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

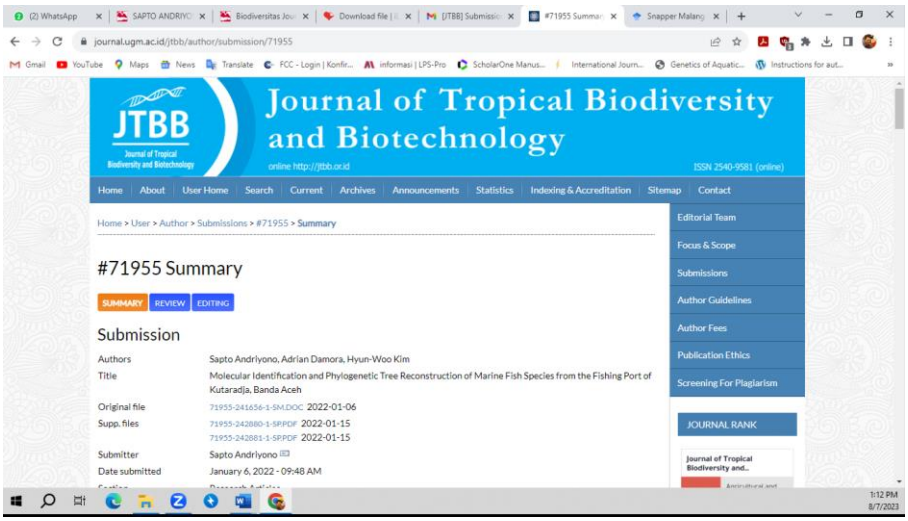
Judul : **Molecular identification and phylogenetic tree reconstruction of marine fish species from the Fishing Port of Kutaradja, Banda Aceh**

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

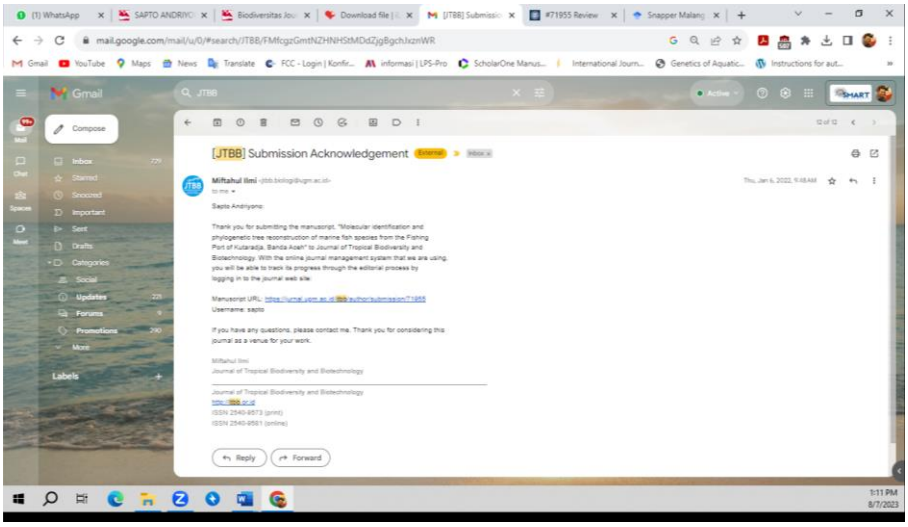
Penulis : **SAPTO ANDRIYONO, ADRIAN DAMORA, HYUN-WOO KIM**

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
3	Bukti Review B	16 April 2022	Page 3, Lampiran 3
4	Bukti Review C	16 April 2022	Page 3, Lampiran 4
5	Bukti Revisi 1	18 Mei 2022	Page 4, Lampiran 5
6	Bukti Revisi 2	18 July 2022	Page 4, Lampiran 6
7	Bukti Accepted	14 September 2022	Page 5
8	Link Artikel terpublikasi	26 Oktober 2022	Page 5, Lampiran 7

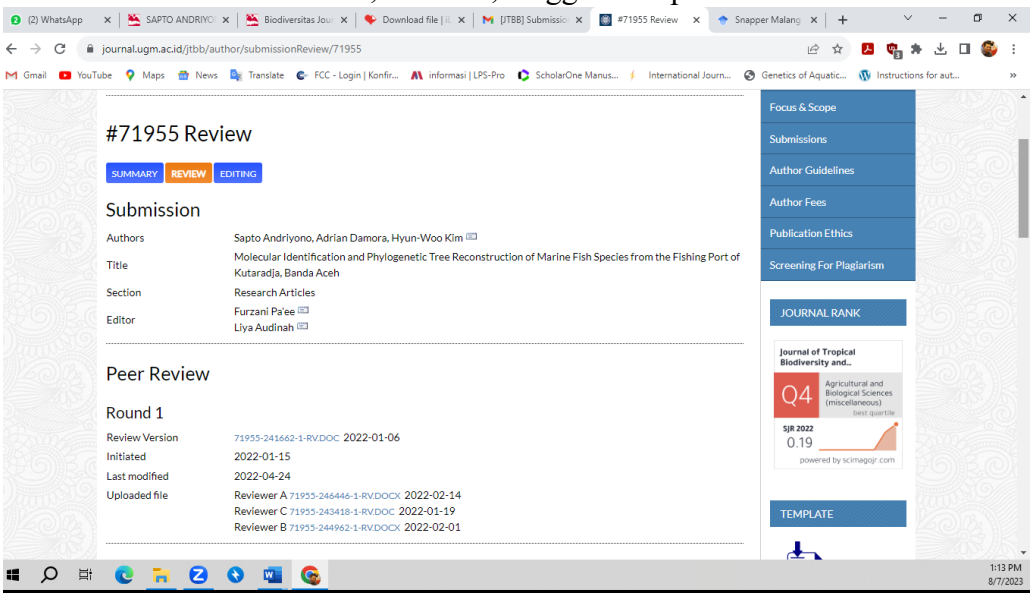
1. Bukti Submit dan Artikel yang disubmit 06 January 2022 disystem OJS dan Email Notifikasi



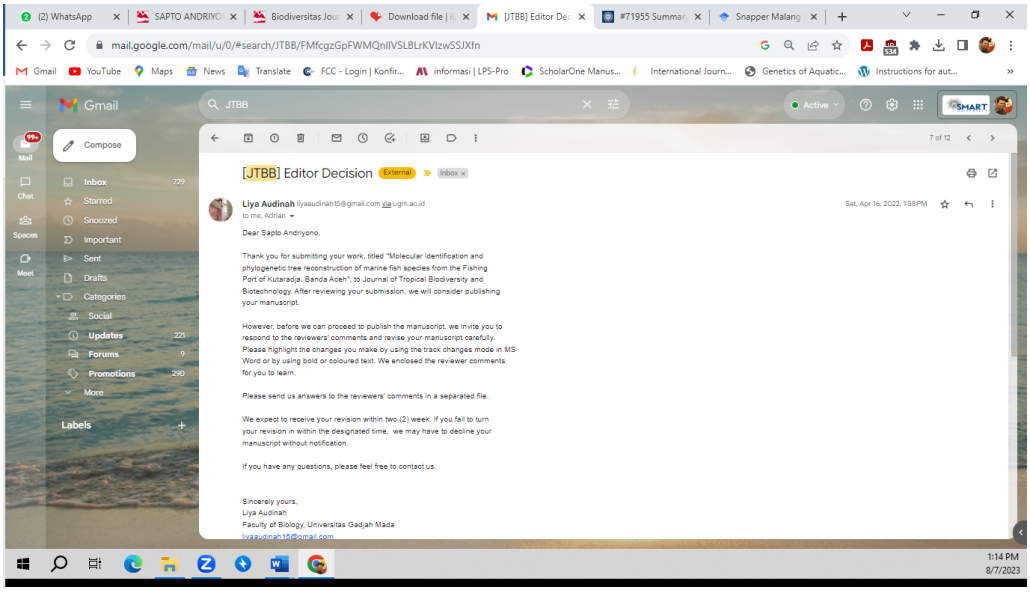
Email Notifikasi



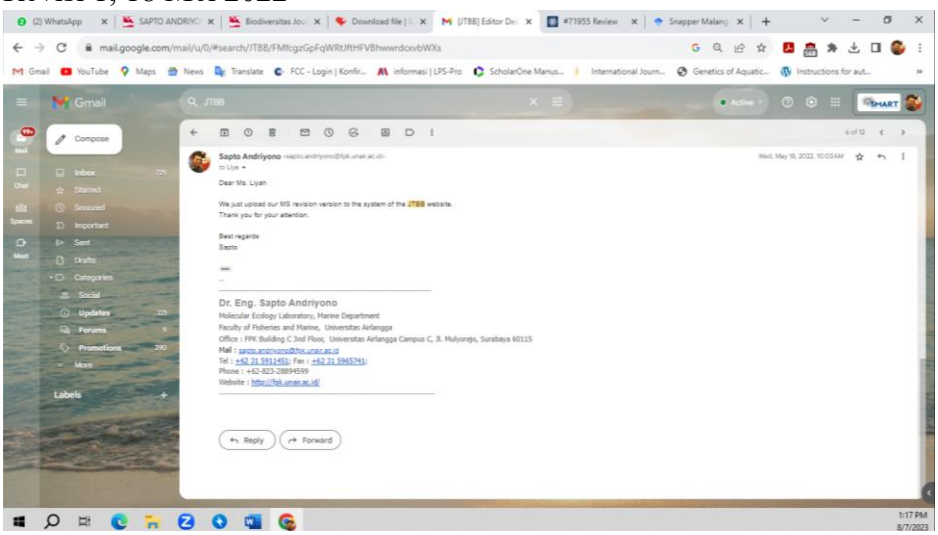
2. Review Roud 1. Reviewer A, B dan C, tanggal 16 April 2022



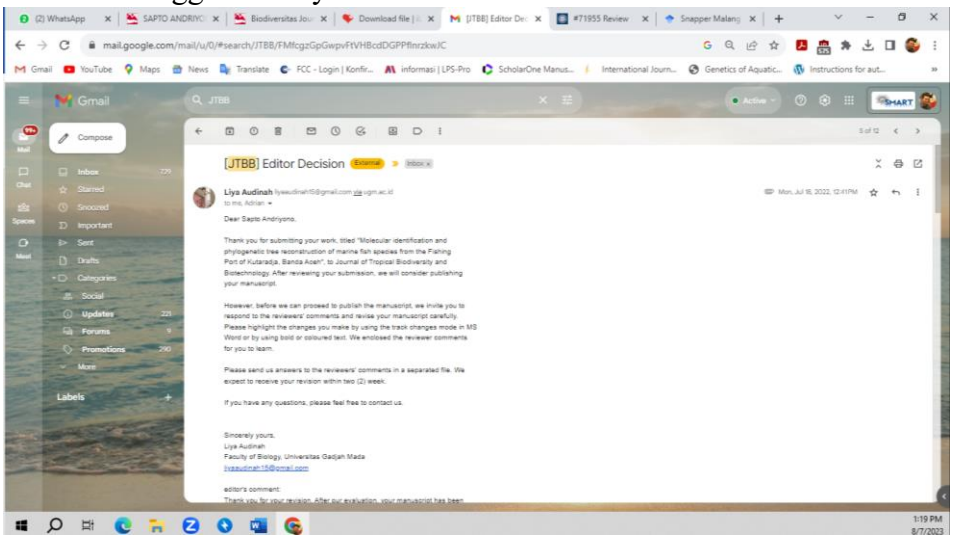
Email Notifikasi 3 Reviewer



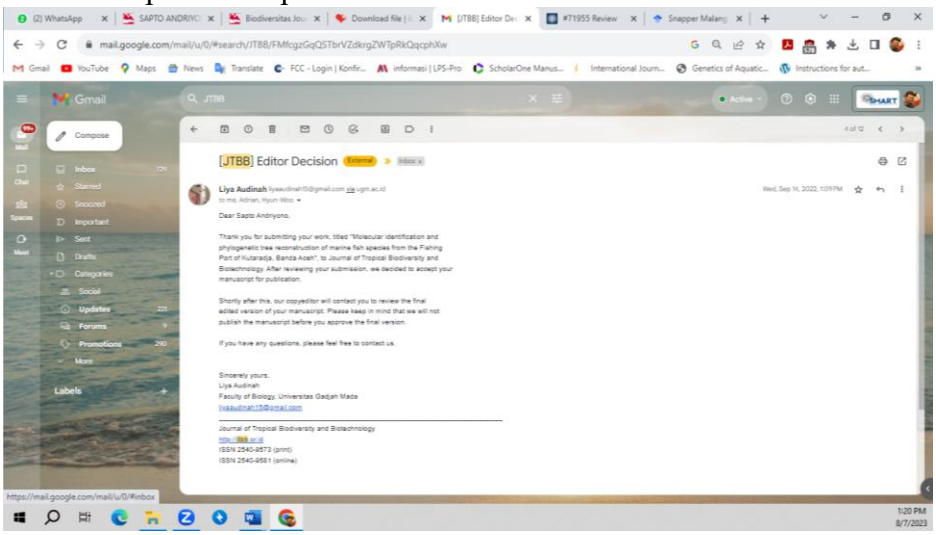
3. Revisi 1, 18 Mei 2022



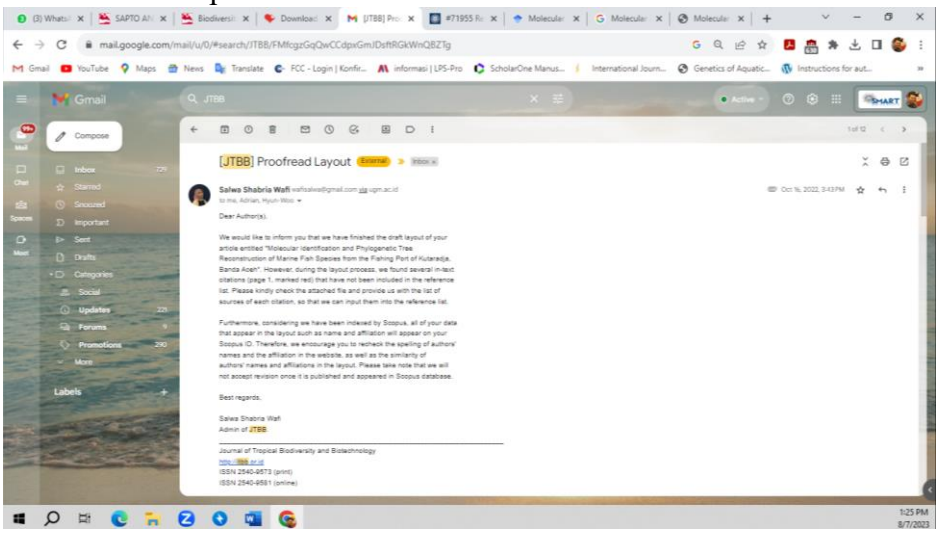
4. Revisi 2. Tanggal 18 July 2022



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

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

Abstract

The enormous potential of marine resources possessed by Banda Aceh Province is expected to be utilized optimally. Accurate in the identification of marine fish resources is a critical requirement to support their utilization and preservation in Banda Aceh Province. In this study, a molecular identification approach carried out in addition to conducting a morphological identification, which several scientists commonly used. The results obtained were 47 COI sequences were generated representing 33 genera, 19 families, and five orders. From the resulting COI partial sequence, there is one haplotype from the Scombridae family (*Auxis thazard*), two haplotypes from the Carangidae family (*Elagatis bipinnulata* and *Decapterus macarellus*), and two haplotypes from the Serranidae family (*Variola albimarginata* and *Cephalopholis sonnerati*). This study is essential in fisheries biology studies and other fisheries studies to support the sustainable utilization 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) known as the 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 considerable fishery potential with waters reaching 295,370 km^2 and with a coastline length reaching 2,666.3 km^2 (Mukhtar, 2017). One of the centers of fishing activities, as well as the most significant fish

landing 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 and Naufal 2017, Mardhatillah, Damora et al. 2019). The fish landing suffered massive damage due to the tsunami that struck Aceh Province and was rebuilt in 2004 (Zulmaidah, Zain 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 zone includes the Indian Ocean, Andaman Sea, and Malacca Strait. Two of the three regions are included in the Fisheries Management Area (FMA) 571 and 572. The level of utilization of pelagic and demersal fish resources in these two FMA is included in the overexploited category (Suman, Irianto et al. 2017, Salmarika and Wisudo 2019).

Previous research on the types of fish landed by many traditional fishers of the Kutaradja fishing port is still being done conventionally. From the inventory carried out at the Kutaradja fishing port, 11 species have been identified (Munawwarah, Sufi et al. 2016). However, another report on the types of marine fish species in Banda Aceh (Simeuleu Island) identified around 77 marine fish species included in 54 genus, 26 families, and seven orders (Batubara, Muchlisin et al. 2017). The reef-associated fishes inventory at Ulee Lheue, Banda Aceh, also mentioned that there were 87 species of reef fishes from 28 families in this location (Fadli, Muchlisin et al. 2019). In different areas, Lhoknga and Lhok Mata Ie Beaches, the eight orders, 11 families, 19 genera, and 25 species were recorded from 51 fish samples (Nur, Batubara et al. 2019). The morphological approach is the most widely used method in many regions in Indonesia, including in Banda Aceh. This research identifies molecular marine fish 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 in molecular identification globally. Research on

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

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

2. Materials and methods

2.1 Sampling site

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

2.2 DNA extraction and PCR

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

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

2.3 PCR condition and Data Analysis

One set universal fish primer targeting cytochrome c oxidase I (COI) region, BCL-BCH (Baldwin, Mounts et al. 2009, Handy, Deeds et al. 2011), 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 carried out under the following setting: 95^{o}C for 5 min in initial denaturation, followed by denaturation at 95^{o}C for 30 s in 40 cycles, 50^{o}C for 30 s in annealing, and 72^{o}C for 45 s in extension step, and a final extension at 72^{o}C for 5 min. The PCR products purified with the AccuPrep®Gel purification kit (Bioneer, Korea).

2.4 Phylogenetic analysis

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

3. Results and Discussion

3.1 Species Identification

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

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

3.2 Perciformes

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) and was lower than the average of transvertional pair (sv=14.86) with an overall transition/transvertion ratio bias is 1.57. The phylogenetic tree was constructed the COI sequences for the Perciformes and shown the average K2P distance within taxonomic levels measured for COI sequences is 0.226 (Figure 1).

3.3 Clupeiformes and Others

The nucleotide frequencies of COI sequences are 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 transvertional pair (sv=22.13) with an average transition/transvertion bias is 8.71. The phylogenetic tree was constructed the COI sequences for 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.

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

In this study, the sample from Aceh had several unique haplotypes compared to the same species from the GenBank database. By aligning the sequence generated with the reference sequence, some different nucleotides produce genetic variations (Table 2). The phylogenetics reconstruction of those sequences shown that several haplotypes 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, and had a genetic distance with an Indian sequence is 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 polymorphic in the sequences obtained.

In the Scombridae family group, haplotypes 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 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 of the Philippines, Australia, and Indonesia with genetic distance 0.00-0.002. In *Epinephelus arelatus* species, there are no haplotypes and sequences obtained from samples from China and Saudi Arabia.

Discussion

Research on molecular identification is now extensive in the field of fisheries and marine sciences. In this study, molecular identification used to completing 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 finds obstacles with the difficulty of getting taxonomists in determining species, in addition

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

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

In this report, family Perciformes are identified as a group that dominates the caught by fishermen in Banda Aceh, who landed at the Kutaradja fishing port. These fish are consumption fish that are essential export commodities with high economic value such as Skipjack tuna (57%) followed by yellowfin tuna (23%) (Lubis, Syaifuddin et al. 2016). The results of identification, the Scombridae family, is a group of pelagic fish that is quite commonly found. The types identified in this report include *Thunnus albacares, Auxis thazar,* and *Katsuwonus pelamis*. Besides, the genus Lutjanidae (snapper) found three species, 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 Grouper and Carangid. 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 on the Clupeiformes order, two families found in the Lampulo, 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 capture fisheries in Banda Aceh use purse seine, which finds a group of pelagic fish in large quantities. Previous studies have explained that the fishermen in Banda Aceh mostly use purse seine (Wiryawan, Wiyono et al. 2016, Hariati 2017). The purse-seine is also generally fishing gear to catch fish of *Euthynnus affinis, Auxis thazard*, and *Auxis rochei* (Salmarika and 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 make coral reef areas as a 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 in the good conditions when compared to the status of coral reefs in the mainland, Sumatra (Edrus, Wijaya et al. 2016). The species of *Epinephelus areolatus*, *Variola albimarginata*, and *Cephalopholis sonnerati* are a group of fish that become coral reefs as their habitat. However, several pelagic fish around the shallow seas of Banda Aceh remains the primary target. The skipjack tuna *Rastrelinger kanagurta* (Hariati, Faizah et al. 2015, Hariati and Fauzi 2017), yellowfin tuna *Thunnus albacares* (Neliyana, Wiyono et al. 2014), Mackerel scad *Decapterus macrosoma*, dan anchovy *Stolephorus* spp) (Kurnia, Purnawan et al. 2016) were also obtained in this study.

In this report, several Acehnese fish sequences also have similarities in some previous studies, and some are unique to other sequences. Species *Auxis Thazard*, identified from the port of Lampulo, may have been collected from the area around the sea waters of Western of Banda Aceh Province with a catch distance of about 50-190 nautical miles (Salmarika and 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 3 prominent marine fish families, namely Scombridae, Serranidae, and Carangidae, was carried out. The results of the investigation found that the Scombridae *Auxis thazard* (Aceh) which separates from the same clade species even though it only has a genetic distance of only 0.002. This haplotype appears likely to occur due to differences in species populations analyzed from India, China (Xu, Van Damme et al. 2019), and Spain (Catanese, Catanese et al. 2008). While other haplotypes found in reef fish are *Variola albimarginata* and *Cephalopholis sonnerati*, the *Variola albimarginata* from Aceh may be a population with 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 carried out in the Andaman Islands and Nicobar Island (Basheer, Vineesh et al. 2017). This area is Indian sea waters, which have the potential to have reef fish, which are almost the same as the species in Aceh. While *Cepalopholis sonnerati* fish species also have similarities with populations from Australia and the Philippines, but slightly different from populations from China (Zhuang, Qu et al. 2013). The study of *Cepalopholis 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 similarities with Indian populations found in the *Cepalopholis sonnerati* species only in previous studies conducted

in the Philippines (Alcantara and Yambot 2016), and Australia (Ward, Zemlak et al. 2005). The speciation process that occurs in coral reef ecosystems occurs with an allopathic pattern that makes geographic isolation 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 a speciation pattern on the main coral reefs (Rocha and Bowen 2008).

4. Conclusions

The 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 becoming fishery commodities and became the main target of the Province of Banda Aceh's exports, including Tuna fish (*Thunnus albacares*) and Cakalang fish (*Katsuwonus pelamis*). In this study, the Scombridae group found one Aceh haplotype in the *Auxis thazard* species (MN257554). In contrast, in the Carangids group, it was known that the *Elagatis bipinnulata* species (MN257553) had similar sequences with the same species from Alabama USA (KF461174). Another species in the Carangidae order, namely *Decapterus macarellus* from Aceh, has similarities with the sequence from China (MH638794) and Malaysia (KY570732). In this type of reef fish, this study found *Variola albimarginata* species (India KM226315), which had similarities with Aceh samples. In contrast, *Cephalopholis sonnerati* (MN257517) had similarities with the same sequence species from the Philippines (KU668631) and Australia (DQ107928). More in-depth research on the haplotypes of the marine fish mentioned above is very much needed to maintain genetic biodiversity in the waters of Banda Aceh, which is a precious asset for Indonesia.

