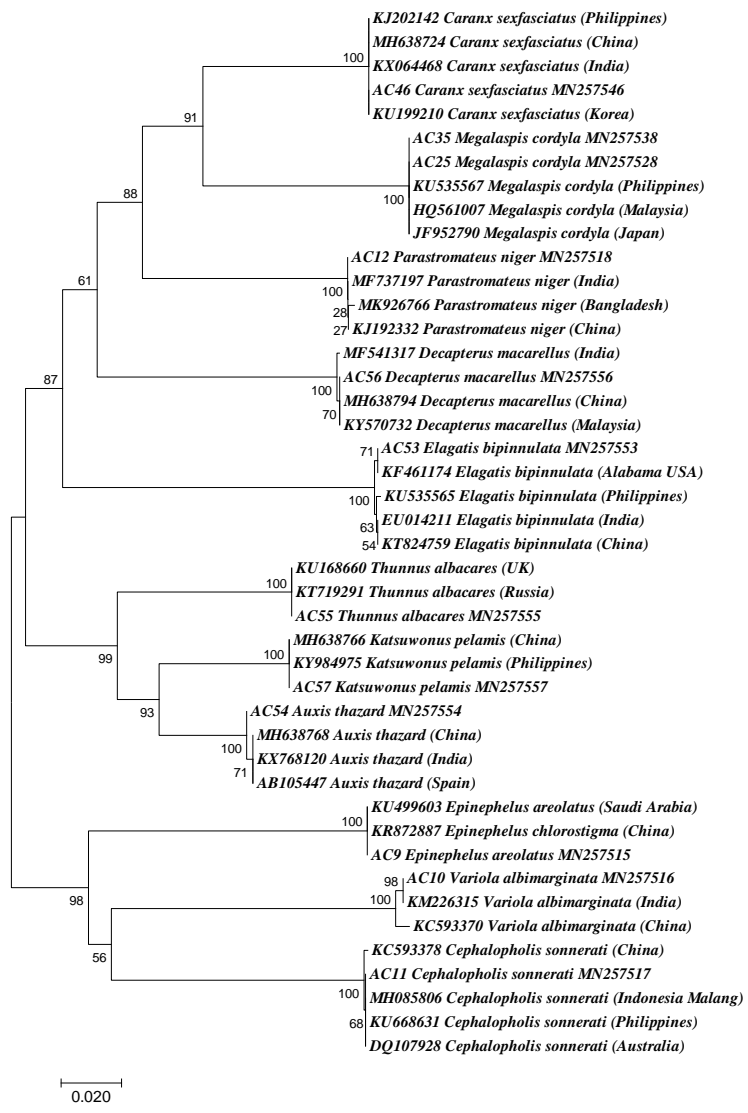


488

489 **Figure 2.** Phylogenetic tree of Clupeiformes including Beryciformes, Pleuronectiformes, and

490 Scorpaeniformes by Neighbor-Joining tree algorithm using MEGA7.





491

492 **Figure 3.** Phylogenetic tree reconstruction of three families (Carangidae, Scombridae, and

493 Serranidae) by Neighbor-Joining algorithm using MEGA7. All sequence on this figure have

494 code with AC, then another sequence has been downloaded from GenBank databased as the

495 reference.

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Commented [U32]: Q18

496 **Table 1.** The marine fish species list was identified by COI region from Lampulo fish