Author contribution

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

Acknowledgments

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

Conflict of Interest

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

References

Alcantara, S. G. and A. V. Yambot (2016). "DNA barcoding of commercially important grouper species (Perciformes, Serranidae) in the Philippines." Mitochondrial DNA Part A **27**(6): 3837-3845.

Baldwin, C. C., J. H. Mounts, D. G. Smith and L. A. Weigt (2009). "Genetic identification and color descriptions of early life-history stages of Belizean Phaeoptyx and Astrapogon (Teleostei: Apogonidae) with comments on identification of adult Phaeoptyx." Zootaxa **2008**: 1-22.

Basheer, V., N. Vineesh, K. Bineesh, R. G. Kumar, C. Mohitha, S. Venu, A. Kathirvelpandian, A. Gopalakrishnan and J. Jena (2017). "Mitochondrial signatures for identification of grouper species from Indian waters." Mitochondrial DNA Part A **28**(4): 451-457.

Batubara, A. S., Z. A. Muchlisin, M. Y. Thamren, U. Usnardi and N. Fadli (2017). "Check list of marine fishes from Simeulue Island waters, Aceh Province, Indonesia." Aceh Journal of Animal Science **2**(2): 77-84.

Bingpeng, X., L. Heshan, Z. Zhilan, W. Chunguang, W. Yanguo and W. Jianjun (2018). "DNA barcoding for identification of fish species in the Taiwan Strait." PloS one **13**(6): e0198109.

Catanese, G., G. Catanese, C. Infante, M. Manchado, G. Catanese, C. Infante and M. Manchado (2008). "Complete mitochondrial DNA sequences of the frigate tuna Auxis thazard and the bullet tuna Auxis rochei: Full Length Research Paper." DNA Sequence **19**(3): 159-166.

Chang, C. H., K. T. Shao, H. Y. Lin, Y. C. Chiu, M. Y. Lee, S. H. Liu and P. L. Lin (2017). "DNA barcodes of the native ray-finned fishes in Taiwan." Molecular ecology resources **17**(4): 796-805.

Edrus, I. N., S. W. Wijaya and I. E. Setiawan (2016). "Struktur komunitas ikan karang di perairan Pulau Raya, Pulau Rusa, Pulau Rondo dan taman laut Rinoi dan Rubiah, Nanggroe Aceh Darussalam." Jurnal Penelitian Perikanan Indonesia **19**(4): 175-186.

Edwarsyah, E., M. Nasir and M. B. Selamat (2019). "The DNA Barcoding of Several Commercial Fish from Simeulue Islands Coast of Aceh Using Cytochrome Oxidase Sub Unit I (COI) Gene Marker." Budapest International Research in Exact Sciences (BirEx) Journal **1**(1): 62-70.

Fadli, N., Z. Muchlisin, F. Pratama, T. Mustari, I. Dewiyanti, S. Purnawan, S. El-Rahimi, H. Sofyan, M. Affan and M. Siti-Azizah (2019). The composition of coral reefs in Ulee Lheue breakwater, Banda Aceh, Aceh, Indonesia. IOP Conference Series: Earth and Environmental Science, IOP Publishing.

299 Handy, S. M., J. R. Deeds, N. V. Ivanova, P. D. Hebert, R. H. Hanner, A. Ormos, L. A.
300 Weigt, M. M. Moore and H. F. Yancy (2011). "A single-laboratory validated method
301 for the generation of DNA barcodes for the identification of fish for regulatory
302 compliance." Journal of AOAC International **94**(1): 201-210.
303 Hariati, T. (2017). "Status dan perkembangan perikanan pukat cincin di Banda Aceh." Jurnal
304 Penelitian Perikanan Indonesia **17**(3): 157-167.
305 Hariati, T., R. Faizah and D. Nugroho (2015). "Umur, Pertumbuhan Dan Laju Pemanfaatan
306 Ikan Banyar (Rastrelliger kanagurta Cuvier, 1816), di Selat Malaka (Wilayah
307 Pengelolaan Perikanan-571)." Jurnal Penelitian Perikanan Indonesia **21**(1): 1-8.
308 Hariati, T. and M. Fauzi (2017). "Aspek reproduksi ikan banyar, Rastrelliger kanagurta (Cuv.
309 1817) di perairan utara Aceh [Reproductive aspect of indian mackerel Rastrelliger
310 kanagurta (Cuv. 1817) of northern Aceh waters]." Jurnal Iktiologi Indonesia **11**(1):
311 47-53.
312 Kamal, M. M., A. A. Hakim, N. A. Butet, Y. Fitrianingsih and R. Astuti (2019). "Autentikasi
313 spesies ikan kerapu berdasarkan marka gen MT-COI dari perairan Peukan Bada,
314 Aceh." Jurnal Biologi Tropis **19**(2): 116-123.
315 KeskİN, E. and H. H. Atar (2013). "DNA barcoding commercially important fish species of T
316 urkey." Molecular ecology resources **13**(5): 788-797.
317 Kumar, S., G. Stecher and K. Tamura (2016). "MEGA7: molecular evolutionary genetics
318 analysis version 7.0 for bigger datasets." Molecular biology and evolution **33**(7):
319 1870-1874.
320 Kurnia, K., S. Purnawan and T. Rizwan (2016). "Pemetaan daerah penangkapan ikan pelagis
321 kecil di Perairan Utara Aceh." Jurnal Ilmiah Mahasiswa Kelautan Perikanan Unsyiah
322 **1**(2).

323 Lakra, W., M. Verma, M. Goswami, K. K. Lal, V. Mohindra, P. Punia, A. Gopalakrishnan,
324 K. Singh, R. D. Ward and P. Hebert (2011). "DNA barcoding Indian marine fishes."
325 Molecular Ecology Resources **11**(1): 60-71.
326 Lubis, A. M., S. Syaifuddin and I. Isnaniah (2016). "The Contribution of the Lampulo
327 Fishing Port, for Fishery Sector in Banda Aceh City, Nanggroe Aceh Darussalam
328 Province." Jurnal Online Mahasiswa Fakultas Perikanan dan Ilmu Kelautan
329 Universitas Riau **3**(1): 1-13.
330 Mardhatillah, I., A. Damora, A. Rahmah, N. Nurfadillah and M. Muhammad (2019).
331 Application of Schaefer model on mackerels fishery in Aceh waters, Indonesia. IOP
332 Conference Series: Earth and Environmental Science, IOP Publishing.
333 Munawwarah, A., I. Sufi and A. Noviyanti (2016). "Identifikasi Jenis-Jenis Ikan Yang
334 Terdapat Di Tempat Pelelangan Ikan (TPI) Di Gampong Lampulo Kecamatan Kuta
335 Alam Banda Aceh." Serambi Saintia: Jurnal Sains dan Aplikasi **4**(1).
336 Neliyana, W. B., E. Wiyono and T. Nurani (2014). "Analisis Kelayakan Usaha Perikanan
337 Pukat Cincin di Pelabuhan Perikanan Pantai (PPP) Lampulo Banda Aceh Propinsi
338 Aceh." Jurnal Marine Fisheries **5**(2): 163-169.
339 Nur, F. M., A. S. Batubara, A. W. Perdana, K. Eriani and Z. A. Muchlisin (2019). Checklist
340 of coral fishes in Lhoknga and Lhok Mata Ie Beaches, Aceh Besar, Indonesia. IOP
341 Conference Series: Earth and Environmental Science, IOP Publishing.
342 Rocha, L. and B. Bowen (2008). "Speciation in coral-reef fishes." Journal of Fish Biology
343 **72**(5): 1101-1121.
344 Salmarika, S. and S. H. Wisudo (2019). "Status Pengelolaan Sumber Daya Ikan Tongkol di
345 Perairan Samudera Hindia Berbasis Pendaratan Pukat Cincin di Pelabuhan
346 Perikanan Samudera Lampulo, Aceh: Suatu Pendekatan Ekosistem." Jurnal
347 Penelitian Perikanan Indonesia **24**(4): 263-272.

348 Suman, A., H. E. Irianto, F. Satria and K. Amri (2017). "Potensi dan tingkat pemanfaatan
349 sumber daya ikan di wilayah pengelolaan perikanan Negara Republik Indonesia
350 (WPP NRI) Tahun 2015 serta Opsi Pengelolaannya." Jurnal Kebijakan Perikanan
351 Indonesia **8**(2): 97-100.
352 Wang, Z.-D., Y.-S. Guo, X.-M. Liu, Y.-B. Fan and C.-W. Liu (2012). "DNA barcoding South
353 China Sea fishes." Mitochondrial Dna **23**(5): 405-410.
354 Ward, R. D., T. S. Zemlak, B. H. Innes, P. R. Last and P. D. Hebert (2005). "DNA barcoding
355 Australia's fish species." Philosophical Transactions of the Royal Society B:
356 Biological Sciences **360**(1462): 1847-1857.
357 Wiryawan, B., E. S. Wiyono and T. W. Nurani (2016). "Analisis Kelayakan Usaha Perikanan
358 Pukat Cincin Di Pelabuhan Perikanan Pantai (Ppp) Lampulo Banda Aceh Propinsi
359 Aceh (Analysis Financial Fisheries of Purse Seine in Lampulo Fishing Port Banda
360 Aceh Provinsi Aceh)." Marine Fisheries: Journal of Marine Fisheries Technology
361 and Management **5**(2): 163-169.
362 Xu, L., K. Van Damme, H. Li, Y. Ji, X. Wang and F. Du (2019). "A molecular approach to
363 the identification of marine fish of the Dongsha Islands (South China Sea)."
364 Fisheries Research **213**: 105-112.
365 Yeni, E. and A. Naufal (2017). Identifikasi Aktivitas Pengembangan Fasilitas Pelabuhan
366 Perikanan Pantai Lampulo Banda Aceh. Prosiding SEMDI-UNAYA (Seminar
367 Nasional Multi Disiplin Ilmu UNAYA).
368 Yusuf, Q. (2003). Empowerment of Panglima Laot in Aceh. International workshop on
369 Marine Science and Resource. Banda Aceh.
370 Zhang, J. and R. Hanner (2012). "Molecular approach to the identification of fish in the South
371 China Sea." PLoS One **7**(2): e30621.

372 Zhuang, X., M. Qu, X. Zhang and S. Ding (2013). "A comprehensive description and
373 evolutionary analysis of 22 grouper (Perciformes, Epinephelidae) mitochondrial
374 genomes with emphasis on two novel genome organizations." PLoS One **8**(8):
375 e73561.
376 Zulmaidah, Z., J. Zain and R. M. Hutauruk (2015). "Facilities Utilization in Lampulo
377 Fisheries Port, District of Kuta Alam, Banda Aceh City, Province Nanggroe Aceh
378 Darussalam." None **2**(2): 1-13.
379
380

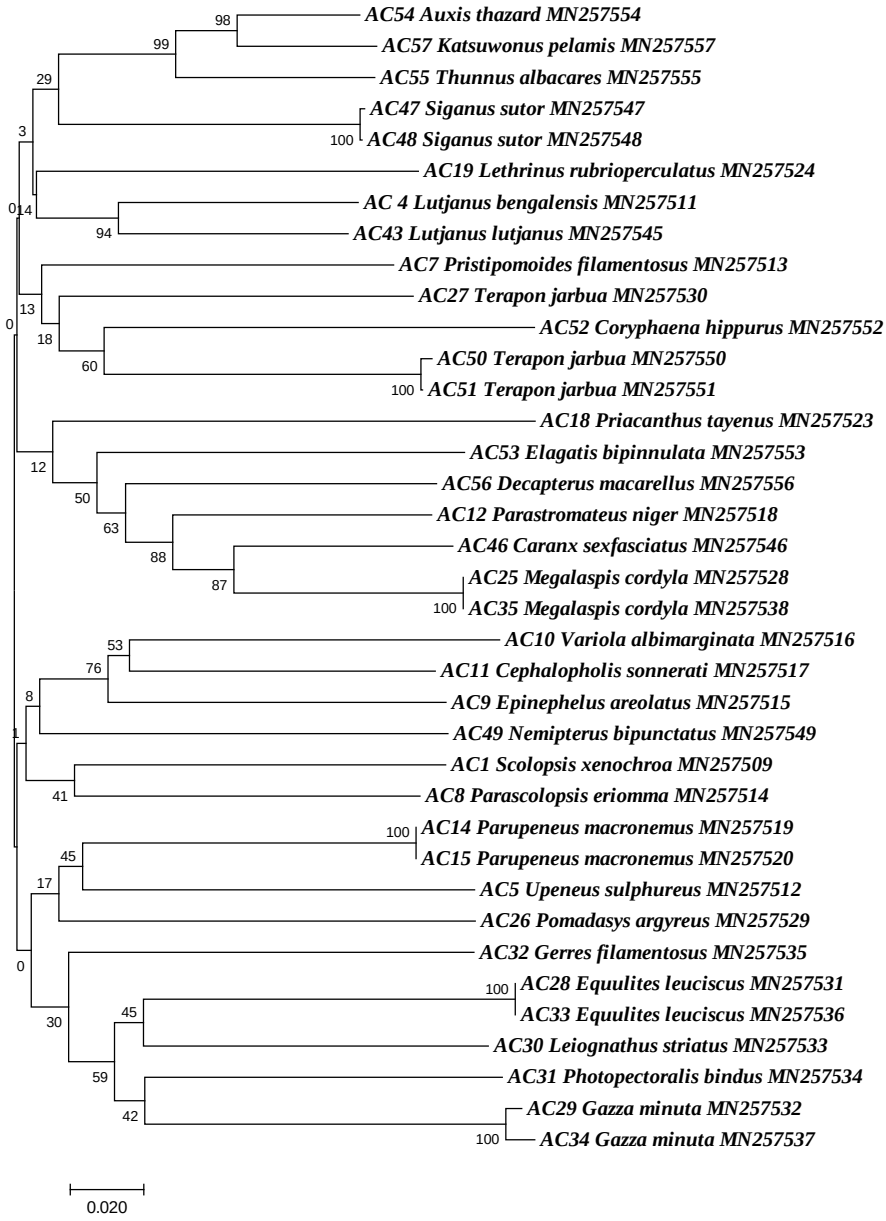


Figure 1. Phylogenetic tree of Perciformes order by Neighbor-Joining tree algorithm using Mega7

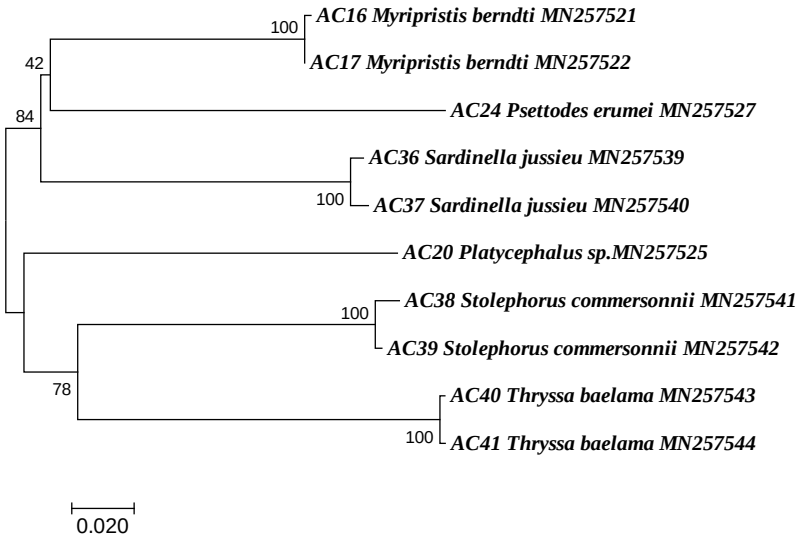
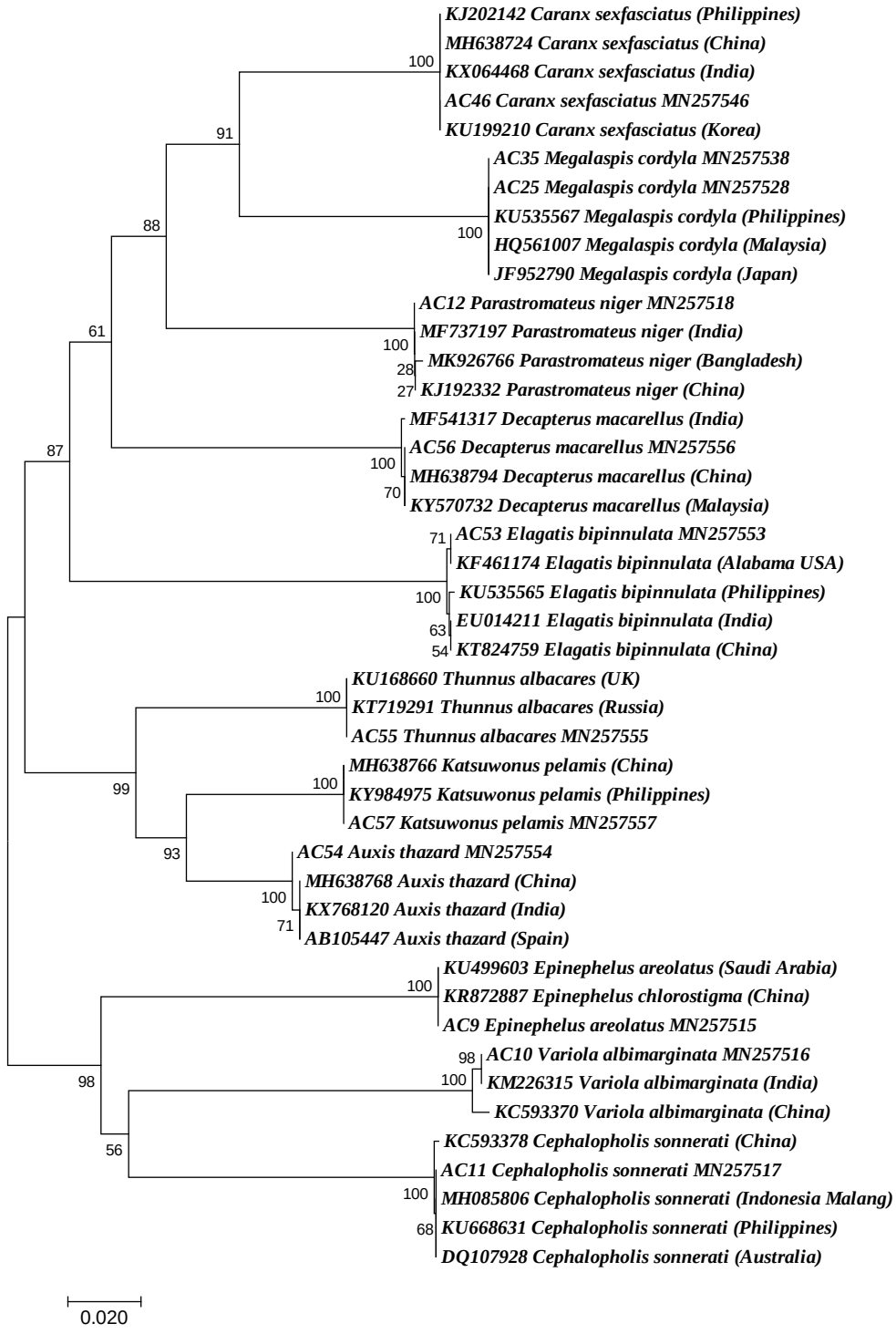


Figure 2. Phylogenetic tree of small number others order in this study including the Clupeiformes, Beryciformes, Pleuronectiformes, and Scorpaeniformes by Neighbor-Joining tree algorithm using Mega7