497 market, Banda Aceh, Indonesia

No.	ID (AC).	Species Name	Family	GenBank Acc No.	Order	Common name	Habitat	IUCN list
1	16	<i>Myripristis berndti</i>	Holocentridae	MN257521	Beryciformes	Blotcheye soldierfish	Indo-Pacific and Eastern Pacific	LC
2	17	<i>Myripristis berndti</i>	Holocentridae	MN257522	Beryciformes	Blotcheye soldierfish	Indo-Pacific and Eastern Pacific	LC
3	36	<i>Sardinella jussieu</i>	Clupeidae	MN257539	Clupeiformes	Mauritian sardinella	Western Indian Ocean	DD
4	37	<i>Sardinella jussieu</i>	Clupeidae	MN257540	Clupeiformes	Mauritian sardinella	Western Indian Ocean	DD
5	38	<i>Stolephorus commersonii</i>	Engraulidae	MN257541	Clupeiformes	Commerson's anchovy	Indo-West Pacific	LC
6	39	<i>Stolephorus commersonii</i>	Engraulidae	MN257542	Clupeiformes	Commerson's anchovy	Indo-West Pacific	LC
7	40	<i>Thryssa baelama</i>	Engraulidae	MN257543	Clupeiformes	Baelama anchovy	Indo-Pacific	LC
8	41	<i>Thryssa baelama</i>	Engraulidae	MN257544	Clupeiformes	Baelama anchovy	Indo-Pacific	LC
9	1	<i>Scolopsis xenochroa</i>	Nemipteridae	MN257509	Perciformes	Oblique-barred monocle bream	Indo-West Pacific	NE
10	4	<i>Lutjanus bengalensis</i>	Lutjanidae	MN257511	Perciformes	Bengal snapper	Indo-West Pacific:	NE
11	5	<i>Upeneus sulphureus</i>	Mullidae	MN257512	Perciformes	Sulphur goatfish	Indo-West Pacific	LC
12	7	<i>Pristipomoides filamentosus</i>	Lutjanidae	MN257513	Perciformes	Crimson jobfish	Indo-Pacific	LC
13	8	<i>Parascolopsis eriomma</i>	Nemipteridae	MN257514	Perciformes	Rosy dwarf monocle bream	Indo-West Pacific	NE
14	9	<i>Epinephelus areolatus</i>	Serranidae	MN257515	Perciformes	Areolate grouper	Indo-Pacific	LC
15	10	<i>Variola albimarginata</i>	Serranidae	MN257516	Perciformes	White-edged lyretail	Indo-Pacific	LC
16	11	<i>Cephalopholis sonnerati</i>	Serranidae	MN257517	Perciformes	Tomato hind	Indo-Pacific	LC
17	12	<i>Parastromateus niger</i>	Carangidae	MN257518	Perciformes	Black pomfret	Indo-West Pacific	LC
18	14	<i>Parupeneus macronemus</i>	Mullidae	MN257519	Perciformes	Long-barbel goatfish	Indo-West Pacific	LC
19	15	<i>Parupeneus macronemus</i>	Mullidae	MN257520	Perciformes	Long-barbel goatfish	Indo-West Pacific	LC
20	18	<i>Priacanthus tayenus</i>	Priacanthidae	MN257523	Perciformes	Purple-spotted bigeye	Indo-West Pacific	LC
21	19	<i>Lethrinus rubrioperculatus</i>	Lethrinidae	MN257524	Perciformes	Spotcheek emperor	Indo-Pacific	LC
22	25	<i>Megalaspis cordyla</i>	Carangidae	MN257528	Perciformes	Torpedo scad	Indo-West Pacific	LC
23	26	<i>Pomadasys argyreus</i>	Haemulidae	MN257529	Perciformes	Bluecheek silver grunt	Indo-West Pacific	NE