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	*Myripristis berndti*	Holocentridae	MN257521	Beryciformes	Blotcheye soldierfish	Indo-Pacific and Eastern Pacific	LC
2	17	*Myripristis berndti*	Holocentridae	MN257522	Beryciformes	Blotcheye soldierfish	Indo-Pacific and Eastern Pacific	LC
3	36	*Sardinella jussieu*	Clupeidae	MN257539	Clupeiformes	Mauritian sardinella	Western Indian Ocean	DD
4	37	*Sardinella jussieu*	Clupeidae	MN257540	Clupeiformes	Mauritian sardinella	Western Indian Ocean	DD
5	38	*Stolephorus commersonnii*	Engraulidae	MN257541	Clupeiformes	Commerson's anchovy	Indo-West Pacific	LC
6	39	*Stolephorus commersonnii*	Engraulidae	MN257542	Clupeiformes	Commerson's anchovy	Indo-West Pacific	LC
7	40	*Thryssa baelama*	Engraulidae	MN257543	Clupeiformes	Baelama anchovy	Indo-Pacific	LC
8	41	*Thryssa baelama*	Engraulidae	MN257544	Clupeiformes	Baelama anchovy	Indo-Pacific	LC
9	1	*Scolopsis xenochroa*	Nemipteridae	MN257509	Perciformes	Oblique-barred monocle bream	Indo-West Pacific	NE
10	4	*Lutjanus bengalensis*	Lutjanidae	MN257511	Perciformes	Bengal snapper	Indo-West Pacific:	NE
11	5	*Upeneus sulphureus*	Mullidae	MN257512	Perciformes	Sulphur goatfish	Indo-West Pacific	LC
12	7	*Pristipomoides filamentosus*	Lutjanidae	MN257513	Perciformes	Crimson jobfish	Indo-Pacific	LC
13	8	*Parascolopsis eriomma*	Nemipteridae	MN257514	Perciformes	Rosy dwarf monocle bream	Indo-West Pacific	NE
14	9	*Epinephelus areolatus*	Serranidae	MN257515	Perciformes	Areolate grouper	Indo-Pacific	LC
15	10	*Variola albimarginata*	Serranidae	MN257516	Perciformes	White-edged lyretail	Indo-Pacific	LC
16	11	*Cephalopholis sonnerati*	Serranidae	MN257517	Perciformes	Tomato hind	Indo-Pacific	LC
17	12	*Parastromateus niger*	Carangidae	MN257518	Perciformes	Black pomfret	Indo-West Pacific	LC
18	14	*Parupeneus macronemus*	Mullidae	MN257519	Perciformes	Long-barbel goatfish	Indo-West Pacific	LC
19	15	*Parupeneus macronemus*	Mullidae	MN257520	Perciformes	Long-barbel goatfish	Indo-West Pacific	LC
20	18	*Priacanthus tayenus*	Priacanthidae	MN257523	Perciformes	Purple-spotted bigeye	Indo-West Pacific	LC
21	19	*Lethrinus rubrioperculatus*	Lethrinidae	MN257524	Perciformes	Spotcheek emperor	Indo-Pacific	LC
22	25	*Megalaspis cordyla*	Carangidae	MN257528	Perciformes	Torpedo scad	Indo-West Pacific	LC
23	26	*Pomadasys argyreus*	Haemulidae	MN257529	Perciformes	Bluecheek silver	Indo-West Pacific	NE

						grunt		
24	27	*Terapon jarbua*	Terapontidae	MN257530	Perciformes	Jarbua terapon	Indo-Pacific	LC
25	28	*Equulites leuciscus*	Leiognathidae	MN257531	Perciformes	Whipfin ponyfish	Indo-West Pacific	LC
26	29	*Gazza minuta*	Leiognathidae	MN257532	Perciformes	Toothpony	Indo-Pacific	LC
27	30	*Leiognathus striatus*	Leiognathidae	MN257533	Perciformes	Toothpony	Western Indian Ocean	NE
28	31	*Photopectoralis bindus*	Leiognathidae	MN257534	Perciformes	Orangefin ponyfish	Indo-West Pacific	NE
29	32	*Gerres filamentosus*	Gerreidae	MN257535	Perciformes	Whipfin silver-biddy	Indo-Pacific	LC
30	33	*Equulites leuciscus*	Leiognathidae	MN257536	Perciformes	Whipfin ponyfish	Indo-West Pacific	LC
31	34	*Gazza minuta*	Leiognathidae	MN257537	Perciformes	Toothpony	Indo-Pacific	LC
32	35	*Megalaspis cordyla*	Carangidae	MN257538	Perciformes	Torpedo scad	Indo-West Pacific	LC
33	43	*Lutjanus lutjanus*	Lutjanidae	MN257545	Perciformes	Bigeye snapper	Indo-West Pacific	LC
34	46	*Caranx sexfasciatus*	Carangidae	MN257546	Perciformes	Bigeye trevally	Indo-Pacific	LC
35	47	*Siganus sutor*	Siganidae	MN257547	Perciformes	Shoemaker spinefoot	Indian Ocean	LC
36	48	*Siganus sutor*	Siganidae	MN257548	Perciformes	Shoemaker spinefoot	Indian Ocean	LC
37	49	*Nemipterus bipunctatus*	Nemipteridae	MN257549	Perciformes	Delagoa threadfin bream	Indian Ocean	NE
38	50	*Terapon jarbua*	Terapontidae	MN257550	Perciformes	Jarbua terapon	Indo-Pacific	LC
39	51	*Terapon jarbua*	Terapontidae	MN257551	Perciformes	Jarbua terapon	Indo-Pacific	LC
40	52	*Coryphaena hippurus*	Coryphaenidae	MN257552	Perciformes	Common dolphinfish	Atlantic, Indian and Pacific	LC
41	53	*Auxis thazard*	Scombridae	MN257553	Perciformes	Frigate tuna	Atlantic, Indian and Pacific (Western Central)	LC
42	54	*Auxis thazard*	Scombridae	MN257554	Perciformes	Frigate tuna	Atlantic, Indian and Pacific (Western Central)	LC
43	55	*Thunnus albacares*	Scombridae	MN257555	Perciformes	Yellowfin tuna	Worldwide in tropical and subtropical seas	NT
44	56	*Decapterus macarellus*	Carangidae	MN257556	Perciformes	Mackerel scad	Circumglobal	LC
45	57	*Katsuwonus pelamis*	Scombridae	MN257557	Perciformes	Skipjack tuna	Cosmopolitan in tropical and warm-temperate waters	LC
46	24	*Psettodes erumei*	Psettodidae	MN257527	Pleuronectiformes	Indian halibut	Indo-West Pacific	NE
47	20	*Platycephalus sp.*	Platycephalidae	MN257525	Scorpaeniformes	Bartail flathead	Indo-West Pacific	DD

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

398

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	*Elagatis bipinnulata*	MN257553	Aceh 53	-	-	A	-	-	T	-	-
		KU535565	Philipinnes	-	-	G	-	-	C	-	-
		KF461174	USA	-	-	A	-	-	T	-	-
		EU014211	India	-	-	A	-	-	C	-	-
		KT824759	China	-	-	A	-	-	C	-	-
2	*Decapterus macarellus*	MN257556	Aceh 6	-	C	-	-	-	-	-	-
		MH638794	China	-	C	-	-	-	-	-	-
		KY570732	Malaysia	-	C	-	-	-	-	-	-
		MF541317	India	-	T	-	-	-	-	-	-
3	*Auxis thazard*	MN257554	Aceh 54	-	-	-	-	-	-	-	-
		MH638768	China	-	-	-	-	-	-	-	-
		KX768120	India	-	-	-	-	-	-	-	-
		AB105447	Spain	-	-	-	-	-	-	-	-
4	*Variola albimarginata*	MN257516	Aceh 10	C	-	-	G	-	-	G	C
		KM226315	India	C	-	-	G	-	-	G	C
		KC593370	China	T	-	-	A	-	-	A	T
5	*Cephalopholis sonnerati*	MN257517	Aceh 11	-	-	-	-	A	-	-	-
		MH085806	Indonesia	-	-	-	-	A	-	-	-
		KU668631	Philipinnes	-	-	-	-	A	-	-	-
		DQ107928	Australia	-	-	-	-	A	-	-	-
		KC593378	China	-	-	-	-	G	-	-	-

401

402

403

Artikel 6, Lampiran 2

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

Abstract

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

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

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

1. Introduction

Aceh is the westernmost province of the Indo-Malaya Archipelago (IMA) known as the 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 considerable fishery potential with waters reaching 295,370 km^2 and with a coastline length reaching 2,666.3 km^2 (Mukhtar, 2017). One of the centers of fishing activities, as well as the most significant fish

landing 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 and Naufal 2017, Mardhatillah, Damora et al. 2019). The fish landing suffered massive damage due to the tsunami that struck Aceh Province and was rebuilt in 2004 (Zulmaidah, Zain 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 zone includes the Indian Ocean, Andaman Sea, and Malacca Strait. Two of the three regions are included in the Fisheries Management Area (FMA) 571 and 572. The level of utilization of pelagic and demersal fish resources in these two FMA is included in the overexploited category (Suman, Irianto et al. 2017, Salmarika and Wisudo 2019).

Previous research on the types of fish landed by many traditional fishers of the Kutaradja fishing port is still being done conventionally. From the inventory carried out at the Kutaradja fishing port, 11 species have been identified (Munawwarah, Sufi et al. 2016). However, another report on the types of marine fish species in Banda Aceh (Simeuleu Island) identified around 77 marine fish species included in 54 genus, 26 families, and seven orders (Batubara, Muchlisin et al. 2017). The reef-associated fishes inventory at Ulee Lheue, Banda Aceh, also mentioned that there were 87 species of reef fishes from 28 families in this location (Fadli, Muchlisin et al. 2019). In different areas, Lhoknga and Lhok Mata Ie Beaches, the eight orders, 11 families, 19 genera, and 25 species were recorded from 51 fish samples (Nur, Batubara et al. 2019). The morphological approach is the most widely used method in many regions in Indonesia, including in Banda Aceh. This research identifies molecular marine fish 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 in molecular identification globally. Research on

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

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

2. Materials and methods

2.1 Sampling site

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

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

2.2 DNA extraction and PCR

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

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

2.3 PCR condition and Data Analysis

One set universal fish primer targeting cytochrome c oxidase I (COI) region, BCL-BCH (Baldwin, Mounts et al. 2009, Handy, Deeds et al. 2011), 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 carried out under the following setting: 95^{o}C for 5 min in initial denaturation, followed by denaturation at 95^{o}C for 30 s in 40 cycles, 50^{o}C for 30 s in annealing, and 72^{o}C for 45 s in extension step, and a final extension at 72^{o}C for 5 min. The PCR products purified with the AccuPrep®Gel purification kit (Bioneer, Korea).

2.4 Phylogenetic analysis

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

3. Results and Discussion

3.1 Species Identification

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

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

3.2 Perciformes

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) and was lower than the average of transvertional pair (sv=14.86) with an overall transition/transvertion ratio bias is 1.57. The phylogenetic tree was constructed the COI sequences for the Perciformes and shown the average K2P distance within taxonomic levels measured for COI sequences is 0.226 (Figure 1).

3.3 Clupeiformes and Others

The nucleotide frequencies of COI sequences are 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 transvertional pair (sv=22.13) with an average transition/transvertion bias is 8.71. The phylogenetic tree was constructed the COI sequences for 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.

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

In this study, the sample from Aceh had several unique haplotypes compared to the same species from the GenBank database. By aligning the sequence generated with the reference sequence, some different nucleotides produce genetic variations (Table 2). The phylogenetics reconstruction of those sequences shown that several haplotypes 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, and had a genetic distance with an Indian sequence is 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 polymorphic in the sequences obtained.

In the Scombridae family group, haplotypes 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 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 of the Philippines, Australia, and Indonesia with genetic distance 0.00-0.002. In *Epinephelus arelatus* species, there are no haplotypes and sequences obtained from samples from China and Saudi Arabia.

Discussion

Research on molecular identification is now extensive in the field of fisheries and marine sciences. In this study, molecular identification used to completing 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 finds obstacles with the difficulty of getting taxonomists in determining species, in addition

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

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

In this report, family Perciformes are identified as a group that dominates the caught by fishermen in Banda Aceh, who landed at the Kutaradja fishing port. These fish are consumption fish that are essential export commodities with high economic value such as Skipjack tuna (57%) followed by yellowfin tuna (23%) (Lubis, Syaifuddin et al. 2016). The results of identification, the Scombridae family, is a group of pelagic fish that is quite commonly found. The types identified in this report include *Thunnus albacares*, *Auxis thazar*, and *Katsuwonus pelamis*. Besides, the genus Lutjanidae (snapper) found three species, 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 Grouper and Carangid. 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 on the Clupeiformes order, two families found in the Lampulo, 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 capture fisheries in Banda Aceh use purse seine, which finds a group of pelagic fish in large quantities. Previous studies have explained that the fishermen in Banda Aceh mostly use purse seine (Wiryawan, Wiyono et al. 2016, Hariati 2017). The purse-seine is also generally fishing gear to catch fish of *Euthynnus affinis*, *Auxis thazard*, and *Auxis rochei* (Salmarika and 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 make coral reef areas as a 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 in the good conditions when compared to the status of coral reefs in the mainland, Sumatra (Edrus, Wijaya et al. 2016). The species of *Epinephelus areolatus*, *Variola albimarginata*, and *Cephalopholis sonnerati* are a group of fish that become coral reefs as their habitat. However, several pelagic fish around the shallow seas of Banda Aceh remains the primary target. The skipjack tuna *Rastrelinger kanagurta* (Hariati, Faizah et al. 2015, Hariati and Fauzi 2017), yellowfin tuna *Thunnus albacares* (Neliyana, Wiyono et al. 2014), Mackerel scad *Decapterus macrosoma*, dan anchovy *Stolephorus* spp) (Kurnia, Purnawan et al. 2016) were also obtained in this study.

In this report, several Acehnese fish sequences also have similarities in some previous studies, and some are unique to other sequences. Species *Auxis Thazard*, identified from the port of Lampulo, may have been collected from the area around the sea waters of Western of Banda Aceh Province with a catch distance of about 50-190 nautical miles (Salmarika and 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 3 prominent marine fish families, namely Scombridae, Serranidae, and Carangidae, was carried out. The results of the investigation found that the Scombridae *Auxis thazard* (Aceh) which separates from the same clade species even though it only has a genetic distance of only 0.002. This haplotype appears likely to occur due to differences in species populations analyzed from India, China (Xu, Van Damme et al. 2019), and Spain (Catanese, Catanese et al. 2008). While other haplotypes found in reef fish are *Variola albimarginata* and *Cephalopholis sonnerati*, the *Variola albimarginata* from Aceh may be a population with 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 carried out in the Andaman Islands and Nicobar Island (Basheer, Vineesh et al. 2017). This area is Indian sea waters, which have the potential to have reef fish, which are almost the same as the species in Aceh. While *Cepalopholis sonnerati* fish species also have similarities with populations from Australia and the Philippines, but slightly different from populations from China (Zhuang, Qu et al. 2013). The study of *Cepalopholis 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 similarities with Indian populations found in the *Cepalopholis sonnerati* species only in previous studies conducted

in the Philippines (Alcantara and Yambot 2016), and Australia (Ward, Zemlak et al. 2005). The speciation process that occurs in coral reef ecosystems occurs with an allopathic pattern that makes geographic isolation 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 a speciation pattern on the main coral reefs (Rocha and Bowen 2008).

4. Conclusions

The 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 becoming fishery commodities and became the main target of the Province of Banda Aceh's exports, including Tuna fish (*Thunnus albacares*) and Cakalang fish (*Katsuwonus pelamis*). In this study, the Scombridae group found one Aceh haplotype in the *Auxis thazard* species (MN257554). In contrast, in the Carangids group, it was known that the *Elagatis bipinnulata* species (MN257553) had similar sequences with the same species from Alabama USA (KF461174). Another species in the Carangidae order, namely *Decapterus macarellus* from Aceh, has similarities with the sequence from China (MH638794) and Malaysia (KY570732). In this type of reef fish, this study found *Variola albimarginata* species (India KM226315), which had similarities with Aceh samples. In contrast, *Cephalopholis sonnerati* (MN257517) had similarities with the same sequence species from the Philippines (KU668631) and Australia (DQ107928). More in-depth research on the haplotypes of the marine fish mentioned above is very much needed to maintain genetic biodiversity in the waters of Banda Aceh, which is a precious asset for Indonesia.

Author contribution

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

Acknowledgments

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

Conflict of Interest

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

References

Alcantara, S. G. and A. V. Yambot (2016). "DNA barcoding of commercially important grouper species (Perciformes, Serranidae) in the Philippines." Mitochondrial DNA Part A **27**(6): 3837-3845.

Baldwin, C. C., J. H. Mounts, D. G. Smith and L. A. Weigt (2009). "Genetic identification and color descriptions of early life-history stages of Belizean Phaeoptyx and Astrapogon (Teleostei: Apogonidae) with comments on identification of adult Phaeoptyx." Zootaxa **2008**: 1-22.

Basheer, V., N. Vineesh, K. Bineesh, R. G. Kumar, C. Mohitha, S. Venu, A. Kathirvelpandian, A. Gopalakrishnan and J. Jena (2017). "Mitochondrial signatures for identification of grouper species from Indian waters." Mitochondrial DNA Part A **28**(4): 451-457.

Batubara, A. S., Z. A. Muchlisin, M. Y. Thamren, U. Usnardi and N. Fadli (2017). "Check list of marine fishes from Simeulue Island waters, Aceh Province, Indonesia." Aceh Journal of Animal Science **2**(2): 77-84.

Bingpeng, X., L. Heshan, Z. Zhilan, W. Chunguang, W. Yanguo and W. Jianjun (2018). "DNA barcoding for identification of fish species in the Taiwan Strait." PloS one **13**(6): e0198109.

Catanese, G., G. Catanese, C. Infante, M. Manchado, G. Catanese, C. Infante and M. Manchado (2008). "Complete mitochondrial DNA sequences of the frigate tuna Auxis thazard and the bullet tuna Auxis rochei: Full Length Research Paper." DNA Sequence **19**(3): 159-166.

Chang, C. H., K. T. Shao, H. Y. Lin, Y. C. Chiu, M. Y. Lee, S. H. Liu and P. L. Lin (2017). "DNA barcodes of the native ray-finned fishes in Taiwan." Molecular ecology resources **17**(4): 796-805.

Edrus, I. N., S. W. Wijaya and I. E. Setiawan (2016). "Struktur komunitas ikan karang di perairan Pulau Raya, Pulau Rusa, Pulau Rondo dan taman laut Rinoi dan Rubiah, Nanggroe Aceh Darussalam." Jurnal Penelitian Perikanan Indonesia **19**(4): 175-186.

Edwarsyah, E., M. Nasir and M. B. Selamat (2019). "The DNA Barcoding of Several Commercial Fish from Simeulue Islands Coast of Aceh Using Cytochrome Oxidase Sub Unit I (COI) Gene Marker." Budapest International Research in Exact Sciences (BirEx) Journal **1**(1): 62-70.

Fadli, N., Z. Muchlisin, F. Pratama, T. Mustari, I. Dewiyanti, S. Purnawan, S. El-Rahimi, H. Sofyan, M. Affan and M. Siti-Azizah (2019). The composition of coral reefs in Ulee Lheue breakwater, Banda Aceh, Aceh, Indonesia. IOP Conference Series: Earth and Environmental Science, IOP Publishing.

Handy, S. M., J. R. Deeds, N. V. Ivanova, P. D. Hebert, R. H. Hanner, A. Ormos, L. A. Weigt, M. M. Moore and H. F. Yancy (2011). "A single-laboratory validated method for the generation of DNA barcodes for the identification of fish for regulatory compliance." Journal of AOAC International **94**(1): 201-210.

Hariati, T. (2017). "Status dan perkembangan perikanan pukat cincin di Banda Aceh." Jurnal Penelitian Perikanan Indonesia **17**(3): 157-167.

Hariati, T., R. Faizah and D. Nugroho (2015). "Umur, Pertumbuhan Dan Laju Pemanfaatan Ikan Banyar (Rastrelliger kanagurta Cuvier, 1816), di Selat Malaka (Wilayah Pengelolaan Perikanan-571)." Jurnal Penelitian Perikanan Indonesia **21**(1): 1-8.

Hariati, T. and M. Fauzi (2017). "Aspek reproduksi ikan banyar, Rastrelliger kanagurta (Cuv. 1817) di perairan utara Aceh [Reproductive aspect of indian mackerel Rastrelliger kanagurta (Cuv. 1817) of northern Aceh waters]." Jurnal Iktiologi Indonesia **11**(1): 47-53.

Kamal, M. M., A. A. Hakim, N. A. Butet, Y. Fitrianingsih and R. Astuti (2019). "Autentikasi spesies ikan kerapu berdasarkan marka gen MT-COI dari perairan Peukan Bada, Aceh." Jurnal Biologi Tropis **19**(2): 116-123.

Keskİn, E. and H. H. Atar (2013). "DNA barcoding commercially important fish species of T urkey." Molecular ecology resources **13**(5): 788-797.

Kumar, S., G. Stecher and K. Tamura (2016). "MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets." Molecular biology and evolution **33**(7): 1870-1874.

Kurnia, K., S. Purnawan and T. Rizwan (2016). "Pemetaan daerah penangkapan ikan pelagis kecil di Perairan Utara Aceh." Jurnal Ilmiah Mahasiswa Kelautan Perikanan Unsyiah **1**(2).

Lakra, W., M. Verma, M. Goswami, K. K. Lal, V. Mohindra, P. Punia, A. Gopalakrishnan, K. Singh, R. D. Ward and P. Hebert (2011). "DNA barcoding Indian marine fishes." Molecular Ecology Resources **11**(1): 60-71.

Lubis, A. M., S. Syaifuddin and I. Isnaniah (2016). "The Contribution of the Lampulo Fishing Port, for Fishery Sector in Banda Aceh City, Nanggroe Aceh Darussalam Province." Jurnal Online Mahasiswa Fakultas Perikanan dan Ilmu Kelautan Universitas Riau **3**(1): 1-13.

Mardhatillah, I., A. Damora, A. Rahmah, N. Nurfadillah and M. Muhammad (2019). Application of Schaefer model on mackerels fishery in Aceh waters, Indonesia. IOP Conference Series: Earth and Environmental Science, IOP Publishing.

Munawwarah, A., I. Sufi and A. Noviyanti (2016). "Identifikasi Jenis-Jenis Ikan Yang Terdapat Di Tempat Pelelangan Ikan (TPI) Di Gampong Lampulo Kecamatan Kuta Alam Banda Aceh." Serambi Saintia: Jurnal Sains dan Aplikasi **4**(1).

Neliyana, W. B., E. Wiyono and T. Nurani (2014). "Analisis Kelayakan Usaha Perikanan Pukat Cincin di Pelabuhan Perikanan Pantai (PPP) Lampulo Banda Aceh Propinsi Aceh." Jurnal Marine Fisheries **5**(2): 163-169.

Nur, F. M., A. S. Batubara, A. W. Perdana, K. Eriani and Z. A. Muchlisin (2019). Checklist of coral fishes in Lhoknga and Lhok Mata Ie Beaches, Aceh Besar, Indonesia. IOP Conference Series: Earth and Environmental Science, IOP Publishing.

Rocha, L. and B. Bowen (2008). "Speciation in coral-reef fishes." Journal of Fish Biology **72**(5): 1101-1121.

Salmarika, S. and S. H. Wisudo (2019). "Status Pengelolaan Sumber Daya Ikan Tongkol di Perairan Samudera Hindia Berbasis Pendaratan Pukat Cincin di Pelabuhan Perikanan Samudera Lampulo, Aceh: Suatu Pendekatan Ekosistem." Jurnal Penelitian Perikanan Indonesia **24**(4): 263-272.

348 Suman, A., H. E. Irianto, F. Satria and K. Amri (2017). "Potensi dan tingkat pemanfaatan
349 sumber daya ikan di wilayah pengelolaan perikanan Negara Republik Indonesia
350 (WPP NRI) Tahun 2015 serta Opsi Pengelolaannya." Jurnal Kebijakan Perikanan
351 Indonesia **8**(2): 97-100.
352 Wang, Z.-D., Y.-S. Guo, X.-M. Liu, Y.-B. Fan and C.-W. Liu (2012). "DNA barcoding South
353 China Sea fishes." Mitochondrial Dna **23**(5): 405-410.
354 Ward, R. D., T. S. Zemlak, B. H. Innes, P. R. Last and P. D. Hebert (2005). "DNA barcoding
355 Australia's fish species." Philosophical Transactions of the Royal Society B:
356 Biological Sciences **360**(1462): 1847-1857.
357 Wiryawan, B., E. S. Wiyono and T. W. Nurani (2016). "Analisis Kelayakan Usaha Perikanan
358 Pukat Cincin Di Pelabuhan Perikanan Pantai (Ppp) Lampulo Banda Aceh Propinsi
359 Aceh (Analysis Financial Fisheries of Purse Seine in Lampulo Fishing Port Banda
360 Aceh Provinsi Aceh)." Marine Fisheries: Journal of Marine Fisheries Technology
361 and Management **5**(2): 163-169.
362 Xu, L., K. Van Damme, H. Li, Y. Ji, X. Wang and F. Du (2019). "A molecular approach to
363 the identification of marine fish of the Dongsha Islands (South China Sea)."
364 Fisheries Research **213**: 105-112.
365 Yeni, E. and A. Naufal (2017). Identifikasi Aktivitas Pengembangan Fasilitas Pelabuhan
366 Perikanan Pantai Lampulo Banda Aceh. Prosiding SEMDI-UNAYA (Seminar
367 Nasional Multi Disiplin Ilmu UNAYA).
368 Yusuf, Q. (2003). Empowerment of Panglima Laot in Aceh. International workshop on
369 Marine Science and Resource. Banda Aceh.
370 Zhang, J. and R. Hanner (2012). "Molecular approach to the identification of fish in the South
371 China Sea." PLoS One **7**(2): e30621.

Zhuang, X., M. Qu, X. Zhang and S. Ding (2013). "A comprehensive description and evolutionary analysis of 22 grouper (Perciformes, Epinephelidae) mitochondrial genomes with emphasis on two novel genome organizations." PLoS One **8**(8): e73561.

Zulmaidah, Z., J. Zain and R. M. Hutauruk (2015). "Facilities Utilization in Lampulo Fisheries Port, District of Kuta Alam, Banda Aceh City, Province Nanggroe Aceh Darussalam." None **2**(2): 1-13.

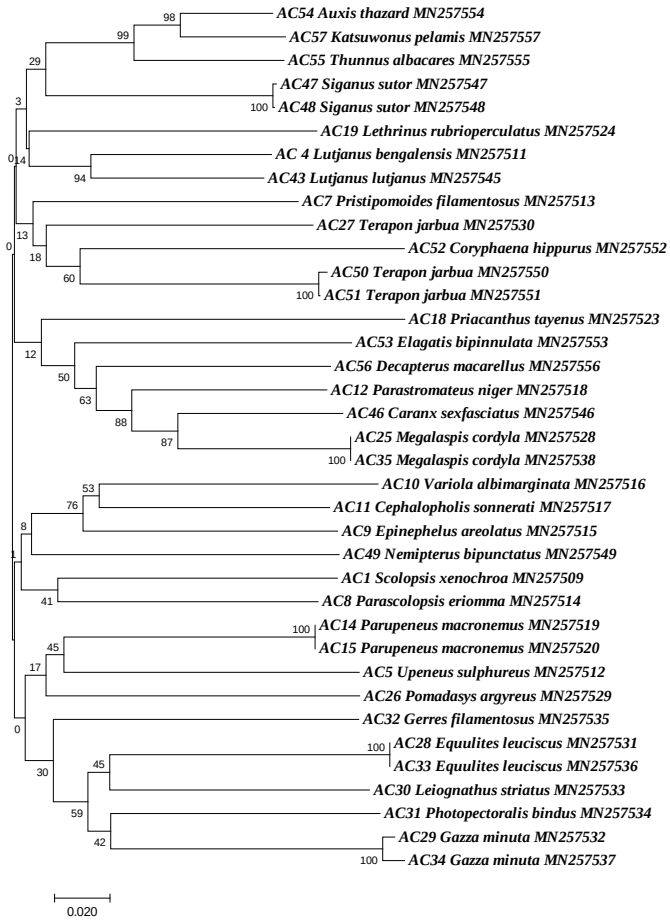


Figure 1. Phylogenetic tree of Perciformes order by Neighbor-Joining tree algorithm using Mega7

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

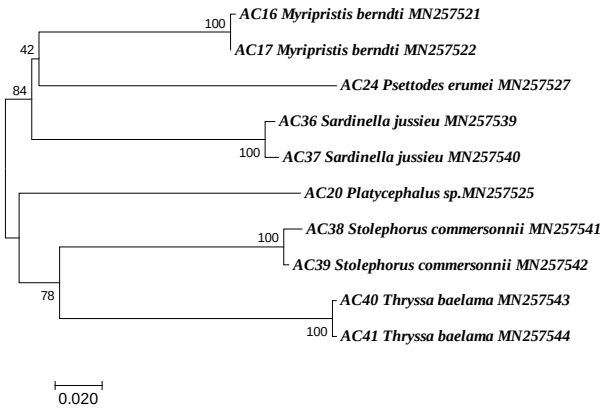
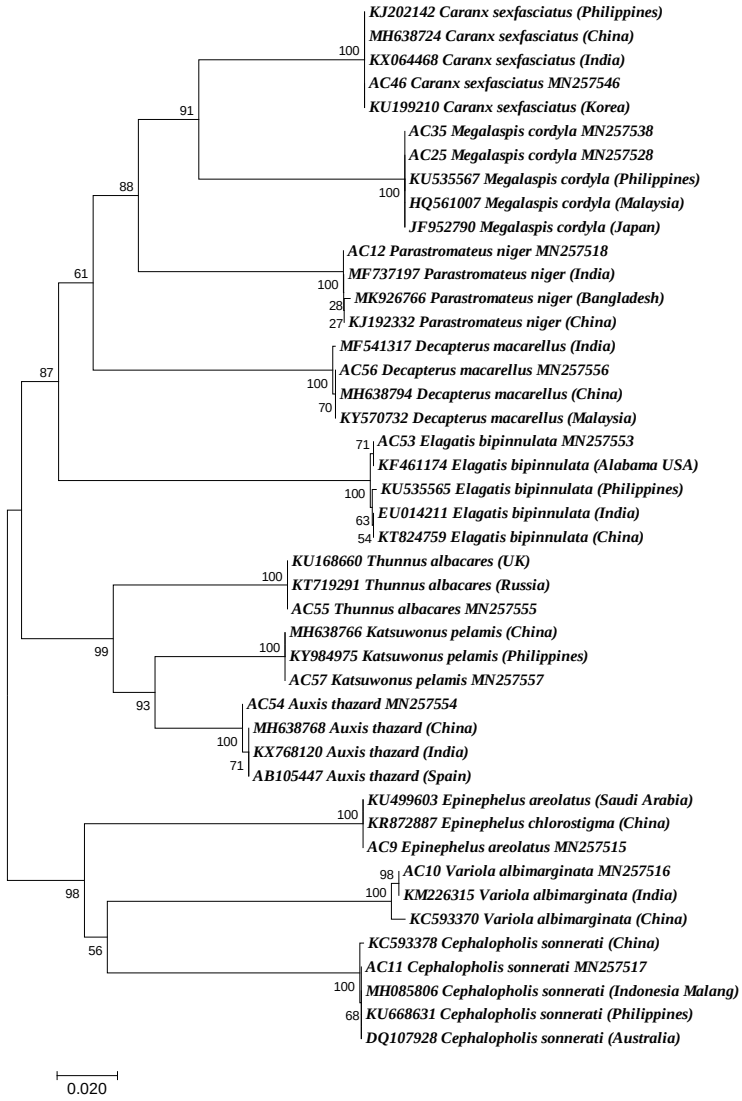


Figure 2. Phylogenetic tree of small number others order in this study including the Clupeiformes, Beryciformes, Pleuronectiformes, and Scorpaeniformes by Neighbor-Joining tree algorithm using Mega7

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	*Myripristis berndti*	Holocentridae	MN257521	Beryciformes	Blotcheye soldierfish	Indo-Pacific and Eastern Pacific	LC
2	17	*Myripristis berndti*	Holocentridae	MN257522	Beryciformes	Blotcheye soldierfish	Indo-Pacific and Eastern Pacific	LC
3	36	*Sardinella jussieu*	Clupeidae	MN257539	Clupeiformes	Mauritian sardinella	Western Indian Ocean	DD
4	37	*Sardinella jussieu*	Clupeidae	MN257540	Clupeiformes	Mauritian sardinella	Western Indian Ocean	DD
5	38	*Stolephorus commersonnii*	Engraulidae	MN257541	Clupeiformes	Commerson's anchovy	Indo-West Pacific	LC
6	39	*Stolephorus commersonnii*	Engraulidae	MN257542	Clupeiformes	Commerson's anchovy	Indo-West Pacific	LC
7	40	*Thryssa baelama*	Engraulidae	MN257543	Clupeiformes	Baelama anchovy	Indo-Pacific	LC
8	41	*Thryssa baelama*	Engraulidae	MN257544	Clupeiformes	Baelama anchovy	Indo-Pacific	LC
9	1	*Scolopsis xenochroa*	Nemipteridae	MN257509	Perciformes	Oblique-barred monocle bream	Indo-West Pacific	NE
10	4	*Lutjanus bengalensis*	Lutjanidae	MN257511	Perciformes	Bengal snapper	Indo-West Pacific:	NE
11	5	*Upeneus sulphureus*	Mullidae	MN257512	Perciformes	Sulphur goatfish	Indo-West Pacific	LC
12	7	*Pristipomoides filamentosus*	Lutjanidae	MN257513	Perciformes	Crimson jobfish	Indo-Pacific	LC
13	8	*Parascolopsis eriomma*	Nemipteridae	MN257514	Perciformes	Rosy dwarf monocle bream	Indo-West Pacific	NE
14	9	*Epinephelus areolatus*	Serranidae	MN257515	Perciformes	Areolate grouper	Indo-Pacific	LC
15	10	*Variola albimarginata*	Serranidae	MN257516	Perciformes	White-edged lyretail	Indo-Pacific	LC
16	11	*Cephalopholis sonnerati*	Serranidae	MN257517	Perciformes	Tomato hind	Indo-Pacific	LC
17	12	*Parastromateus niger*	Carangidae	MN257518	Perciformes	Black pomfret	Indo-West Pacific	LC
18	14	*Parupeneus macronemus*	Mullidae	MN257519	Perciformes	Long-barbel goatfish	Indo-West Pacific	LC
19	15	*Parupeneus macronemus*	Mullidae	MN257520	Perciformes	Long-barbel goatfish	Indo-West Pacific	LC
20	18	*Priacanthus tayenus*	Priacanthidae	MN257523	Perciformes	Purple-spotted bigeye	Indo-West Pacific	LC
21	19	*Lethrinus rubrioperculatus*	Lethrinidae	MN257524	Perciformes	Spotcheek emperor	Indo-Pacific	LC
22	25	*Megalaspis cordyla*	Carangidae	MN257528	Perciformes	Torpedo scad	Indo-West Pacific	LC
23	26	*Pomadasys argyreus*	Haemulidae	MN257529	Perciformes	Bluecheek silver	Indo-West Pacific	NE

						grunt		
24	27	*Terapon jarbua*	Terapontidae	MN257530	Perciformes	Jarbua terapon	Indo-Pacific	LC
25	28	*Equulites leuciscus*	Leiognathidae	MN257531	Perciformes	Whipfin ponyfish	Indo-West Pacific	LC
26	29	*Gazza minuta*	Leiognathidae	MN257532	Perciformes	Toothpony	Indo-Pacific	LC
27	30	*Leiognathus striatus*	Leiognathidae	MN257533	Perciformes	Toothpony	Western Indian Ocean	NE
28	31	*Photopectoralis bindus*	Leiognathidae	MN257534	Perciformes	Orangefin ponyfish	Indo-West Pacific	NE
29	32	*Gerres filamentosus*	Gerreidae	MN257535	Perciformes	Whipfin silver-biddy	Indo-Pacific	LC
30	33	*Equulites leuciscus*	Leiognathidae	MN257536	Perciformes	Whipfin ponyfish	Indo-West Pacific	LC
31	34	*Gazza minuta*	Leiognathidae	MN257537	Perciformes	Toothpony	Indo-Pacific	LC
32	35	*Megalaspis cordyla*	Carangidae	MN257538	Perciformes	Torpedo scad	Indo-West Pacific	LC
33	43	*Lutjanus lutjanus*	Lutjanidae	MN257545	Perciformes	Bigeye snapper	Indo-West Pacific	LC
34	46	*Caranx sexfasciatus*	Carangidae	MN257546	Perciformes	Bigeye trevally	Indo-Pacific	LC
35	47	*Siganus sutor*	Siganidae	MN257547	Perciformes	Shoemaker spinefoot	Indian Ocean	LC
36	48	*Siganus sutor*	Siganidae	MN257548	Perciformes	Shoemaker spinefoot	Indian Ocean	LC
37	49	*Nemipterus bipunctatus*	Nemipteridae	MN257549	Perciformes	Delagoa threadfin bream	Indian Ocean	NE
38	50	*Terapon jarbua*	Terapontidae	MN257550	Perciformes	Jarbua terapon	Indo-Pacific	LC
39	51	*Terapon jarbua*	Terapontidae	MN257551	Perciformes	Jarbua terapon	Indo-Pacific	LC
40	52	*Coryphaena hippurus*	Coryphaenidae	MN257552	Perciformes	Common dolphinfish	Atlantic, Indian and Pacific	LC
41	53	*Auxis thazard*	Scombridae	MN257553	Perciformes	Frigate tuna	Atlantic, Indian and Pacific (Western Central)	LC
42	54	*Auxis thazard*	Scombridae	MN257554	Perciformes	Frigate tuna	Atlantic, Indian and Pacific (Western Central)	LC
43	55	*Thunnus albacares*	Scombridae	MN257555	Perciformes	Yellowfin tuna	Worldwide in tropical and subtropical seas	NT
44	56	*Decapterus macarellus*	Carangidae	MN257556	Perciformes	Mackerel scad	Circumglobal	LC
45	57	*Katsuwonus pelamis*	Scombridae	MN257557	Perciformes	Skipjack tuna	Cosmopolitan in tropical and warm-temperate waters	LC
46	24	*Psettodes erumei*	Psettodidae	MN257527	Pleuronectiformes	Indian halibut	Indo-West Pacific	NE
47	20	*Platycephalus sp.*	Platycephalidae	MN257525	Scorpaeniformes	Bartail flathead	Indo-West Pacific	DD

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

398

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

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

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^2 and a coastline length corresponding to 6.3 km^2 (Mukhtar, 2017). 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 (Mardhatillah et al. 2019, Yeni and Naufal 2017, Yusuf 2003). The fish landing site suffered massive damage due to the tsunami that struck Aceh Province and was 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 Indonesian Fisheries Management Areas (FMA), namely FMA 571 and 572. 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 (Salmarika and Wisudo 2019, Suman et al. 2017).

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 genus, 26 families, and seven orders (Batubara et al. 2017). The reef-associated fish inventory at Ulee Lheue, Banda Aceh, also mentioned that there were 87 species of reef fishes from 28 families in this location (Fadli et al. 2019). In different areas (i.e. Lhoknga and Lhok Mata Ie Beaches) 25 fish species which are members of eight orders, 11 families, and 19 genera were recorded from 51 fish samples (Nur et al. 2019); 71 species were identified in Pusong Bay, Lhokseumawe belonging to 54 genus, 37 families and 15 orders (Damora et al. 2020); 50 species were identified in 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 identifies 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 (KeskİN and Atar 2013), marine fish in China (Wang et al. 2012, Zhang and Hanner 2012), and marine fish in Taiwan (Bingpeng et al. 2018, Chang et al. 2017). 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 fishing port of Kutaradja.

The purpose of this research is to identify marine fish down to species level by using a molecular approach to increase the accuracy of species level identification. 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 value. 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.

2. Materials and methods

2.1 Sampling site

A total of 47 fish samples were collected from the Kutaradja fish market close to the Kutaradja fish landing 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. 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.

2.2 DNA extraction

Each specimen were 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.

2.3 PCR amplification and sequencing

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). All PCR products were sent to Macrogen (Seoul, Korea) for sequencing.

2.4 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) 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 transition/transversion bias after phylogenetic trees reconstruction was conducted.

3. Results

3.1 Species Identification

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

the Kutaradja 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 was 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”.

3.2 Perciformes

From the total of 37 samples, we successfully identified 31 species from 14 families under Perciformes. 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 transvertional pair (sv=14.86) with an overall transition/transvertion 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 levels measured for COI sequences is 0.226 (Figure 1).

3.3 Clupeiformes and Others

In addition to Perciformes, Clupeiformes 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 transvertional pair (sv=22.13) with an average transition/transvertion 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.

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

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

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

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

Discussion

Research on molecular identification is now extensive in the field of fisheries and marine sciences. In this study, molecular identification is used to complete 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 face 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 is 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 (Lampulo). There are still inaccurate information regarding marine fish identification in some reports. Some identification 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 this Kutaradja (Lampulo) 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, 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 capture 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 (Hariati 2017, Wiryawan et al. 2016). The purse-seine is also a fishing gear generally used to catch *Euthynnus affinis, Auxis thazard,* and *Auxis rochei* (Salmarika and 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 a group of fish that utilize coral reefs as their habitat. However, several pelagic fish found around the shallow seas of Banda Aceh is still the primary target. The skipjack tuna *Rastrelliger kanagurta* (Hariati and Fauzi 2017, Hariati et al. 2015), 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 port of 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 and 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 appears 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 may 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 *Cepalopholis 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 *Cepalopholis 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 similarities with Indian populations were found in the *Cepalopholis sonnerati* sample species, similarities were only found with 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 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).

4. Conclusions

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

Author 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.

Acknowledgments

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

Conflict of Interest

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

References

Afriyie G, Huang S, Dong Z, Guo Y, Kuebutornye FK, Larbi CA, Asiedu B, Wang Z. 2019. DNA barcoding of Ghanaian fish species: Status and prospects. African Journal of Biotechnology 18: 659-663. http://doi.org.10.5897/AJB2019.16792.

298 Alcantara SG, Yambot AV. 2016. DNA barcoding of commercially important grouper species
299 (Perciformes, Serranidae) in the Philippines. Mitochondrial DNA Part A 27: 3837-3845.
300 http://doi.org/10.3109/19401736.2014.958672.
301 Baldwin CC, Mounts JH, Smith DG, Weigt LA. 2009. Genetic identification and color
302 descriptions of early life-history stages of Belizean Phaeoptyx and Astrapogon (Teleostei:
303 Apogonidae) with comments on identification of adult Phaeoptyx. Zootaxa 2008: 1-22.
304 https://doi.org/10.11646/zootaxa.2008.1.1.
305 Basheer V, Vineesh N, Bineesh K, Kumar RG, Mohitha C, Venu S, Kathirvelpandian A,
306 Gopalakrishnan A, Jena J. 2017. Mitochondrial signatures for identification of grouper
307 species from Indian waters. Mitochondrial DNA Part A 28: 451-457.
308 http://doi.org/10.3109/19401736.2015.1137899.
309 Batubara AS, Muchlisin ZA, Thamren MY, Usnardi U, Fadli N. 2017. Check list of marine
310 fishes from Simeulue Island waters, Aceh Province, Indonesia. Aceh Journal of Animal
311 Science 2: 77-84. https://doi.org/10.13170/ajas.2.2.9584
312 Bingpeng X, Heshan L, Zhilan Z, Chunguang W, Yanguo W, Jianjun W. 2018. DNA barcoding
313 for identification of fish species in the Taiwan Strait. PloS one 13: e0198109.
314 https://doi.org/10.1371/journal.pone.0198109
315 Catanese G, Catanese G, Infante C, Manchado M, Catanese G, Infante C, Manchado M. 2008.
316 Complete mitochondrial DNA sequences of the frigate tuna Auxis thazard and the bullet
317 tuna Auxis rochei: Full Length Research Paper. DNA Sequence 19: 159-166.
318 http://doi.org/10.1080.10425170701207117
319 Chang CH, Shao KT, Lin HY, Chiu YC, Lee MY, Liu SH, Lin PL. 2017. DNA barcodes of the
320 native ray-finned fishes in Taiwan. Molecular ecology resources 17: 796-805.
321 https://doi.org/10.1111/1755-0998.12601
322 Damanik MRS, Lubis MRK, Astuti AJD. 2016. Kajian pendekatan ekosistem dalam
323 pengelolaan perikanan di wilayah pengelolaan perikanan (WPP) 571 Selat Malaka
324 Provinsi Sumatera Utara. Jurnal Geografi 8: 165-176.
325 https://doi.org/10.24114/jg.v8i2.5780
326 Damora A, Batubara AS, Zuhdi Z, Restiangsih Y, Amir F, Irham M, Fadli N, Nur F, Rizal R.
327 2020. Diversity of marine fish and their conservation status in Pusong Bay, Lhokseumawe
328 City, Aceh Province, Indonesia. European Journal of Environmental Sciences 10: 115-
329 123. https://doi.org/10.14712/23361964.2020.13
330 Edrus IN, Wijaya SW, Setiawan IE. 2016. Struktur komunitas ikan karang di perairan Pulau
331 Raya, Pulau Rusa, Pulau Rondo dan taman laut Rinoi dan Rubiah, Nanggroe Aceh

Darussalam. Jurnal Penelitian Perikanan Indonesia 19: 175-186. http://dx.doi.org/10.15578/jppi.19.4.2013.175-186

Edwarsyah E, Nasir M, Selamat MB. 2019. The DNA Barcoding of Several Commercial Fish from Simeulue Islands Coast of Aceh Using Cytochrome Oxidase Sub Unit I (COI) Gene Marker. Budapest International Research in Exact Sciences (BirEx) Journal 1: 62-70. https://doi.org/10.33258/birex.v1i1.140

Fadli N, Muchlisin Z, Pratama F, Mustari T, Dewiyanti I, Purnawan S, El-Rahimi S, Sofyan H, Affan M, Siti-Azizah M. 2019. The composition of coral reefs in Ulee Lheue breakwater, Banda Aceh, Aceh, Indonesia. Pages 012077. IOP Conference Series: Earth and Environmental Science: IOP Publishing. https://doi.org/10.1088/1755-1315/348/1/012077

Handy SM, Deeds JR, Ivanova NV, Hebert PD, Hanner RH, Ormos A, Weigt LA, Moore MM, Yancy HF. 2011. A single-laboratory validated method for the generation of DNA barcodes for the identification of fish for regulatory compliance. Journal of AOAC International 94: 201-210. https://doi.org/10.1093/jaoac/94.1.201

Hariati T. 2017. Status dan perkembangan perikanan pukat cincin di Banda Aceh. Jurnal Penelitian Perikanan Indonesia 17: 157-167. http://dx.doi.org/10.15578/jppi.17.3.2011. 157-167

Hariati T, Fauzi M. 2017. Aspek reproduksi ikan banyar, Rastrelliger kanagurta (Cuv. 1817) di perairan utara Aceh [Reproductive aspect of indian mackerel Rastrelliger kanagurta (Cuv. 1817) of northern Aceh waters]. Jurnal Iktiologi Indonesia 11: 47-53. https://doi.org/10.32491/jii.v11i1.155

Hariati T, Faizah R, Nugroho D. 2015. Umur, Pertumbuhan Dan Laju Pemanfaatan Ikan Banyar (Rastrelliger kanagurta Cuvier, 1816), di Selat Malaka (Wilayah Pengelolaan Perikanan-571). Jurnal Penelitian Perikanan Indonesia 21: 1-8. http://dx.doi.org/10.15578/jppi.21.1.2015.1-8

Kamal MM, Hakim AA, Butet NA, Fitrianingsih Y, Astuti R. 2019. Autentikasi spesies ikan kerapu berdasarkan marka gen MT-COI dari perairan Peukan Bada, Aceh. Jurnal Biologi Tropis 19: 116-123. https://doi.org/10.29303/jtb.v19i2.1245

Keskİn E, Atar HH. 2013. DNA barcoding commercially important fish species of T urkey. Molecular ecology resources 13: 788-797. https://doi.org/10.1111/1755-0998.12120

Kumar S, Stecher G, Tamura K. 2016. MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. Molecular biology and evolution 33: 1870-1874. https://doi.org/10.1093/molbev/msw054