24	27	<i>Terapon jarbua</i>	Terapontidae	MN257530	Perciformes	Jarbua terapon	Indo-Pacific	LC
25	28	<i>Equulites leuciscus</i>	Leiognathidae	MN257531	Perciformes	Whipfin ponyfish	Indo-West Pacific	LC
26	29	<i>Gazza minuta</i>	Leiognathidae	MN257532	Perciformes	Toothpony	Indo-Pacific	LC
27	30	<i>Leiognathus striatus</i>	Leiognathidae	MN257533	Perciformes	Toothpony	Western Indian Ocean	NE
28	31	<i>Photopectoralis bindus</i>	Leiognathidae	MN257534	Perciformes	Orangefin ponyfish	Indo-West Pacific	NE
29	32	<i>Gerres filamentosus</i>	Gerreidae	MN257535	Perciformes	Whipfin silver-biddy	Indo-Pacific	LC
30	33	<i>Equulites leuciscus</i>	Leiognathidae	MN257536	Perciformes	Whipfin ponyfish	Indo-West Pacific	LC
31	34	<i>Gazza minuta</i>	Leiognathidae	MN257537	Perciformes	Toothpony	Indo-Pacific	LC
32	35	<i>Megalaspis cordyla</i>	Carangidae	MN257538	Perciformes	Torpedo scad	Indo-West Pacific	LC
33	43	<i>Lutjanus lutjanus</i>	Lutjanidae	MN257545	Perciformes	Bigeye snapper	Indo-West Pacific	LC
34	46	<i>Caranx sexfasciatus</i>	Carangidae	MN257546	Perciformes	Bigeye trevally	Indo-Pacific	LC
35	47	<i>Siganus sutor</i>	Siganidae	MN257547	Perciformes	Shoemaker spinefoot	Indian Ocean	LC
36	48	<i>Siganus sutor</i>	Siganidae	MN257548	Perciformes	Shoemaker spinefoot	Indian Ocean	LC
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>Katsuwonus pelamis</i>	Scombridae	MN257557	Perciformes	Skipjack tuna	Cosmopolitan in tropical and warm-temperate waters	LC
46	24	<i>Psettodes erumei</i>	Psettodidae	MN257527	Pleuronectiformes	Indian halibut	Indo-West Pacific	NE
47	20	<i>Platycephalus sp.</i>	Platycephalidae	MN257525	Scorpaeniformes	Bartail flathead	Indo-West Pacific	DD

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

500 **Table 2.** Alignment result of several marine fish species from Aceh showing nucleotides  
 501 different from the references (GenBank database) based on Cluastal 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	-	-	-