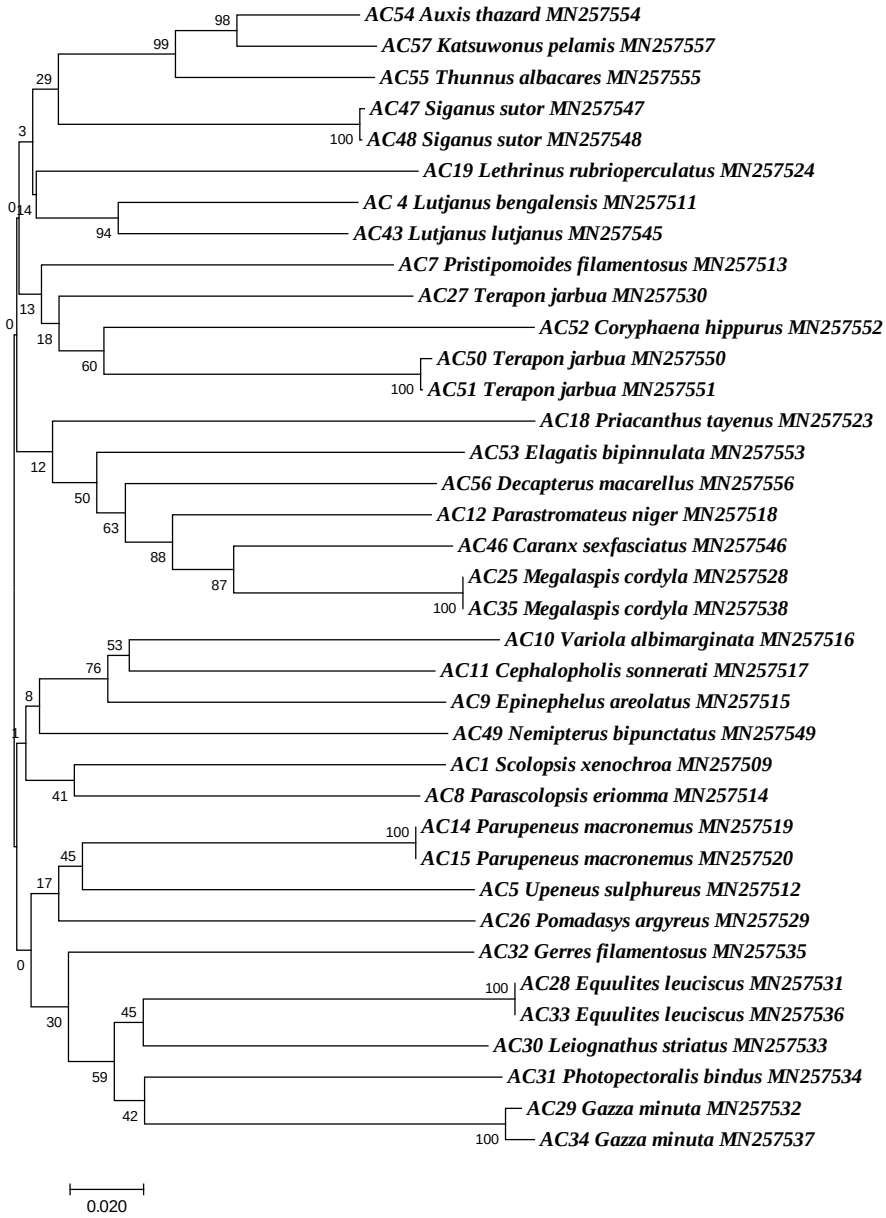
366 Kurnia K, Purnawan S, Rizwan T. 2016. Pemetaan daerah penangkapan ikan pelagis kecil di
367 Perairan Utara Aceh. Jurnal Ilmiah Mahasiswa Kelautan Perikanan Unsyiah 1.
368 http://www.jim.unsyiah.ac.id/fkp/article/view/204
369 Lakra W, Verma M, Goswami M, Lal KK, Mohindra V, Punia P, Gopalakrishnan A, Singh K,
370 Ward RD, Hebert P. 2011. DNA barcoding Indian marine fishes. Molecular Ecology
371 Resources 11: 60-71. https://doi.org/10.1111/j.1755-0998.2010.02894.x
372 Lubis AM, Syaifuddin S, Isnaniah I. 2016. The Contribution of the Lampulo Fishing Port, for
373 Fishery Sector in Banda Aceh City, Nanggroe Aceh Darussalam Province. Jurnal Online
374 Mahasiswa Fakultas Perikanan dan Ilmu Kelautan Universitas Riau 3: 1-13.
375 http://jom.unri.ac.id/index.php/JOMFAPERIKA/article/view/9183
376 Mardhatillah I, Damora A, Rahmah A, Nurfadillah N, Muhammad M. 2019. Application of
377 Schaefer model on mackerels fishery in Aceh waters, Indonesia. Pages 012114. IOP
378 Conference Series: Earth and Environmental Science: IOP Publishing.
379 https://doi.org/10.1088/1755-1315/348/1/012114
380 Munawwarah A, Sufi I, Noviyanti A. 2016. Identifikasi Jenis-Jenis Ikan Yang Terdapat Di
381 Tempat Pelelangan Ikan (TPI) Di Gampong Lampulo Kecamatan Kuta Alam Banda
382 Aceh. Serambi Saintia: Jurnal Sains dan Aplikasi 4. https://doi.org/10.32672/jss.v4i1.115
383 Nasution MH, Anwar S, Fitri A, Zohra AF. 2019. Forecasting The Amount of
384 Tuna/Madidihang (Yellowfin tuna) Landed in PPS Kutaraja Banda Aceh City With The
385 Triple Exponential Smoothing Method. Samakia: Jurnal Ilmu Perikanan 10: 08-14.
386 https://doi.org/10.35316/jsapi.v10i1.231
387 Neliyana WB, Wiyono E, Nurani T. 2014. Analisis Kelayakan Usaha Perikanan Pukat Cincin
388 di Pelabuhan Perikanan Pantai (PPP) Lampulo Banda Aceh Propinsi Aceh. Jurnal Marine
389 Fisheries 5: 163-169. https://doi.org/10.1088/1755-1315/348/1/012104
390 Nur FM, Batubara AS, Perdana AW, Eriani K, Muchlisin ZA. 2019. Checklist of coral fishes
391 in Lhoknga and Lhok Mata Ie Beaches, Aceh Besar, Indonesia. Pages 012104. IOP
392 Conference Series: Earth and Environmental Science: IOP Publishing.
393 https://doi.org/10.29244/jmf.5.2.163-169
394 Pringgenis D, Susilowati R. 2016. Highly commercial fisheries tawar fish: molecular analysis
395 DNA mitochondrial COI gene sequence and proximate analysis from malacca Strait,
396 Riau. Jurnal Teknologi 78. https://doi.org/10.11113/jt.v78.8149
397 Rocha L, Bowen B. 2008. Speciation in coral-reef fishes. Journal of Fish Biology 72: 1101-
398 1121. https://doi.org/10.1111/j.1095-8649.2007.01770.x

399 Salmarika S, Wisudo SH. 2019. Status Pengelolaan Sumber Daya Ikan Tongkol di Perairan
400 Samudera Hindia Berbasis Pendaratan Pukat Cincin di Pelabuhan Perikanan Samudera
401 Lampulo, Aceh: Suatu Pendekatan Ekosistem. Jurnal Penelitian Perikanan Indonesia 24:
402 263-272. http://ejournal-balitbang.kkp.go.id/index.php/jppi/article/view/7417
403 Serdiati N, Arfiati D, Widodo MS, Lelono TJ, Ndobe S, Saranga R. 2020. Morphological
404 variations and phylogenetic analysis of Oryzias nigrimas Kottelat, 1990 (rice fish) from
405 Lake Poso, Central Sulawesi, Indonesia. Biodiversitas Journal of Biological Diversity 21.
406 https://doi.org/10.13057/biodiv/d210305
407 Suman A, Irianto HE, Satria F, Amri K. 2017. Potensi dan tingkat pemanfaatan sumber daya
408 ikan di wilayah pengelolaan perikanan Negara Republik Indonesia (WPP NRI) Tahun
409 2015 serta Opsi Pengelolaannya. Jurnal Kebijakan Perikanan Indonesia 8: 97-100.
410 http://dx.doi.org/10.15578/jkpi.8.2.2016.97-100
411 Wang Z-D, Guo Y-S, Liu X-M, Fan Y-B, Liu C-W. 2012. DNA barcoding South China Sea
412 fishes. Mitochondrial Dna 23: 405-410. https://doi.org/10.3109/19401736.2012.710204.
413 Ward RD, Zemlak TS, Innes BH, Last PR, Hebert PD. 2005. DNA barcoding Australia's fish
414 species. Philosophical Transactions of the Royal Society B: Biological Sciences 360:
415 1847-1857. https://doi.org/10.1098/rstb.2005.1716
416 Wiryawan B, Wiyono ES, Nurani TW. 2016. Analisis Kelayakan Usaha Perikanan Pukat
417 Cincin Di Pelabuhan Perikanan Pantai (Ppp) Lampulo Banda Aceh Propinsi Aceh
418 (Analysis Financial Fisheries of Purse Seine in Lampulo Fishing Port Banda Aceh
419 Provinsi Aceh). Marine Fisheries: Journal of Marine Fisheries Technology and
420 Management 5: 163-169. https://doi.org/10.29244/jmf.5.2.163-169
421 Xu L, Van Damme K, Li H, Ji Y, Wang X, Du F. 2019. A molecular approach to the
422 identification of marine fish of the Dongsha Islands (South China Sea). Fisheries Research
423 213: 105-112. https://doi.org/10.1016/j.fishres.2019.01.011
424 Yeni E, Naufal A. 2017. Identifikasi Aktivitas Pengembangan Fasilitas Pelabuhan Perikanan
425 Pantai Lampulo Banda Aceh. Pages 355-363. Prosiding SEMDI-UNAYA (Seminar
426 Nasional Multi Disiplin Ilmu UNAYA). http://jurnal.abulyatama.ac.id/index.php/
427 semdiunaya/article/view/219
428 Yusuf Q. 2003. Empowerment of Panglima Laot in Aceh. Pages 11-13. International workshop
429 on Marine Science and Resource. Banda Aceh.
430 Zhang J, Hanner R. 2012. Molecular approach to the identification of fish in the South China
431 Sea. PLoS One 7: e30621. https://doi.org/10.1371/journal.pone.0030621

Zhuang X, Qu M, Zhang X, Ding S. 2013. A comprehensive description and evolutionary analysis of 22 grouper (Perciformes, Epinephelidae) mitochondrial genomes with emphasis on two novel genome organizations. PLoS One 8: e73561. https ://doi.org/10.1371/journal.pone.0073561

Zulfahmi I, Apriansyah M, Batubara AS, Kautsari N, Sumon KA, Rahman MM, Nur FM. 2022. Commercial marine fish species from Weh Island, Indonesia: Checklist, distribution pattern and conservation status. Biodiversitas Journal of Biological Diversity 23. https:// 10.13057/biodiv/d230432

Zulmaidah Z, Zain J, Hutauruk RM. 2015. Facilities Utilization in Lampulo Fisheries Port, District of Kuta Alam, Banda Aceh City, Province Nanggroe Aceh Darussalam. None 2: 1-13. http://jom.unri.ac.id/index.php/JOMFAPERIKA/article/view/6854



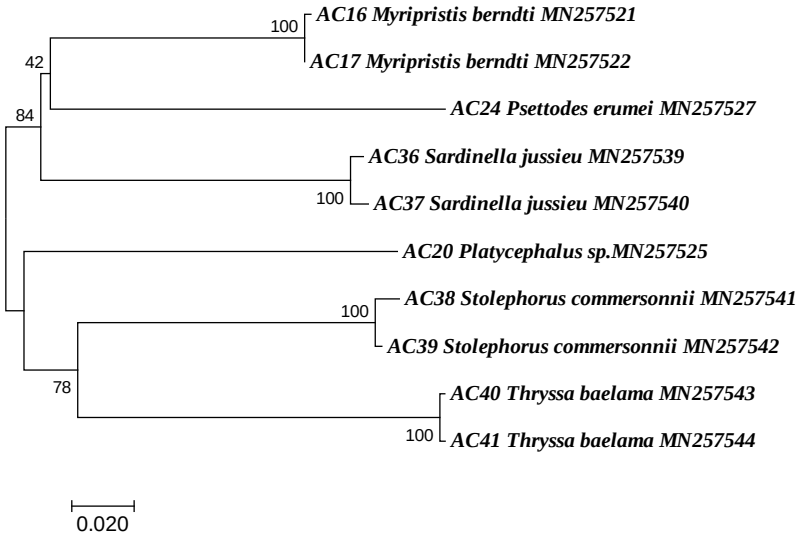
443

444

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

446 using Mega7

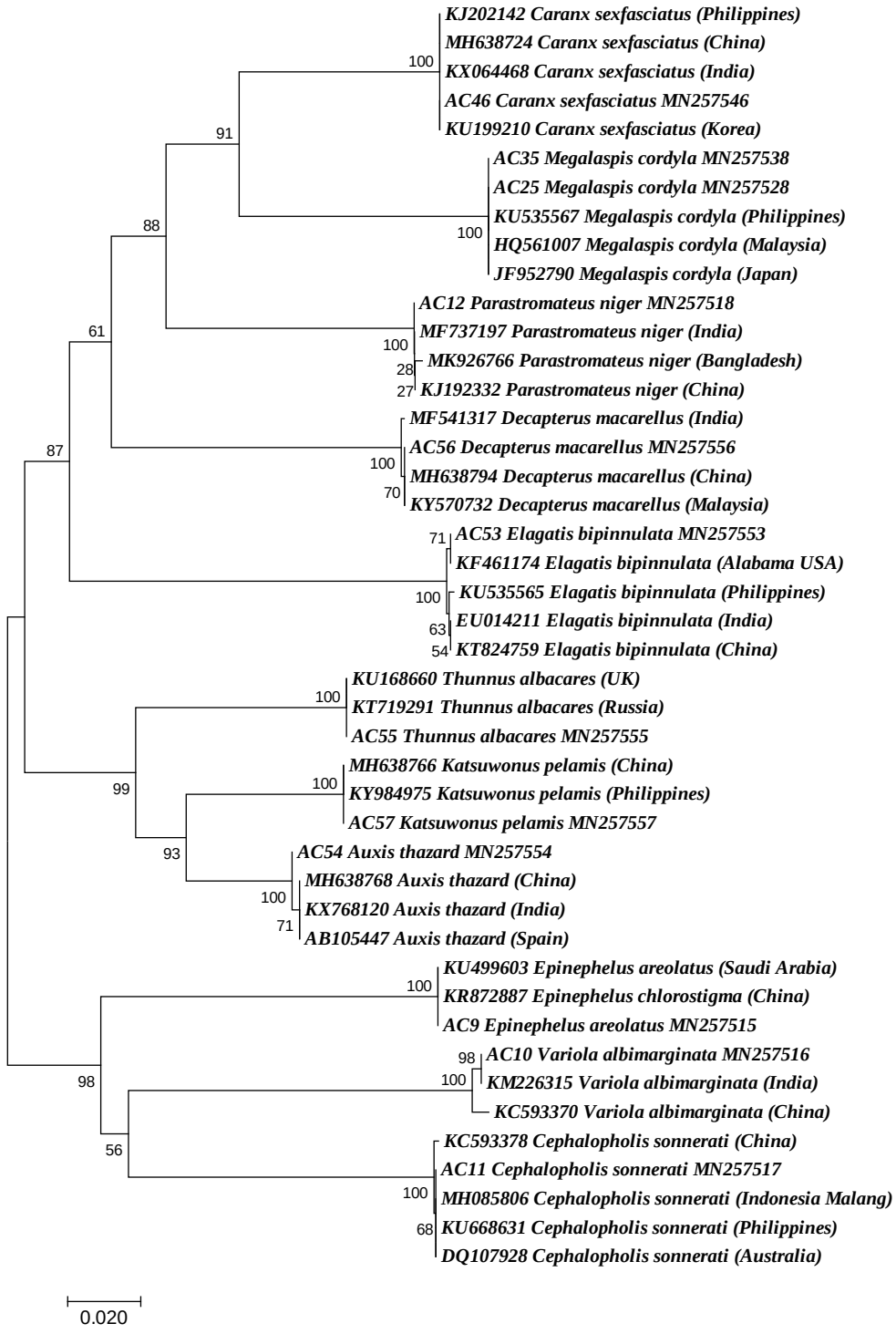
447



448

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

450 Scorpaeniformes by Neighbor-Joining tree algorithm using Mega7



451

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	*Myripristis berndti*	Holocentridae	MN257521	Beryciformes	Blotcheye soldierfish	Indo-Pacific and Eastern Pacific	LC
2	17	*Myripristis berndti*	Holocentridae	MN257522	Beryciformes	Blotcheye soldierfish	Indo-Pacific and Eastern Pacific	LC
3	36	*Sardinella jussieu*	Clupeidae	MN257539	Clupeiformes	Mauritian sardinella	Western Indian Ocean	DD
4	37	*Sardinella jussieu*	Clupeidae	MN257540	Clupeiformes	Mauritian sardinella	Western Indian Ocean	DD
5	38	*Stolephorus commersonnii*	Engraulidae	MN257541	Clupeiformes	Commerson's anchovy	Indo-West Pacific	LC
6	39	*Stolephorus commersonnii*	Engraulidae	MN257542	Clupeiformes	Commerson's anchovy	Indo-West Pacific	LC
7	40	*Thryssa baelama*	Engraulidae	MN257543	Clupeiformes	Baelama anchovy	Indo-Pacific	LC
8	41	*Thryssa baelama*	Engraulidae	MN257544	Clupeiformes	Baelama anchovy	Indo-Pacific	LC
9	1	*Scolopsis xenochroa*	Nemipteridae	MN257509	Perciformes	Oblique-barred monocle bream	Indo-West Pacific	NE
10	4	*Lutjanus bengalensis*	Lutjanidae	MN257511	Perciformes	Bengal snapper	Indo-West Pacific:	NE
11	5	*Upeneus sulphureus*	Mullidae	MN257512	Perciformes	Sulphur goatfish	Indo-West Pacific	LC
12	7	*Pristipomoides filamentosus*	Lutjanidae	MN257513	Perciformes	Crimson jobfish	Indo-Pacific	LC
13	8	*Parascolopsis eriomma*	Nemipteridae	MN257514	Perciformes	Rosy dwarf monocle bream	Indo-West Pacific	NE
14	9	*Epinephelus areolatus*	Serranidae	MN257515	Perciformes	Areolate grouper	Indo-Pacific	LC
15	10	*Variola albimarginata*	Serranidae	MN257516	Perciformes	White-edged lyretail	Indo-Pacific	LC
16	11	*Cephalopholis sonnerati*	Serranidae	MN257517	Perciformes	Tomato hind	Indo-Pacific	LC
17	12	*Parastromateus niger*	Carangidae	MN257518	Perciformes	Black pomfret	Indo-West Pacific	LC
18	14	*Parupeneus macronemus*	Mullidae	MN257519	Perciformes	Long-barbel goatfish	Indo-West Pacific	LC
19	15	*Parupeneus macronemus*	Mullidae	MN257520	Perciformes	Long-barbel goatfish	Indo-West Pacific	LC
20	18	*Priacanthus tayenus*	Priacanthidae	MN257523	Perciformes	Purple-spotted bigeye	Indo-West Pacific	LC
21	19	*Lethrinus rubrioperculatus*	Lethrinidae	MN257524	Perciformes	Spotcheek emperor	Indo-Pacific	LC
22	25	*Megalaspis cordyla*	Carangidae	MN257528	Perciformes	Torpedo scad	Indo-West Pacific	LC
23	26	*Pomadasys argyreus*	Haemulidae	MN257529	Perciformes	Bluecheek silver grunt	Indo-West Pacific	NE

24	27	*Terapon jarbua*	Terapontidae	MN257530	Perciformes	Jarbua terapon	Indo-Pacific	LC
25	28	*Equulites leuciscus*	Leiognathidae	MN257531	Perciformes	Whipfin ponyfish	Indo-West Pacific	LC
26	29	*Gazza minuta*	Leiognathidae	MN257532	Perciformes	Toothpony	Indo-Pacific	LC
27	30	*Leiognathus striatus*	Leiognathidae	MN257533	Perciformes	Toothpony	Western Indian Ocean	NE
28	31	*Photopectoralis bindus*	Leiognathidae	MN257534	Perciformes	Orangefin ponyfish	Indo-West Pacific	NE
29	32	*Gerres filamentosus*	Gerreidae	MN257535	Perciformes	Whipfin silver-biddy	Indo-Pacific	LC
30	33	*Equulites leuciscus*	Leiognathidae	MN257536	Perciformes	Whipfin ponyfish	Indo-West Pacific	LC
31	34	*Gazza minuta*	Leiognathidae	MN257537	Perciformes	Toothpony	Indo-Pacific	LC
32	35	*Megalaspis cordyla*	Carangidae	MN257538	Perciformes	Torpedo scad	Indo-West Pacific	LC
33	43	*Lutjanus lutjanus*	Lutjanidae	MN257545	Perciformes	Bigeye snapper	Indo-West Pacific	LC
34	46	*Caranx sexfasciatus*	Carangidae	MN257546	Perciformes	Bigeye trevally	Indo-Pacific	LC
35	47	*Siganus sutor*	Siganidae	MN257547	Perciformes	Shoemaker spinefoot	Indian Ocean	LC
36	48	*Siganus sutor*	Siganidae	MN257548	Perciformes	Shoemaker spinefoot	Indian Ocean	LC
37	49	*Nemipterus bipunctatus*	Nemipteridae	MN257549	Perciformes	Delagoa threadfin bream	Indian Ocean	NE
38	50	*Terapon jarbua*	Terapontidae	MN257550	Perciformes	Jarbua terapon	Indo-Pacific	LC
39	51	*Terapon jarbua*	Terapontidae	MN257551	Perciformes	Jarbua terapon	Indo-Pacific	LC
40	52	*Coryphaena hippurus*	Coryphaenidae	MN257552	Perciformes	Common dolphinfish	Atlantic, Indian and Pacific	LC
41	53	*Auxis thazard*	Scombridae	MN257553	Perciformes	Frigate tuna	Atlantic, Indian and Pacific (Western Central)	LC
42	54	*Auxis thazard*	Scombridae	MN257554	Perciformes	Frigate tuna	Atlantic, Indian and Pacific (Western Central)	LC
43	55	*Thunnus albacares*	Scombridae	MN257555	Perciformes	Yellowfin tuna	Worldwide in tropical and subtropical seas	NT
44	56	*Decapterus macarellus*	Carangidae	MN257556	Perciformes	Mackerel scad	Circumglobal	LC
45	57	*Katsuwonus pelamis*	Scombridae	MN257557	Perciformes	Skipjack tuna	Cosmopolitan in tropical and warm-temperate waters	LC
46	24	*Psettodes erumei*	Psettodidae	MN257527	Pleuronectiformes	Indian halibut	Indo-West Pacific	NE
47	20	*Platycephalus sp.*	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	*Elagatis bipinnulata*	MN257553	Aceh 53	-	-	A	-	-	T	-	-
		KU535565	Philipinnes	-	-	G	-	-	C	-	-
		KF461174	USA	-	-	A	-	-	T	-	-
		EU014211	India	-	-	A	-	-	C	-	-
		KT824759	China	-	-	A	-	-	C	-	-
2	*Decapterus macarellus*	MN257556	Aceh 6	-	C	-	-	-	-	-	-
		MH638794	China	-	C	-	-	-	-	-	-
		KY570732	Malaysia	-	C	-	-	-	-	-	-
		MF541317	India	-	T	-	-	-	-	-	-
3	*Auxis thazard*	MN257554	Aceh 54	-	-	-	-	-	-	-	-
		MH638768	China	-	-	-	-	-	-	-	-
		KX768120	India	-	-	-	-	-	-	-	-
		AB105447	Spain	-	-	-	-	-	-	-	-
4	*Variola albimarginata*	MN257516	Aceh 10	C	-	-	G	-	-	G	C
		KM226315	India	C	-	-	G	-	-	G	C
		KC593370	China	T	-	-	A	-	-	A	T
5	*Cephalopholis sonnerati*	MN257517	Aceh 11	-	-	-	-	A	-	-	-
		MH085806	Indonesia	-	-	-	-	A	-	-	-
		KU668631	Philipinnes	-	-	-	-	A	-	-	-
		DQ107928	Australia	-	-	-	-	A	-	-	-
		KC593378	China	-	-	-	-	G	-	-	-

460

Artikel 6, Lampiran 6

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

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

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, 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*), and 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 fisheries potential, with a water area reaching 295,370 km^2 and a coastline length of 2,666.3 km (Mukhtar 2017). 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[3]

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

Fishing Port of Kutaradja. Marine fisheries production at this fishing port increased from 8,922 tons in 2013 to 12,305 tons in 2017 (Mardhatillah et al. 2019, Yeni and Naufal 2017, Yusuf 2003). The fish landing site suffered massive damage due to the tsunami that struck Aceh Province and was 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 Indonesian 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 (Salmarika and Wisudo 2019, Suman et al. 2017).