## 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 le 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

No.	ID (AC)	Species Name	Family	GenBank Acc No.	Order	Common name	Habitat	IUCN list
1	16	<i>Myripristis berndti</i>	Holocentridae	MN257521	Beryciformes	Blotcheye soldierfish	Indo-Pacific and Eastern Pacific	LC
2	17	<i>Myripristis berndti</i>	Holocentridae	MN257522	Beryciformes	Blotcheye soldierfish	Indo-Pacific and Eastern Pacific	LC
3	36	<i>Sardinella jussieu</i>	Clupeidae	MN257539	Clupeiformes	Mauritian sardinella	Western Indian Ocean	DD
4	37	<i>Sardinella jussieu</i>	Clupeidae	MN257540	Clupeiformes	Mauritian sardinella	Western Indian Ocean	DD
5	38	<i>Stolephorus commersonnii</i>	Engraulidae	MN257541	Clupeiformes	Commerson's anchovy	Indo-West Pacific	LC
6	39	<i>Stolephorus commersonnii</i>	Engraulidae	MN257542	Clupeiformes	Commerson's anchovy	Indo-West Pacific	LC
7	40	<i>Thryssa baelama</i>	Engraulidae	MN257543	Clupeiformes	Baelama anchovy	Indo-Pacific	LC
8	41	<i>Thryssa baelama</i>	Engraulidae	MN257544	Clupeiformes	Baelama anchovy	Indo-Pacific	LC
9	1	<i>Scolopsis xenochroa</i>	Nemipteridae	MN257509	Perciformes	Oblique-barred monocle bream	Indo-West Pacific	NE
10	4	<i>Lutjanus bengalensis</i>	Lutjanidae	MN257511	Perciformes	Bengal snapper	Indo-West Pacific:	NE
11	5	<i>Upeneus sulphureus</i>	Mullidae	MN257512	Perciformes	Sulphur goatfish	Indo-West Pacific	LC
12	7	<i>Pristipomoides filamentosus</i>	Lutjanidae	MN257513	Perciformes	Crimson jobfish	Indo-Pacific	LC
13	8	<i>Parascolopsis eriomma</i>	Nemipteridae	MN257514	Perciformes	Rosy dwarf monocle bream	Indo-West Pacific	NE
14	9	<i>Epinephelus areolatus</i>	Serranidae	MN257515	Perciformes	Areolate grouper	Indo-Pacific	LC
15	10	<i>Variola albimarginata</i>	Serranidae	MN257516	Perciformes	White-edged lyretail	Indo-Pacific	LC
16	11	<i>Cephalopholis sonnerati</i>	Serranidae	MN257517	Perciformes	Tomato hind	Indo-Pacific	LC
17	12	<i>Parastromateus niger</i>	Carangidae	MN257518	Perciformes	Black pomfret	Indo-West Pacific	LC
18	14	<i>Parupeneus macronemus</i>	Mullidae	MN257519	Perciformes	Long-barbel goatfish	Indo-West Pacific	LC
19	15	<i>Parupeneus macronemus</i>	Mullidae	MN257520	Perciformes	Long-barbel goatfish	Indo-West Pacific	LC
20	18	<i>Priacanthus tayenus</i>	Priacanthidae	MN257523	Perciformes	Purple-spotted bigeye	Indo-West Pacific	LC
21	19	<i>Lethrinus rubrioperculatus</i>	Lethrinidae	MN257524	Perciformes	Spotcheek emperor	Indo-Pacific	LC
22	25	<i>Megalaspis cordyla</i>	Carangidae	MN257528	Perciformes	Torpedo scad	Indo-West Pacific	LC
23	26	<i>Pomadasyus argyreus</i>	Haemulidae	MN257529	Perciformes	Bluecheek silver grunt	Indo-West Pacific	NE
24	27	<i>Terapon jarbua</i>	Terapontidae	MN257530	Perciformes	Jarbua terapon	Indo-Pacific	LC
25	28	<i>Equulites leuciscus</i>	Leiognathidae	MN257531	Perciformes	Whipfin ponyfish	Indo-West Pacific	LC
26	29	<i>Gazza minuta</i>	Leiognathidae	MN257532	Perciformes	Toothpony	Indo-Pacific	LC
27	30	<i>Leiognathus striatus</i>	Leiognathidae	MN257533	Perciformes	Toothpony	Western Indian Ocean	NE
28	31	<i>Photopectoralis bindus</i>	Leiognathidae	MN257534	Perciformes	Orangefin ponyfish	Indo-West Pacific	NE
29	32	<i>Gerres filamentosus</i>	Gerreidae	MN257535	Perciformes	Whipfin silver-biddy	Indo-Pacific	LC
30	33	<i>Equulites leuciscus</i>	Leiognathidae	MN257536	Perciformes	Whipfin ponyfish	Indo-West Pacific	LC
31	34	<i>Gazza minuta</i>	Leiognathidae	MN257537	Perciformes	Toothpony	Indo-Pacific	LC
32	35	<i>Megalaspis cordyla</i>	Carangidae	MN257538	Perciformes	Torpedo scad	Indo-West Pacific	LC
33	43	<i>Lutjanus lutjanus</i>	Lutjanidae	MN257545	Perciformes	Bigeye snapper	Indo-West Pacific	LC
34	46	<i>Caranx sexfasciatus</i>	Carangidae	MN257546	Perciformes	Bigeye trevally	Indo-Pacific	LC
35	47	<i>Siganus sutor</i>	Siganidae	MN257547	Perciformes	Shoemaker spinefoot	Indian Ocean	LC
36	48	<i>Siganus sutor</i>	Siganidae	MN257548	Perciformes	Shoemaker spinefoot	Indian Ocean	LC