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

Commented [U4]: Q1 regarding FMA

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

location (Fadli et al. 2019). In different areas (i.e. Lhoknga and Lhok Mata Ie Beaches) 25 fish species which are members of eight orders, 11 families, and 19 genera were recorded from 51 fish samples (Nur et al. 2019); 71 species were identified in Pusong Bay, Lhokseumawe belonging to 54 genus, 37 families and 15 orders (Damora et al. 2020); 50 species were identified in 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 identifies 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 (KeskİN and Atar 2013), marine fish in China (Wang Zhong-Duo et al. 2012, Zhang and Hanner 2012), and marine fish in Taiwan (Bingpeng et al. 2018, Chang et al. 2017). 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

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

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

2. Materials and methods

2.1 Sampling site

A total of 47 fish samples were collected from the Kutaradja Fishing Port on 19 July 2019 (5º35'09"N -95º19'06"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.

Commented [U7]: Q6 Reference for morphological identification

Commented [U8]: Q7 Sample code

2.2 DNA extraction and PCR condition

Each specimen were 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 -70ºC for further analysis.

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

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

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

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: 95ºC for 5 min in initial denaturation, followed by denaturation at 95ºC for 30 s in 40 cycles, 50ºC for 30 s in annealing, and 72ºC for 45 s in extension step, and a final extension at 72ºC for 5 min. 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.

Commented [U11]: Q8 Lab for experiments

2.4 Sequence alignment and data analyses

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.

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) 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.

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

3. Results

3.1 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 and 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 was 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”.

3.2 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 transvertional pair (sv=14.86) with an overall transition/transvertion ratio 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 identity 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)

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

3.3 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 transvertional pair (sv=22.13) with an average transition/transvertion 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.

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)

3.4 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

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.

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

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

Discussion

Research on molecular identification is now extensive in the field of fisheries and marine sciences. In this study, molecular identification is used to complete 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 face 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 is 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 capture 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 (Hariati 2017, Wiryawan et al. 2016). The purse-seine is also a fishing gear generally used to catch *Thunnus tonggol*, *Euthynnus affinis*, *Auxis thazard*, and *Auxis rochei* (Salmarika and Wisudo 2019). Add reference: (Rahmah et al. 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 a group of fish that utilize coral reefs as their habitat. However, several pelagic fish found around the shallow seas of Banda Aceh is still the primary target. The Indian mackerel *Rastrelliger kanagurta* (Hariati and Fauzi 2017, Hariati et al. 2015), yellowfin tuna *Thunnus albacares* (Neliyana et al. 2014), mackerel scad *Decapterus macrosoma*, and the anchovy *Stolephorus* spp. (Kurnia et al. 2016) were also obtained in this study.

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

caught from the area around the seas of Western Banda Aceh Province, indicating a catch distance of about 50-190 nautical miles (Salmarika and 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 appears 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 may 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 *Cepalopholis 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 *Cepalopholis 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 similarities with Indian populations were found in the *Cepalopholis sonnerati* sample species, similarities were only found with 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 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 (Mullins et al. 2018, Pecoraro et al. 2017), migration (Wang Xinyun 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).

4. Conclusions

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 important 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 present in the waters of Banda Aceh, Indonesia.

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

Author 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. research design and manuscript finalization

Acknowledgments

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

Conflict of Interest

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

References

Commented [a26]: Please follow our template

Commented [U27R26]: Reference was formatted according journal style

Afriyie, G., Huang, S., Dong, Z., Guo, Y., Kuebutornye, F.K., Larbi, C.A, Asiedu B. & Wang Z. 2019. DNA barcoding of Ghanaian fish species: Status and prospects. African Journal of Biotechnology 18: 659-663. doi:10.5897/AJB2019.16792.

Alcantara, S.G. & Yambot, A.V., 2016. DNA barcoding of commercially important grouper species (Perciformes, Serranidae) in the Philippines. Mitochondrial DNA Part A 27: 3837-3845. doi:10.3109/19401736.2014.958672.

Baldwin, C.C., Mounts, J.H., Smith, D.G. & Weigt, L.A., 2009. Genetic identification and color descriptions of early life-history stages of Belizean Phaeoptyx and Astrapogon (Teleostei: Apogonidae) with comments on identification of adult Phaeoptyx. Zootaxa 2008: 1-22. doi:10.11646/zootaxa.2008.1.1.

Basheer, V., Vineesh, N., Bineesh, K., Kumar, R.G., Mohitha, C., Venu, S., Kathirvelpandian, A., Gopalakrishnan, A., & Jena, J., 2017. Mitochondrial signatures for identification of grouper species from Indian waters. Mitochondrial DNA Part A 28: 451-457. doi:10.3109/19401736.2015.1137899.

Batubara, A.S., Muchlisin, Z.A., Thamren, M.Y., Usnardi, U. & Fadli, N., 2017. Check list of marine fishes from Simeulue Island waters, Aceh Province, Indonesia. Aceh Journal of Animal Science 2: 77-84. doi:10.13170/ajas.2.2.9584

Bingpeng, X., Heshan, L., Zhilan, Z., Chunguang, W., Yanguo, W. & Jianjun, W., 2018. DNA barcoding for identification of fish species in the Taiwan Strait. PloS one 13: e0198109. doi:10.1371/journal.pone.0198109

Carpenter, K.E. & Niem, V.H., 2001. FAO species identification guide for fishery purposes. The living marine resources of the Western Central Pacific. Volume 6. Bony fishes part 4 (Labridae to Latimeriidae), estuarine crocodiles, sea turtles, sea snakes and marine mammals: FAO Library.

Catanese, G., Catanese, G., Infante, C., Manchado, M., Catanese, G., Infante, C. & Manchado, M., 2008. Complete mitochondrial DNA sequences of the frigate tuna Auxis thazard and the bullet tuna Auxis rochei: Full Length Research Paper. DNA Sequence 19: 159-166. doi:10.1080.10425170701207117

Chang, CH., Shao, K.T., Lin, H.Y., Chiu, Y.C., Lee, M.Y., Liu, S.H. & Lin, P.L., 2017. DNA barcodes of the native ray-finned fishes in Taiwan. Molecular ecology resources 17: 796-805. doi:10.1111/1755-0998.12601

Damanik, M.R.S., Lubis, M.R.K. & Astuti, A.J.D., 2016. Kajian pendekatan ekosistem dalam pengelolaan perikanan di wilayah pengelolaan perikanan (WPP) 571 Selat Malaka Provinsi Sumatera Utara. Jurnal Geografi 8: 165-176. doi:10.24114/jg.v8i2.5780

Damora, A., Batubara, A.S., Zuhdi, Z., Restiangsih, Y., Amir, F., Irham, M., Fadli, N., Nur, F. & Rizal, R., 2020. Diversity of marine fish and their conservation status in Pusong Bay, Lhokseumawe City, Aceh Province, Indonesia. European Journal of Environmental Sciences 10: 115-123. doi:10.14712/23361964.2020.13

Edrus, I.N., Wijaya, S.W. & Setiawan, I.E., 2016. Struktur komunitas ikan karang di perairan Pulau Raya, Pulau Rusa, Pulau Rondo dan taman laut Rinoi dan Rubiah, Nanggroe Aceh Darussalam. Jurnal Penelitian Perikanan Indonesia 19: 175-186. doi:10.15578/jppi.19.4.2013.175-186

Edwarsyah, E., Nasir, M. & Selamat, M.B., 2019. The DNA Barcoding of Several Commercial Fish from Simeulue Islands Coast of Aceh Using Cytochrome Oxidase Sub Unit I (COI) Gene Marker. Budapest International Research in Exact Sciences (BirEx) Journal 1: 62-70. doi:10.33258/birex.v1i1.140

Fadli, N., Muchlisin, Z., Pratama, F., Mustari, T., Dewiyanti, I., Purnawan, S., El-Rahimi, S., Sofyan, H., Affan, M. & Siti-Azizah, M., 2019. The composition of coral reefs in Ulee

Lheue breakwater, Banda Aceh, Aceh, Indonesia. Pages 012077. IOP Conference Series: Earth and Environmental Science: IOP Publishing. doi:10.1088/1755-1315/348/1/012077

Handy, S.M., Deeds, J.R., Ivanova, N.V., Hebert, P.D., Hanner, R.H., Ormos, A., Weigt, L.A., Moore, M.M. & Yancy, H.F., 2011. A single-laboratory validated method for the generation of DNA barcodes for the identification of fish for regulatory compliance. Journal of AOAC International 94: 201-210. doi:10.1093/jaoac/94.1.201

Hariati, T., 2017. Status dan perkembangan perikanan pukat cincin di Banda Aceh. Jurnal Penelitian Perikanan Indonesia 17: 157-167. doi:10.15578/jppi.17.3.2011. 157-167

Hariati, T. & Fauzi, M., 2017. Aspek reproduksi ikan banyar, Rastrelliger kanagurta (Cuv. 1817) di perairan utara Aceh [Reproductive aspect of indian mackerel Rastrelliger kanagurta (Cuv. 1817) of northern Aceh waters]. Jurnal Iktiologi Indonesia 11: 47-53. doi:10.32491/jii.v11i1.155

Hariati, T., Faizah, R.& Nugroho, D., 2015. Umur, Pertumbuhan Dan Laju Pemanfaatan Ikan Banyar (Rastrelliger kanagurta Cuvier, 1816), di Selat Malaka (Wilayah Pengelolaan Perikanan-571). Jurnal Penelitian Perikanan Indonesia 21: 1-8. doi:10.15578/jppi.21.1.2015.1-8

Kamal, M.M., Hakim, A.A., Butet, N.A., Fitrianingsih, Y.& Astuti, R., 2019. Autentikasi spesies ikan kerapu berdasarkan marka gen MT-COI dari perairan Peukan Bada, Aceh. Jurnal Biologi Tropis 19: 116-123. doi:10.29303/jtb.v19i2.1245

KeskİN, E. & Atar, H.H., 2013. DNA barcoding commercially important fish species of T urkey. Molecular ecology resources 13: 788-797. doi:10.1111/1755-0998.12120

Krčmář, P., Piskata, Z. & Servusová, E., 2019. Identification of tuna species Thunnus albacares and Katsuwonus pelamis in canned products by real-time PCR method. Acta Veterinaria Brno 88: 323-328 doi: 10.2754/avb201988030323

Kumar, S., Stecher, G. & Tamura, K., 2016. MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. Molecular biology and evolution 33: 1870-1874. doi:10.1093/molbev/msw054

Kurnia, K., Purnawan, S. & Rizwan, T., 2016. Pemetaan daerah penangkapan ikan pelagis kecil di Perairan Utara Aceh. Jurnal Ilmiah Mahasiswa Kelautan Perikanan Unsyiah 1. http://www.jim.unsyiah.ac.id/fkp/article/view/204

Lakra, W., Verma, M., Goswami, M., Lal, K.K., Mohindra, V., Punia, P., Gopalakrishnan, A., Singh, K., Ward, R.D. & Hebert, P., 2011. DNA barcoding Indian marine fishes. Molecular Ecology Resources 11: 60-71. doi:10.1111/j.1755-0998.2010.02894.x

Lubis, A.M., Syaifuddin, S. & Isnaniah, I., 2016. The Contribution of the Lampulo Fishing Port, for Fishery Sector in Banda Aceh City, Nanggroe Aceh Darussalam Province. Jurnal Online Mahasiswa Fakultas Perikanan dan Ilmu Kelautan Universitas Riau 3: 1-13. http://jom.unri.ac.id/index.php/JOMFAPERIKA/article/view/9183

Mardhatillah, I., Damora, A., Rahmah, A., Nurfadillah, N. & Muhammad, M., 2019. Application of Schaefer model on mackerels fishery in Aceh waters, Indonesia. Pages 012114. IOP Conference Series: Earth and Environmental Science: IOP Publishing. doi:10.1088/1755-1315/348/1/012114

Mullins, R.B., McKeown, N,J,, Sauer, W.H. & Shaw, P.W., 2018. Genomic analysis reveals multiple mismatches between biological and management units in yellowfin tuna (Thunnus albacares). ICES Journal of Marine Science 75: 2145-2152. doi: 10.1093/icesjms/fsy102

Munawwarah, A., Sufi, I. & Noviyanti, A., 2016. Identifikasi Jenis-Jenis Ikan Yang Terdapat Di Tempat Pelelangan Ikan (TPI) Di Gampong Lampulo Kecamatan Kuta Alam Banda Aceh. Serambi Saintia: Jurnal Sains dan Aplikasi 4. doi:10.32672/jss.v4i1.115

Nasution, M.H., Anwar, S., Fitri, A. & Zohra, A.F., 2019. Forecasting The Amount of Tuna/Madidihang (Yellowfin tuna) Landed in PPS Kutaraja Banda Aceh City With The Triple Exponential Smoothing Method. Samakia: Jurnal Ilmu Perikanan 10: 08-14. doi:10.35316/jsapi.v10i1.231

Neliyana, W.B., Wiyono, E. & Nurani, T., 2014. Analisis Kelayakan Usaha Perikanan Pukat Cincin di Pelabuhan Perikanan Pantai (PPP) Lampulo Banda Aceh Propinsi Aceh. Jurnal Marine Fisheries 5: 163-169. doi:10.1088/1755-1315/348/1/012104

Nur, F.M., Batubara, A.S., Perdana, A.W., Eriani, K. & Muchlisin, Z.A., 2019. Checklist of coral fishes in Lhoknga and Lhok Mata Ie Beaches, Aceh Besar, Indonesia. Pages 012104. IOP Conference Series: Earth and Environmental Science: IOP Publishing. doi:10.29244/jmf.5.2.163-169

Pecoraro, C., Zudaire, I., Bodin, N., Murua, H., Taconet, P., Díaz-Jaimes, P., Cariani, A., Tinti F. & Chassot, E., 2017. Putting all the pieces together: integrating current knowledge of the biology, ecology, fisheries status, stock structure and management of yellowfin tuna (Thunnus albacares). Reviews in Fish Biology and Fisheries 27: 811-841. doi:10.1007/s11160-016-9460-z

Primyastanto, M., Lestariadi, R.A. & Haris, A.K., 2021. Sustainable operational analysis of the cultivation of Indonesian thunnus albacares by bioeconomic approach. Croatian Journal of Fisheries: Ribarstvo 79: 61-70. https://hrcak.srce.hr/257456

Pringgenis, D. & Susilowati, R., 2016. Highly commercial fisheries tawar fish: molecular analysis DNA mitochondrial COI gene sequence and proximate analysis from malacca Strait, Riau. Jurnal Teknologi 78. doi:10.11113/jt.v78.8149

Rocha, L. & Bowen, B., 2008. Speciation in coral-reef fishes. Journal of Fish Biology 72: 1101-1121. doi:10.1111/j.1095-8649.2007.01770.x

Salmarika, S. & Wisudo, S.H., 2019. Status Pengelolaan Sumber Daya Ikan Tongkol di Perairan Samudera Hindia Berbasis Pendaratan Pukat Cincin di Pelabuhan Perikanan Samudera Lampulo, Aceh: Suatu Pendekatan Ekosistem. Jurnal Penelitian Perikanan Indonesia 24: 263-272. http://ejournal-balitbang.kkp.go.id/index.php/jppi/article/view/7417

Serdiati, N., Arfiati, D., Widodo, M.S., Lelono, T.J., Ndobe, S. & Saranga, R., 2020. Morphological variations and phylogenetic analysis of Oryzias nigrimas Kottelat, 1990 (rice fish) from Lake Poso, Central Sulawesi, Indonesia. Biodiversitas Journal of Biological Diversity 21. doi:10.13057/biodiv/d210305

Suman, A., Irianto, H.E., Satria, F. & Amri, K., 2017. Potensi dan tingkat pemanfaatan sumber daya ikan di wilayah pengelolaan perikanan Negara Republik Indonesia (WPP NRI) Tahun 2015 serta Opsi Pengelolaannya. Jurnal Kebijakan Perikanan Indonesia 8: 97-100. doi:10.15578/jkpi.8.2.2016.97-100

Wang, X., Geng, L., Xie, J. & Qian, Y-F., 2018. Relationship between water migration and quality changes of yellowfin tuna (Thunnus albacares) during storage at 0 C and 4 C by LF-NMR. Journal of aquatic food product technology 27: 35-47. doi:10.1080/10498850.2017.1400630

Wang, Z-D., Guo, Y-S., Liu, X-M., Fan, Y-B. & Liu, C-W., 2012. DNA barcoding South China Sea fishes. Mitochondrial Dna 23: 405-410. doi:10.3109/19401736.2012.710204.

Ward, R.D., Zemlak, T.S., Innes, B.H., Last, P.R. & Hebert P.D., 2005. DNA barcoding Australia's fish species. Philosophical Transactions of the Royal Society B: Biological Sciences 360: 1847-1857. doi:10.1098/rstb.2005.1716

Wiryawan, B., Wiyono, E.S. & Nurani, T.W., 2016. Analisis Kelayakan Usaha Perikanan Pukat Cincin Di Pelabuhan Perikanan Pantai (Ppp) Lampulo Banda Aceh Propinsi Aceh (Analysis Financial Fisheries of Purse Seine in Lampulo Fishing Port Banda Aceh Provinsi Aceh). Marine Fisheries: Journal of Marine Fisheries Technology and Management 5: 163-169. doi:10.29244/jmf.5.2.163-169

Xu, L., Van- Damme, K., Li, H., Ji, Y., Wang, X. & Du, F., 2019. A molecular approach to the identification of marine fish of the Dongsha Islands (South China Sea). Fisheries Research 213: 105-112. doi:10.1016/j.fishres.2019.01.011

Yeni, E. & Naufal, A., 2017. Identifikasi Aktivitas Pengembangan Fasilitas Pelabuhan Perikanan Pantai Lampulo Banda Aceh. Pages 355-363. Prosiding SEMDI-UNAYA (Seminar Nasional Multi Disiplin Ilmu UNAYA). http://jurnal.abulyatama.ac.id/index.php/ semdiunaya/article/view/219

Yusuf, Q., 2003. Empowerment of Panglima Laot in Aceh. Pages 11-13. International workshop on Marine Science and Resource. Banda Aceh.

Zhang, J. & Hanner, R., 2012. Molecular approach to the identification of fish in the South China Sea. PLoS One 7: e30621. doi:10.1371/journal.pone.0030621

Zhuang, X., Qu, M., Zhang, X. & Ding, S., 2013. A comprehensive description and evolutionary analysis of 22 grouper (Perciformes, Epinephelidae) mitochondrial genomes with emphasis on two novel genome organizations. PLoS One 8: e73561. doi:10.1371/journal.pone.0073561

Zulfahmi, I., Apriansyah, M., Batubara, A.S., Kautsari, N., Sumon, K.A., Rahman, M.M. & Nur, F.M., 2022. Commercial marine fish species from Weh Island, Indonesia: Checklist, distribution pattern and conservation status. Biodiversitas Journal of Biological Diversity 23. doi:10.13057/biodiv/d230432

Zulmaidah, Z., Zain, J. & Hutauruk, R.M., 2015. Facilities Utilization in Lampulo Fisheries Port, District of Kuta Alam, Banda Aceh City, Province Nanggroe Aceh Darussalam. None 2: 1-13. http://jom.unri.ac.id/index.php/JOMFAPERIKA/article/view/6854.

Commented [a28]:

Commented [U29R28]: Q19. All reference in Bahasa useful and support of this MS.