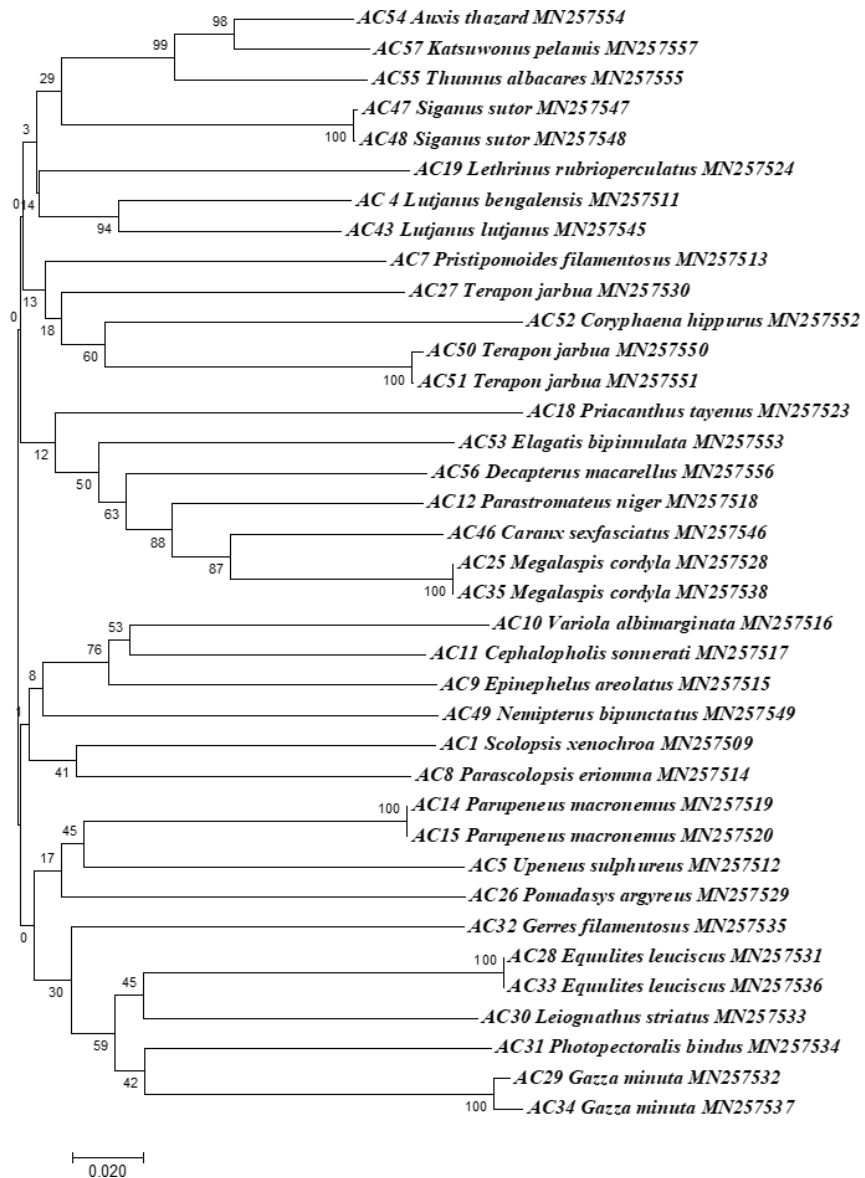
**Table 1.** Contd.

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>Katsuwonus pelamis</i>	Scombridae	MN257557	Perciformes	Skipjack tuna	Cosmopolitan in tropical and warm-temperate waters	LC
46	24	<i>Psettodes erumei</i>	Psettodidae	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).

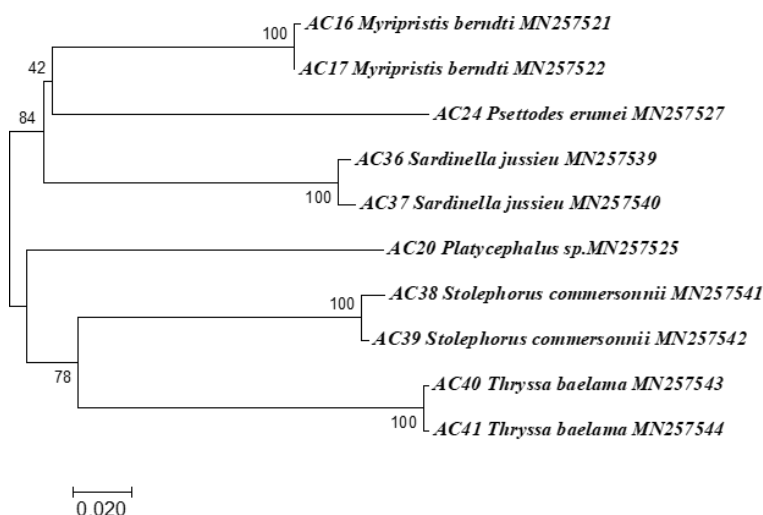


**Figure 1.** Phylogenetic tree of several Perciformes order by Neighbor-Joining tree algorithm using Mega7

### 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.



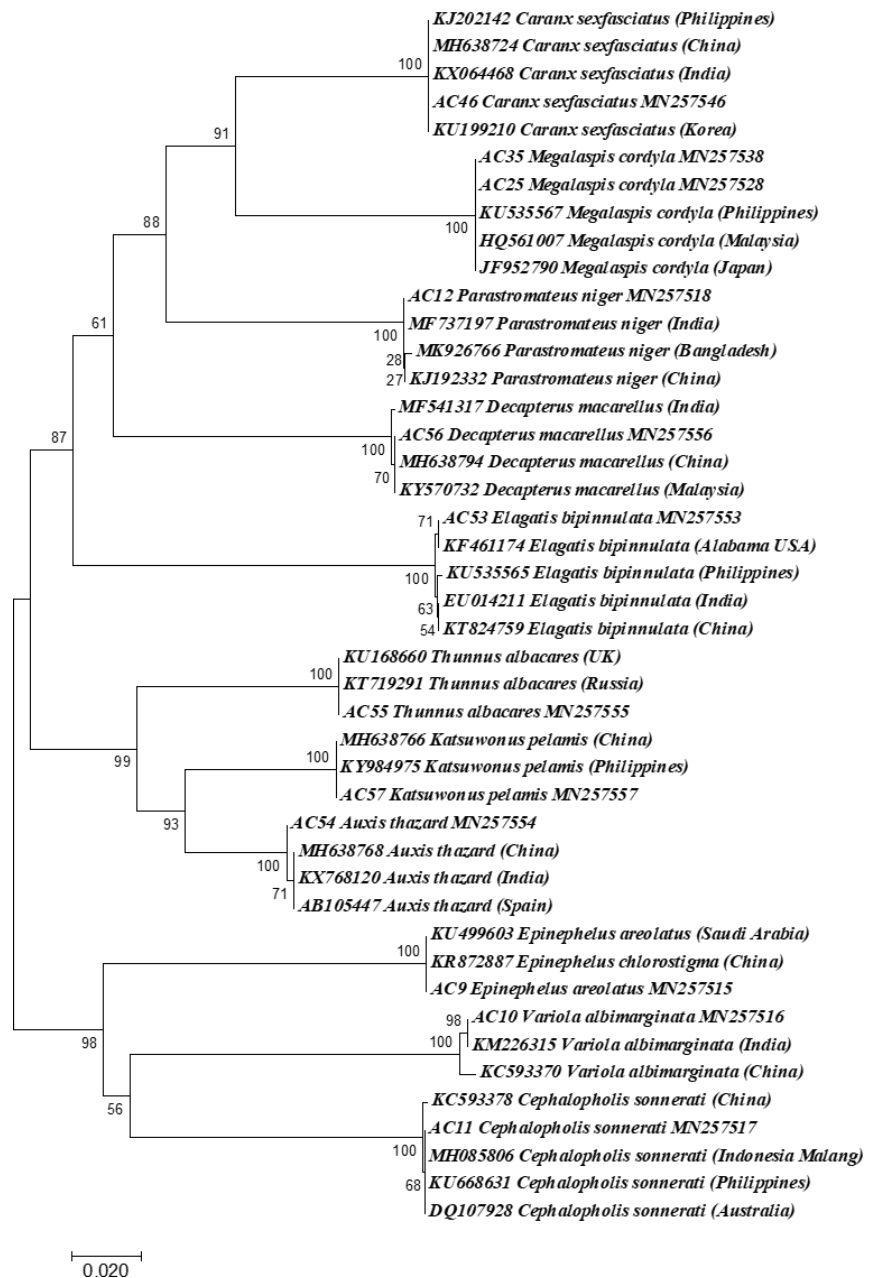
**Figure 2.** Phylogenetic tree of Clupeiformes including Beryciformes, Pleuronectiformes, and Scorpaeniformes by Neighbor-Joining tree algorithm using MEGA7.

### 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 areolatus* 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 *Parastromateus 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 (*Stelephorus 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 Kutardja 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.

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