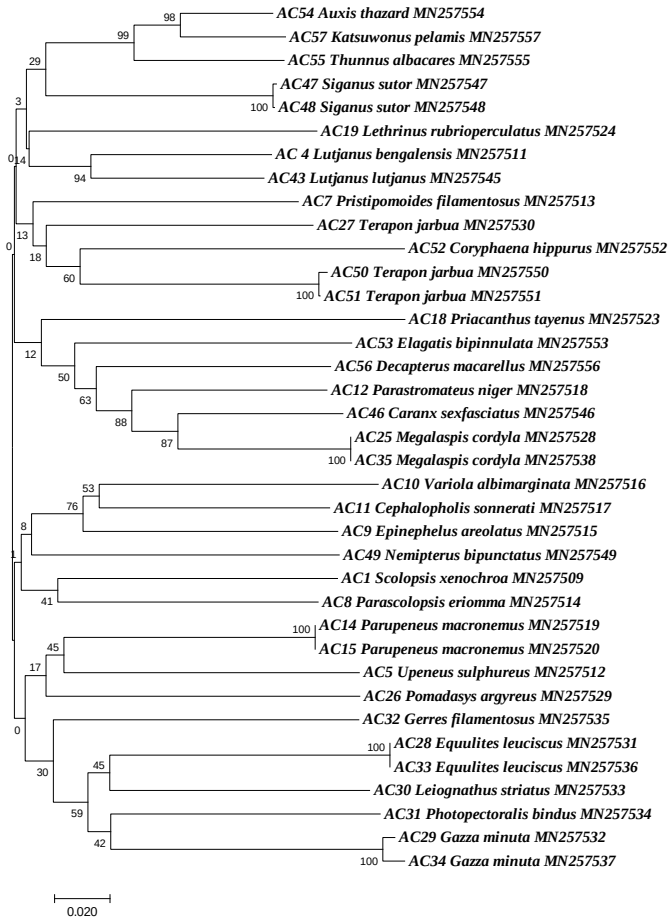
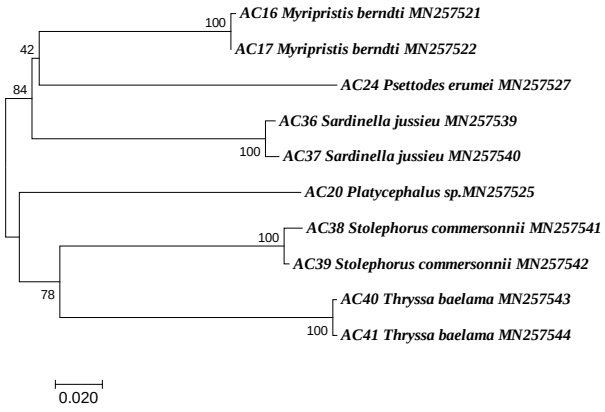


Figure 1. Phylogenetic tree of several Perciformes order by Neighbor-Joining tree algorithm using Mega7

Commented [a30]: Replace Mega 7 -> MEGA7 or MEGA 7



488

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

490 Scorpaeniformes by Neighbor-Joining tree algorithm using MEGA7.

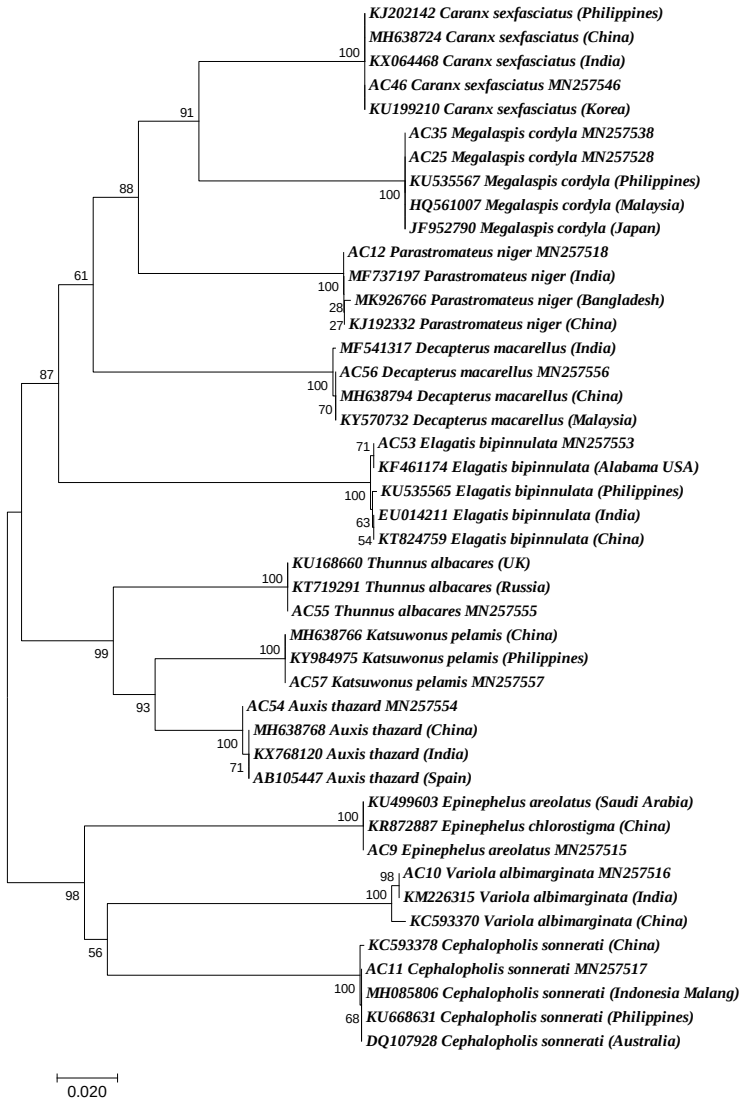


Figure 3. Phylogenetic tree reconstruction of three families (Carangidae, Scombridae, and Serranidae) by Neighbor-Joining algorithm using MEGA7. All sequence on this figure have code with AC, then another sequence has been downloaded from GenBank databased as the reference.

Commented [a31]: Please Replace Mega 7 -> MEGA7 or MEGA 7

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	*Myripristis berndti*	Holocentridae	MN257521	Beryciformes	Blotcheye soldierfish	Indo-Pacific and Eastern Pacific	LC
2	17	*Myripristis berndti*	Holocentridae	MN257522	Beryciformes	Blotcheye soldierfish	Indo-Pacific and Eastern Pacific	LC
3	36	*Sardinella jussieu*	Clupeidae	MN257539	Clupeiformes	Mauritian sardinella	Western Indian Ocean	DD
4	37	*Sardinella jussieu*	Clupeidae	MN257540	Clupeiformes	Mauritian sardinella	Western Indian Ocean	DD
5	38	*Stolephorus commersonnii*	Engraulidae	MN257541	Clupeiformes	Commerson's anchovy	Indo-West Pacific	LC
6	39	*Stolephorus commersonnii*	Engraulidae	MN257542	Clupeiformes	Commerson's anchovy	Indo-West Pacific	LC
7	40	*Thryssa baelama*	Engraulidae	MN257543	Clupeiformes	Baelama anchovy	Indo-Pacific	LC
8	41	*Thryssa baelama*	Engraulidae	MN257544	Clupeiformes	Baelama anchovy	Indo-Pacific	LC
9	1	*Scolopsis xenochroa*	Nemipteridae	MN257509	Perciformes	Oblique-barred monocle bream	Indo-West Pacific	NE
10	4	*Lutjanus bengalensis*	Lutjanidae	MN257511	Perciformes	Bengal snapper	Indo-West Pacific:	NE
11	5	*Upeneus sulphureus*	Mullidae	MN257512	Perciformes	Sulphur goatfish	Indo-West Pacific	LC
12	7	*Pristipomoides filamentosus*	Lutjanidae	MN257513	Perciformes	Crimson jobfish	Indo-Pacific	LC
13	8	*Parascolopsis eriomma*	Nemipteridae	MN257514	Perciformes	Rosy dwarf monocle bream	Indo-West Pacific	NE
14	9	*Epinephelus areolatus*	Serranidae	MN257515	Perciformes	Areolate grouper	Indo-Pacific	LC
15	10	*Variola albimarginata*	Serranidae	MN257516	Perciformes	White-edged lyretail	Indo-Pacific	LC
16	11	*Cephalopholis sonnerati*	Serranidae	MN257517	Perciformes	Tomato hind	Indo-Pacific	LC
17	12	*Parastromateus niger*	Carangidae	MN257518	Perciformes	Black pomfret	Indo-West Pacific	LC
18	14	*Parupeneus macronemus*	Mullidae	MN257519	Perciformes	Long-barbel goatfish	Indo-West Pacific	LC
19	15	*Parupeneus macronemus*	Mullidae	MN257520	Perciformes	Long-barbel goatfish	Indo-West Pacific	LC
20	18	*Priacanthus tayenus*	Priacanthidae	MN257523	Perciformes	Purple-spotted bigeye	Indo-West Pacific	LC
21	19	*Lethrinus rubrioperculatus*	Lethrinidae	MN257524	Perciformes	Spotcheek emperor	Indo-Pacific	LC
22	25	*Megalaspis cordyla*	Carangidae	MN257528	Perciformes	Torpedo scad	Indo-West Pacific	LC
23	26	*Pomadasys argyreus*	Haemulidae	MN257529	Perciformes	Bluecheek silver grunt	Indo-West Pacific	NE

24	27	*Terapon jarbua*	Terapontidae	MN257530	Perciformes	Jarbua terapon	Indo-Pacific	LC
25	28	*Equulites leuciscus*	Leiognathidae	MN257531	Perciformes	Whipfin ponyfish	Indo-West Pacific	LC
26	29	*Gazza minuta*	Leiognathidae	MN257532	Perciformes	Toothpony	Indo-Pacific	LC
27	30	*Leiognathus striatus*	Leiognathidae	MN257533	Perciformes	Toothpony	Western Indian Ocean	NE
28	31	*Photopectoralis bindus*	Leiognathidae	MN257534	Perciformes	Orangefin ponyfish	Indo-West Pacific	NE
29	32	*Gerres filamentosus*	Gerreidae	MN257535	Perciformes	Whipfin silver-biddy	Indo-Pacific	LC
30	33	*Equulites leuciscus*	Leiognathidae	MN257536	Perciformes	Whipfin ponyfish	Indo-West Pacific	LC
31	34	*Gazza minuta*	Leiognathidae	MN257537	Perciformes	Toothpony	Indo-Pacific	LC
32	35	*Megalaspis cordyla*	Carangidae	MN257538	Perciformes	Torpedo scad	Indo-West Pacific	LC
33	43	*Lutjanus lutjanus*	Lutjanidae	MN257545	Perciformes	Bigeye snapper	Indo-West Pacific	LC
34	46	*Caranx sexfasciatus*	Carangidae	MN257546	Perciformes	Bigeye trevally	Indo-Pacific	LC
35	47	*Siganus sutor*	Siganidae	MN257547	Perciformes	Shoemaker spinefoot	Indian Ocean	LC
36	48	*Siganus sutor*	Siganidae	MN257548	Perciformes	Shoemaker spinefoot	Indian Ocean	LC
37	49	*Nemipterus bipunctatus*	Nemipteridae	MN257549	Perciformes	Delagoa threadfin bream	Indian Ocean	NE
38	50	*Terapon jarbua*	Terapontidae	MN257550	Perciformes	Jarbua terapon	Indo-Pacific	LC
39	51	*Terapon jarbua*	Terapontidae	MN257551	Perciformes	Jarbua terapon	Indo-Pacific	LC
40	52	*Coryphaena hippurus*	Coryphaenidae	MN257552	Perciformes	Common dolphinfish	Atlantic, Indian and Pacific	LC
41	53	*Auxis thazard*	Scombridae	MN257553	Perciformes	Frigate tuna	Atlantic, Indian and Pacific (Western Central)	LC
42	54	*Auxis thazard*	Scombridae	MN257554	Perciformes	Frigate tuna	Atlantic, Indian and Pacific (Western Central)	LC
43	55	*Thunnus albacares*	Scombridae	MN257555	Perciformes	Yellowfin tuna	Worldwide in tropical and subtropical seas	NT
44	56	*Decapterus macarellus*	Carangidae	MN257556	Perciformes	Mackerel scad	Circumglobal	LC
45	57	*Katsuwonus pelamis*	Scombridae	MN257557	Perciformes	Skipjack tuna	Cosmopolitan in tropical and warm-temperate waters	LC
46	24	*Psettodes erumei*	Psettodidae	MN257527	Pleuronectiformes	Indian halibut	Indo-West Pacific	NE
47	20	*Platycephalus sp.*	Platycephalidae	MN257525	Scorpaeniformes	Bartail flathead	Indo-West Pacific	DD

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

499

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	*Elagatis bipinnulata*	MN257553	Aceh 53	-	-	A	-	-	T	-	-
		KU535565	Philipinnes	-	-	G	-	-	C	-	-
		KF461174	USA	-	-	A	-	-	T	-	-
		EU014211	India	-	-	A	-	-	C	-	-
		KT824759	China	-	-	A	-	-	C	-	-
2	*Decapterus macarellus*	MN257556	Aceh 6	-	C	-	-	-	-	-	-
		MH638794	China	-	C	-	-	-	-	-	-
		KY570732	Malaysia	-	C	-	-	-	-	-	-
		MF541317	India	-	T	-	-	-	-	-	-
3	*Auxis thazard*	MN257554	Aceh 54	-	-	-	-	-	-	-	-
		MH638768	China	-	-	-	-	-	-	-	-
		KX768120	India	-	-	-	-	-	-	-	-
		AB105447	Spain	-	-	-	-	-	-	-	-
4	*Variola albimarginata*	MN257516	Aceh 10	C	-	-	G	-	-	G	C
		KM226315	India	C	-	-	G	-	-	G	C
		KC593370	China	T	-	-	A	-	-	A	T
5	*Cephalopholis sonnerati*	MN257517	Aceh 11	-	-	-	-	A	-	-	-
		MH085806	Indonesia	-	-	-	-	A	-	-	-
		KU668631	Philipinnes	-	-	-	-	A	-	-	-
		DQ107928	Australia	-	-	-	-	A	-	-	-
		KC593378	China	-	-	-	-	G	-	-	-

502

Journal of Tropical Biodiversity and Biotechnology
Volume 07, Issue 03 (2022): jtbb71955
DOI: 10.22146/jtbb.71955

Research Article

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

Sapto Andriyono[1]*, Adrian Damora[2], Hyun-Woo Kim[1,3]
1) Department of Marine, Faculty of Fisheries and Marine, Universitas Airlangga, Indonesia
2) Faculty of Marine and Fisheries, Universitas Syiah Kuala, Banda Aceh, Indonesia
3) Departmen of Marine Biology, School of Fisheries Science, Pukyong National University, Busan, Republic of Korea.
* Corresponding author, email: sapto.andriyono@fpk.unair.ac.id

Keywords:
Aceh
DNA barcoding
haplotype analysis
marine fish
molecular
Submitted:
06 January 2022
Accepted:
14 September 2022
Published:
26 October 2022
Editor:
Furzani binti Pa'ee

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.

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^2 and a coastline length of 2,666.3 km (Fikri 2013). One of the centres of fishing activity and the most significant fish landing site in Aceh is the Fishing Port of Kutaradja. Marine fisheries production at this fishing port increased from 8,922 tons in 2013 to 12,305 tons in 2017 (Yusuf 2003; Yeni & Naufal 2017; Mardhatillah et al. 2019) The fish landing site suffered massive damage due to the tsunami that struck Aceh Province and rebuilt in 2004 (Zulmaidah et al. 2015). The rebuilding of the Kutaradja fishing port has revived the economy and fisheries activities in the Banda Aceh region.

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

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

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

This research identified marine fish at the molecular level in the Cytochrome C Oxidase subunit I (COI) region of the mitochondrial gene to complete the morphological identification that was also carried out. This COI Region is the region that some gene markers have agreed on globally for molecular identification. Research on barcoding of several aquatic biota has been carried out such as for marine fish in Australia (Ward et al. 2005), marine fish in India (Lakra et al. 2011), marine fish in Turkey (Keskİn & 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 wascollected 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) 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	*Myripristis berndti*	Holocentridae	MN257521	Beryciformes	Blotcheye soldierfish	Indo-Pacific and Eastern Pacific	LC
2	17	*Myripristis berndti*	Holocentridae	MN257522	Beryciformes	Blotcheye soldierfish	Indo-Pacific and Eastern Pacific	LC
3	36	*Sardinella jussieu*	Clupeidae	MN257539	Clupeiformes	Mauritian sardinella	Western Indian Ocean	DD
4	37	*Sardinella jussieu*	Clupeidae	MN257540	Clupeiformes	Mauritian sardinella	Western Indian Ocean	DD
5	38	*Stolephorus commersonnii*	Engraulidae	MN257541	Clupeiformes	Commerson's anchovy	Indo-West Pacific	LC
6	39	*Stolephorus commersonnii*	Engraulidae	MN257542	Clupeiformes	Commerson's anchovy	Indo-West Pacific	LC
7	40	*Thryssa baelama*	Engraulidae	MN257543	Clupeiformes	Baelama anchovy	Indo-Pacific	LC
8	41	*Thryssa baelama*	Engraulidae	MN257544	Clupeiformes	Baelama anchovy	Indo-Pacific	LC
9	1	*Scolopsis xenochroa*	Nemipteridae	MN257509	Perciformes	Oblique-barred monocle bream	Indo-West Pacific	NE
10	4	*Lutjanus bengalensis*	Lutjanidae	MN257511	Perciformes	Bengal snapper	Indo-West Pacific:	NE
11	5	*Upeneus sulphureus*	Mullidae	MN257512	Perciformes	Sulphur goatfish	Indo-West Pacific	LC
12	7	*Pristipomoides filamentosus*	Lutjanidae	MN257513	Perciformes	Crimson jobfish	Indo-Pacific	LC
13	8	*Parascolopsis eriomma*	Nemipteridae	MN257514	Perciformes	Rosy dwarf monocle bream	Indo-West Pacific	NE
14	9	*Epinephelus areolatus*	Serranidae	MN257515	Perciformes	Areolate grouper	Indo-Pacific	LC
15	10	*Variola albimarginata*	Serranidae	MN257516	Perciformes	White-edged lyretail	Indo-Pacific	LC
16	11	*Cephalopholis sonnerati*	Serranidae	MN257517	Perciformes	Tomato hind	Indo-Pacific	LC
17	12	*Parastromateus niger*	Carangidae	MN257518	Perciformes	Black pomfret	Indo-West Pacific	LC
18	14	*Parupeneus macronemus*	Mullidae	MN257519	Perciformes	Long-barbel goatfish	Indo-West Pacific	LC
19	15	*Parupeneus macronemus*	Mullidae	MN257520	Perciformes	Long-barbel goatfish	Indo-West Pacific	LC
20	18	*Priacanthus tayenus*	Priacanthidae	MN257523	Perciformes	Purple-spotted bigeye	Indo-West Pacific	LC
21	19	*Lethrinus rubrioperculatus*	Lethrinidae	MN257524	Perciformes	Spotcheek emperor	Indo-Pacific	LC
22	25	*Megalaspis cordyla*	Carangidae	MN257528	Perciformes	Torpedo scad	Indo-West Pacific	LC
23	26	*Pomadasys argyreus*	Haemulidae	MN257529	Perciformes	Bluecheek silver grunt	Indo-West Pacific	NE
24	27	*Terapon jarbua*	Terapontidae	MN257530	Perciformes	Jarbua terapon	Indo-Pacific	LC
25	28	*Equulites leuciscus*	Leiognathidae	MN257531	Perciformes	Whipfin ponyfish	Indo-West Pacific	LC
26	29	*Gazza minuta*	Leiognathidae	MN257532	Perciformes	Toothpony	Indo-Pacific	LC
27	30	*Leiognathus striatus*	Leiognathidae	MN257533	Perciformes	Toothpony	Western Indian Ocean	NE
28	31	*Photopectoralis bindus*	Leiognathidae	MN257534	Perciformes	Orangefin ponyfish	Indo-West Pacific	NE
29	32	*Gerres filamentosus*	Gerreidae	MN257535	Perciformes	Whipfin silver-biddy	Indo-Pacific	LC
30	33	*Equulites leuciscus*	Leiognathidae	MN257536	Perciformes	Whipfin ponyfish	Indo-West Pacific	LC
31	34	*Gazza minuta*	Leiognathidae	MN257537	Perciformes	Toothpony	Indo-Pacific	LC
32	35	*Megalaspis cordyla*	Carangidae	MN257538	Perciformes	Torpedo scad	Indo-West Pacific	LC
33	43	*Lutjanus lutjanus*	Lutjanidae	MN257545	Perciformes	Bigeye snapper	Indo-West Pacific	LC
34	46	*Caranx sexfasciatus*	Carangidae	MN257546	Perciformes	Bigeye trevally	Indo-Pacific	LC
35	47	*Siganus sutor*	Siganidae	MN257547	Perciformes	Shoemaker spinefoot	Indian Ocean	LC
36	48	*Siganus sutor*	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	*Nemipterus bipunctatus*	Nemipteridae	MN257549	Perciformes	Delagoa threadfin bream	Indian Ocean	NE
38	50	*Terapon jarbua*	Terapontidae	MN257550	Perciformes	Jarbua terapon	Indo-Pacific	LC
39	51	*Terapon jarbua*	Terapontidae	MN257551	Perciformes	Jarbua terapon	Indo-Pacific	LC
40	52	*Coryphaena hippurus*	Coryphaenidae	MN257552	Perciformes	Common dolphinfish	Atlantic, Indian and Pacific	LC
41	53	*Auxis thazard*	Scombridae	MN257553	Perciformes	Frigate tuna	Atlantic, Indian and Pacific (Western Central)	LC
42	54	*Auxis thazard*	Scombridae	MN257554	Perciformes	Frigate tuna	Atlantic, Indian and Pacific (Western Central)	LC
43	55	*Thunnus albacares*	Scombridae	MN257555	Perciformes	Yellowfin tuna	Worldwide in tropical and subtropical seas	NT
44	56	*Decapterus macarellus*	Carangidae	MN257556	Perciformes	Mackerel scad	Circumglobal	LC
45	57	*Katsuwonus pelamis*	Scombridae	MN257557	Perciformes	Skipjack tuna	Cosmopolitan in tropical and warm-temperate waters	LC
46	24	*Psettodes erumei*	Psettodidae	MN257527	Pleuronectiformes	Indian halibut	Indo-West Pacific	NE
47	20	*Platycephalus sp.*	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 transvertional pair (sv=14.86) with an overall transition/transvertion 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 levels measured for COI sequences is 0.226 (Figure 1).

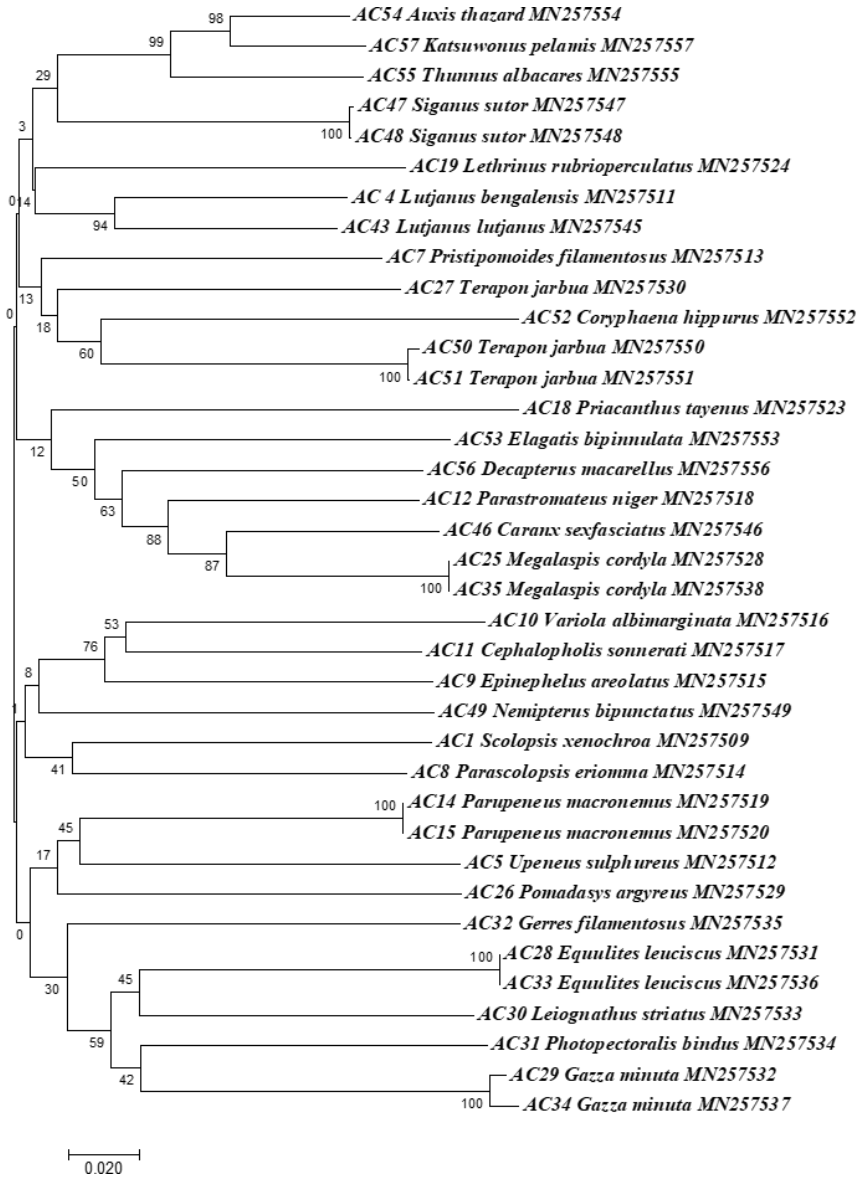


Figure 1. Phylogenetic tree of several Perciformes order by Neighbor-Joining tree algorithm using Mega7

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 transvertional pair (sv=22.13) with an average transition/transvertion bias of 8.71. The phylogenetic tree was constructed using the COI sequences for the small number order, including the Clupeiformes, Beryciformes, Pleuronectiformes, and Scorpaeniformes (Figure 2). The average K2P distance within taxonomic levels measured for COI sequences is 0.214.

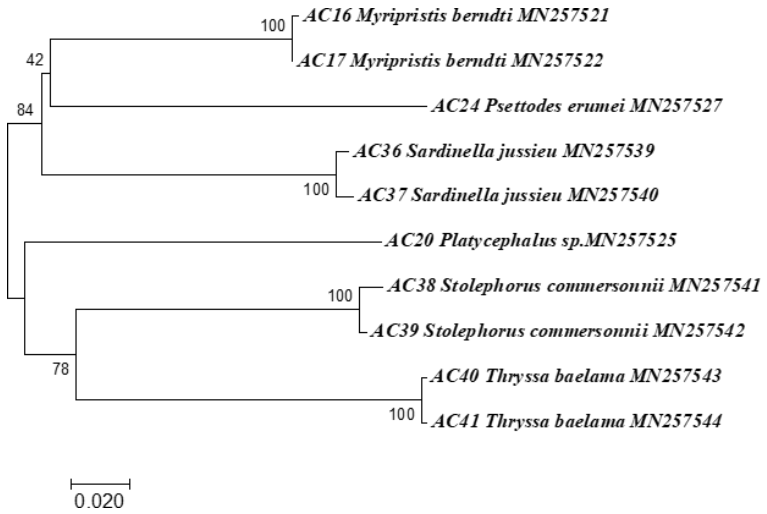


Figure 2. Phylogenetic tree of Clupeiformes including Beryciformes, Pleuronectiformes, and Scorpaeniformes by Neighbor-Joining tree algorithm using MEGA7.

The haplotype of Scombridae, Serranidae, and Carangidae from Aceh

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

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

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

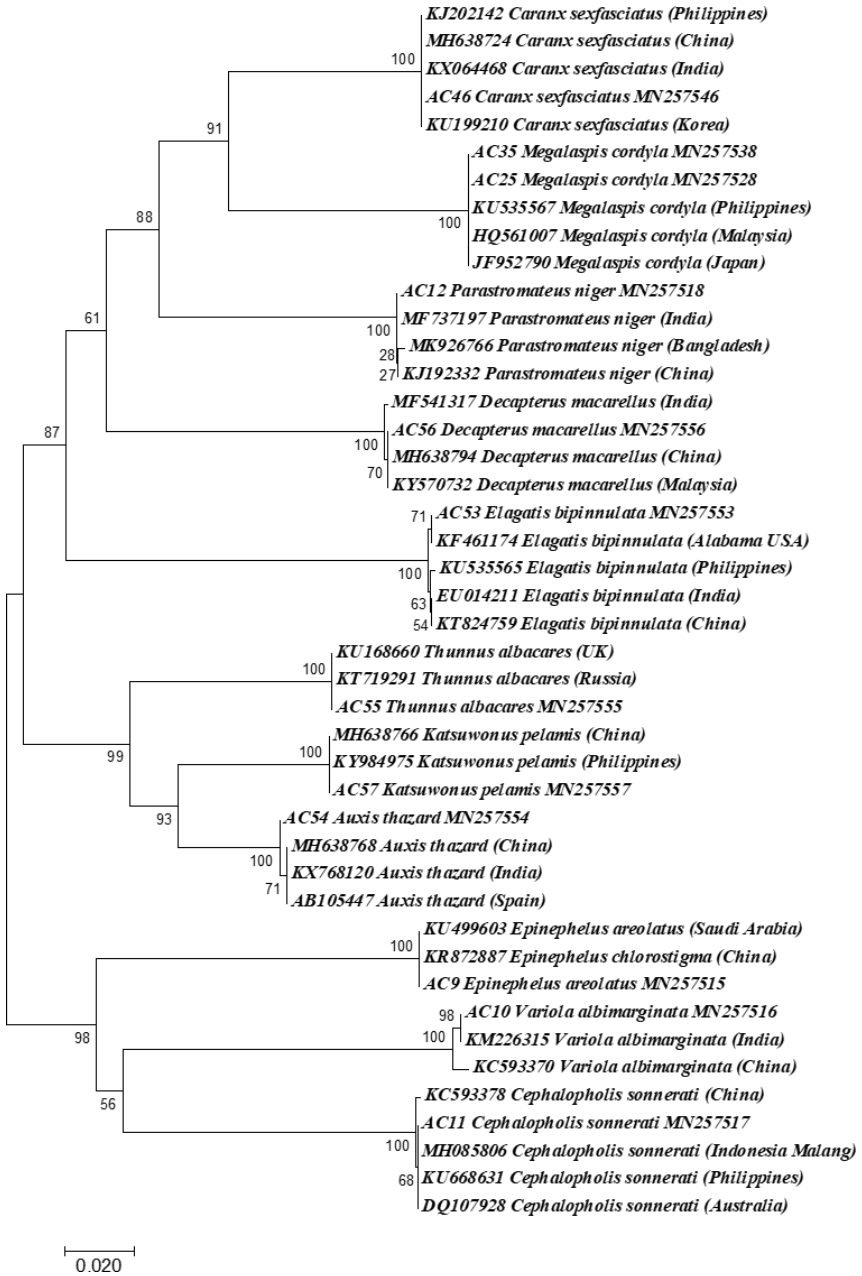


Figure 3. Phylogenetic tree reconstruction of three families (Carangidae, Scombridae, and Serranidae) by Neighbor-Joining algorithm using MEGA7. All sequence on this figure have code with AC, then another sequence has been downloaded from GenBank databased as the reference.

Discussion

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

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

No.	Species name	GenBank Acc Number	Origin	Sequence number							
				123	171	213	249	258	328	408	471
1	*Elagatis bipinnulata*	MN257553	Aceh 53	-	-	A	-	-	T	-	-
		KU535565	Philippines	-	-	G	-	-	C	-	-
		KF461174	USA	-	-	A	-	-	T	-	-
		EU014211	India	-	-	A	-	-	C	-	-
		KT824759	China	-	-	A	-	-	C	-	-
2	*Decapterus macarellus*	MN257556	Aceh 6	-	C	-	-	-	-	-	-
		MH638794	China	-	C	-	-	-	-	-	-
		KY570732	Malaysia	-	C	-	-	-	-	-	-
		MF541317	India	-	T	-	-	-	-	-	-
3	*Auxis thazard*	MN257554	Aceh 54	-	-	-	-	-	-	-	-
		MH638768	China	-	-	-	-	-	-	-	-
		KX768120	India	-	-	-	-	-	-	-	-
		AB105447	Spain	-	-	-	-	-	-	-	-
4	*Variola albimarginata*	MN257516	Aceh 10	C	-	-	G	-	-	G	C
		KM226315	India	C	-	-	G	-	-	G	C
		KC593370	China	T	-	-	A	-	-	A	T
5	*Cephalopholis sonnerati*	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 Kutaradja Fishing Port at Lampulo, may have been caught from the area around the seas of Western Banda Aceh Province, indicating a catch distance of about 50-190 nautical miles (Salmarika & Wisudo 2019). Although it is still in the Indian Ocean region, there may be specialization in this species so that the Aceh haplotype separated from the same species in the resulting phylogenetic tree analysis.

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

out. The results of the investigation found that the Scombridae *Auxis thazard* (Aceh) became separated from the same clade species even though it only has a genetic distance of only 0.002. This haplotype appeared likely to occur due to differences compared to species populations analyzed from India, China (Xu et al. 2019), and Spain (Catanese et al. 2008). While for other haplotypes found from the reef fish *Variola albimarginata* and *Cephalopholis sonnerati*, the *Variola albimarginata* from Aceh might be from a population previously described from the results of a study conducted in India that allows the sharing of habitats in the Indian Ocean in the Western part of Sumatra Island. Previous studies on molecular identification of *Variola albimarginata* species have been carried out in the Andaman Islands and Nicobar Island (Basheer et al. 2017). This area is part of Indian sea territory, which may potentially have reef fish that are of almost the same as the species in Aceh. While *Cepalopholis 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 *Cepalopholis 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 *Cepalopholis 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

ACKNOWLEDGMENTS

This work was supported by an educational grant from the LPDP BUDI-LN batch I 2016 and Molecular Physiology Laboratory, Department of Marine Biology, Pukyong National University, Korea. This paper is the initiation of the first research collaboration between Universitas Airlangga and Syiah Kuala University. This research was supported by Research Grant No. 439/ UN3.1.12/ PT/2022 on DNA Barcoding fish from inland and marine water ecosystem.

CONFLICT OF INTEREST

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

REFERENCES

Afriyie, G. et al., 2019. DNA barcoding of Ghanaian fish species: Status and prospects. *African Journal of Biotechnology*, 18, pp.659-663. doi: 10.5897/AJB2019.16792

Alcantara, S.G. & Yambot, A.V., 2016. DNA barcoding of commercially important grouper species (Perciformes, Serranidae) in the Philippines. *Mitochondrial DNA Part A*, 27, pp.3837-3845. doi: 10.3109/19401736.2014.958672

Baldwin, C.C. et al., 2009. Genetic identification and color descriptions of early life-history stages of Belizean Phaeoptyx and Astrapogon (Teleostei: Apogonidae) with comments on identification of adult Phaeoptyx. *Zootaxa*, 2008, pp.1-22. doi: 10.11646/zootaxa.2008.1.1

Basheer, V. et al., 2017. Mitochondrial signatures for identification of grouper species from Indian waters. *Mitochondrial DNA Part A*, 28, pp.451-457. doi: 10.3109/19401736.2015.1137899

Batubara, A.S. et al., 2017. Check list of marine fishes from Simeulue Island waters, Aceh Province, Indonesia. *Aceh Journal of Animal Science*, 2, pp.77-84. doi: 10.13170/ajas.2.2.9584.

Bellwood, D.R. & Meyer, C.P. 2009. Searching for heat in a marine biodiversity hotspot. Journal of Biogeography, 36, 569–576. doi: 10.1111/j.1365-2699.2008.02029.x

Bingpeng, X. et al., 2018. DNA barcoding for identification of fish species in the Taiwan Strait. *PloS one*, 13, e0198109. doi: 10.1371/journal.pone.0198109

Briggs, J.C., 2005. The marine East Indies : diversity and speciation. *Biogeography*, 32, pp.1517–1522. doi: 10.1111/j.1365-2699.2005.01266.x

Carpenter, K.E. & Niem, V.H., 2001. FAO species identification guide for fishery purposes. The living marine resources of the Western Central Pacific. Volume 6. Bony fishes part 4 (Labridae to Latimeriidae), estuarine crocodiles, sea turtles, sea snakes and marine mammals. FAO Library.

Catanese, G. et al., 2008. Complete mitochondrial DNA sequences of the frigate tuna Auxis thazard and the bullet tuna *Auxis rochei*: Full Length Research Paper. *DNA Sequence*, 19, pp.159-166. doi: 10.1080/10425170701207117

Chang, C.H. et al., 2017. DNA barcodes of the native ray-finned fishes in Taiwan. *Molecular ecology resources*, 17, pp.796-805. doi: 10.1111/1755-0998.12601

Damanik, M.R.S. et al., 2016. Kajian pendekatan ekosistem dalam pengelolaan perikanan di wilayah pengelolaan perikanan (WPP) 571 Selat Malaka Provinsi Sumatera Utara. *Jurnal Geografi*, 8, pp.165-176. doi:10.24114/jg.v8i2.5780

Damora, A. et al., 2020. Diversity of marine fish and their conservation status in Pusong Bay, Lhokseumawe City, Aceh Province, Indonesia. *European Journal of Environmental Sciences*, 10, pp.115-123. doi:10.14712/23361964.2020.13

Edrus, I.N. et al., 2016. Struktur komunitas ikan karang di perairan Pulau Raya, Pulau Rusa, Pulau Rondo dan taman laut Rinoi dan Rubiah, Nanggroe Aceh Darussalam. *Jurnal Penelitian Perikanan Indonesia*, 19, pp.175-186. Doi: 10.15578/jppi.19.4.2013.175-186

Edwarsyah, E. et al., 2019. The DNA Barcoding of Several Commercial Fish from Simeulue Islands Coast of Aceh Using Cytochrome Oxidase Sub Unit I (COI) Gene Marker. *Budapest International Research in Exact Sciences (BirEx) Journal*, 1, pp.62-70. doi:10.33258/birex.v1i1.140

Fadli, N. et al., 2019. The composition of coral reefs in Ulee Lheue breakwater, Banda Aceh, Aceh, Indonesia. *IOP Conference Series: Earth and Environmental Science*, 012077. doi:10.1088/1755-1315/348/1/012077

Fikri, F., 2013. Pelaksanaan Perlindungan Terhadap Sumberdaya Perikanan dalam Laut Teritorial dan Zona Ekonomi Ekslusif Indonesia di Provinsi Aceh. *Kanun Jurnal Ilmu Hukum*, 15(3), 415-435. http://www.jurnal.unsyiah.ac.id/kanun/article/view/6181

Gaither, M. et al., 2011. Phylogeography of the reef fish Cephalopholis argus (Epinephelidae) indicates Pleistocene isolation across the indo-pacific barrier with contemporary overlap in the coral triangle. *BMC Evolutionary Biology*, 11, 189. doi:10.1186/1471-2148-11-189

Handy, S.M. et al., 2011. A single-laboratory validated method for the generation of DNA barcodes for the identification of fish for regulatory compliance. *Journal of AOAC International*, 94, pp.201-210. doi: 10.1093/jaoac/94.1.201

Hariati, T. et al., 2015. Umur, Pertumbuhan Dan Laju Pemanfaatan Ikan Banyar (*Rastrelliger kanagurta* Cuvier, 1816), di Selat Malaka (Wilayah Pengelolaan Perikanan-571). *Jurnal Penelitian Perikanan Indonesia*, 21, pp.1-8. doi: 10.15578/jppi.21.1.2015.1-8

Hariati, T., 2017. Status dan perkembangan perikanan pukat cincin di Banda Aceh. *Jurnal Penelitian Perikanan Indonesia*, 17, pp.157-167. doi: 10.15578/jppi.17.3.2011.157-167

Hariati, T. & Fauzi, M., 2017. Aspek reproduksi ikan banyar, *Rastrelliger kanagurta* (Cuv. 1817) di perairan utara Aceh [Reproductive aspect of indian mackerel *Rastrelliger kanagurta* (Cuv. 1817) of northern Aceh waters]. *Jurnal Iktiologi Indonesia*, 11, pp.47-53. doi: 10.32491/jii.v11i1.155

Hoeksema, B.W., 2007. *Delineation of the Indo-Malayan centre of maximum marine biodiversity: the Coral Triangle*. In Biogeography, time, and place: distributions, barriers, and islands (pp. 117-178). Springer, Dordrecht. doi: 10.1007/978-1-4020-6374-9_5

Kamal, M.M. et al., 2019. Autentikasi spesies ikan kerapu berdasarkan marka gen MT-COI dari perairan Peukan Bada, Aceh. *Jurnal Biologi Tropis*, 19, pp.116-123. doi: 10.29303/jbt.v19i2.1245

KeskİN, E. & Atar, H.H., 2013. DNA barcoding commercially important fish species of Turkey. *Molecular ecology resources*, 13, pp.788-797. doi: 10.1111/1755-0998.12120

Krčmář, P. et al., 2019. Identification of tuna species Thunnus albacares and Katsuwonus pelamis in canned products by real-time PCR method. *Acta Veterinaria Brno*, 88, pp.323-328. doi:10.2754/avb201988030323

Kumar, S. at al., 2016. MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Molecular biology and evolution*, 33, pp.1870-1874. doi:10.1093/molbev/msw054

Kurnia, K. et al., 2016. Pemetaan daerah penangkapan ikan pelagis kecil di Perairan Utara Aceh. *Jurnal Ilmiah Mahasiswa Kelautan Perikanan Unsyiah*, 1.

Lakra, W. et al., 2011. DNA barcoding Indian marine fishes. *Molecular Ecology Resources*, 11, pp.60-71. 10.1111/j.1755-0998.2010.02894.x

Lubis, A.M. et al., 2016. The Contribution of the Lampulo Fishing Port, for Fishery Sector in Banda Aceh City, Nanggroe Aceh Darussalam Province. *Jurnal Online Mahasiswa Fakultas Perikanan dan Ilmu Kelautan Universitas Riau*, 3, pp.1-13.

Mardhatillah, I. et al., 2019. Application of Schaefer model on mackerels fishery in Aceh waters, Indonesia. *IOP Conference Series: Earth and Environmental Science*, 012114. doi: 10.1088/1755-1315/348/1/012114

Mullins, R.B. et al., 2018. Genomic analysis reveals multiple mismatches between biological and management units in yellowfin tuna (*Thunnus albacares*). *ICES Journal of Marine Science*, 75, pp.2145-2152. doi: 10.1093/icesjms/fsy102

Munawwarah, A. et al., 2016. Identifikasi Jenis-Jenis Ikan Yang Terdapat Di Tempat Pelelangan Ikan (TPI) Di Gampong Lampulo Kecamatan Kuta Alam Banda Aceh. *Serambi Saintia: Jurnal Sains dan Aplikasi*, 4. doi: 10.32672/jss.v4i1.115

Nasution, M.H. et al., 2019. Forecasting The Amount of Tuna/Madidihang (Yellowfin tuna) Landed in PPS Kutaraja Banda Aceh City With The Triple Exponential Smoothing Method. *Samakia: Jurnal Ilmu Perikanan*, 10, pp.08-14. doi: 10.35316/jsapi.v10i1.231

Neliyana, W.B. et al., 2014. Analisis Kelayakan Usaha Perikanan Pukat Cincin di Pelabuhan Perikanan Pantai (PPP) Lampulo Banda Aceh Propinsi Aceh. *Jurnal Marine Fisheries*, 5, pp.163-169.

Nur, F.M. et al., 2019. Checklist of coral fishes in Lhoknga and Lhok Mata Ie Beaches, Aceh Besar, Indonesia. *IOP Conference Series: Earth and Environmental Science*, 012104. doi: 10.1088/1755-1315/348/1/012104

Pecoraro, C. et al., 2017. Putting all the pieces together: integrating current knowledge of the biology, ecology, fisheries status, stock structure and management of yellowfin tuna (*Thunnus albacares*). *Reviews in Fish Biology and Fisheries*, 27, pp.811-841. doi: 10.1007/s11160-016-9460-z

Primyastanto, M. et al., 2021. Sustainable operational analysis of the cultivation of Indonesian *Thunnus albacares* by bioeconomic approach. *Croatian Journal of Fisheries: Ribarstvo*, 79, pp.61-70. doi: 10.2478cjf-2021-0007

Pringgenis, D. & Susilowati, R., 2016. Highly commercial fisheries tawar fish: molecular analysis DNA mitochondrial COI gene sequence and proximate analysis from malacca Strait, Riau. *Jurnal Teknologi*, 78. doi: 10.11113/jt.v78.8149

Rocha, L. & Bowen, B. 2008. Speciation in coral-reef fishes. *Journal of Fish Biology*, 72, pp.1101-1121. doi: 10.1111/j.1095-8649.2007.01770.x

Salmarika, S. & Wisudo, S.H., 2019. Status Pengelolaan Sumber Daya Ikan Tongkol di Perairan Samudera Hindia Berbasis Pendaratan Pukat Cincin di Pelabuhan Perikanan Samudera Lampulo, Aceh: Suatu Pendekatan Ekosistem. *Jurnal Penelitian Perikanan Indonesia*, 24, pp.263-272.

Serdiati, N. et al., 2020. Morphological variations and phylogenetic analysis of Oryzias nigrimas Kottelat, 1990 (rice fish) from Lake Poso, Central Sulawesi, Indonesia. *Biodiversitas Journal of Biological Diversity*, 21. doi: 10.13057/biodiv/d210305

Suman, A. et al., 2017. Potensi dan tingkat pemanfaatan sumber daya ikan di wilayah pengelolaan perikanan Negara Republik Indonesia (WPP NRI) Tahun 2015 serta Opsi Pengelolaannya. *Jurnal Kebijakan Perikanan Indonesia*, 8, pp.97-100. doi: 10.15578/jkpi.8.2.2016.97-100

Veron, J.E.N. et al., 2009. Delineating the Coral Triangle. Galaxea. *Journal of Cournal Reef Studies*, 11, pp.91-100. doi:10.3755/galaxea.11.91

Wang, X. et al., 2018. Relationship between water migration and quality changes of yellowfin tuna (*Thunnus albacares*) during storage at 0 C and 4 C by LF-NMR. *Journal of aquatic food product technology*, 27, pp.35-47. doi: 10.1080/10498850.2017.1400630

Wang, Z-D. et al., 2012. DNA barcoding South China Sea fishes. *Mitochondrial DNA*, 23, pp.405-410. doi: 10.3109/19401736.2012.710204

Ward, R.D. et al., 2005. DNA barcoding Australia's fish species. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 360, pp.1847-1857. doi: 10.1098/rstb.2005.1716

Wiryawan, B. et al., 2016. Analisis Kelayakan Usaha Perikanan Pukat Cincin Di Pelabuhan Perikanan Pantai (Ppp) Lampulo Banda Aceh Propinsi Aceh (Analysis Financial Fisheries of Purse Seine in Lampulo Fishing Port Banda Aceh Provinsi Aceh). *Marine Fisheries: Journal of Marine Fisheries Technology and Management*, 5, pp.163-169. doi: 10.29244/jmf.5.2.163-169

Xu, L. et al., 2019. A molecular approach to the identification of marine fish of the Dongsha Islands (South China Sea). *Fisheries Research*, 213, pp.105-112. doi: 10.1016/j.fishres.2019.01.011

Yeni, E. & Naufal, A., 2017. Identifikasi Aktivitas Pengembangan Fasilitas Pelabuhan Perikanan Pantai Lampulo Banda Aceh. *Prosiding SEMDI-UNAYA (Seminar Nasional Multi Disiplin Ilmu UNAYA)*, pp.355-363.

Yusuf, Q., 2003. Empowerment of Panglima Laot in Aceh. *International workshop on Marine Science and Resource*, pp.11-13.

Zhang, J. & Hanner, R., 2012. Molecular approach to the identification of fish in the South China Sea. *PLoS One*, 7, e30621. doi: 10.1371/journal.pone.0030621

Zhuang, X. et al., 2013. A comprehensive description and evolutionary analysis of 22 grouper (Perciformes, Epinephelidae) mitochondrial genomes with emphasis on two novel genome organizations. *PLoS One*, 8, e73561. doi: 10.1371/journal.pone.0073561

Zulfahmi, I. et al., 2022. Commercial marine fish species from Weh Island, Indonesia: Checklist, distribution pattern and conservation status. *Biodiversitas Journal of Biological Diversity*, 23. doi: 10.13057/biodiv/d230432

Zulmaidah Z, Zain J, Hutauruk RM. 2015. Facilities Utilization in Lampulo Fisheries Port, District of Kuta Alam, Banda Aceh City, Province Nanggroe Aceh Darussalam. *Jurnal Online Mahasiswa*, 2, pp.1-13.