

Marine Fish Detection by Environmental DNA (eDNA) Metabarcoding Approach in the Pelabuhan Ratu Bay, Indonesia

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Abstract— Diversity studies in marine ecosystems have experienced many developments with molecular applications through the environmental DNA (eDNA) metabarcoding approach. This method has widely used in identifying rare species, invasive species, and biodiversity information in both freshwater and marine water environments. In this study, the eDNA metabarcoding approach was the first applied to estimate fish diversity surrounding the Pelabuhan Ratu Bay waters. Five liters of seawater samples have been collected from five spots of the surrounding port area. The metabarcoding technique was applied out by using combination DNA barcoding and the next-generation sequencing (NGS) method by MiSeq platform. The MiFish Pipeline for data analyzing to get species estimation and reads numbers for quantifying the fish abundance in this location. DNA successfully identified 20 species, which commercially in the traditional fish market in Pelabuhan Ratu. Then, the eDNA metabarcoding revealed that 44 marine fish species representing 36 genera, 24 families, and ten orders exist in Pelabuhan Ratu Bay's surrounding water. Several marine fish not included in economically but have ecological value in marine fish diversity. Furthermore, fish fall into the category of vulnerable fish (*Thunnus obesus*), poisonous fish (*Acanthurus lineatus*, *Acanthurus xanthopterus*, *Siganus vermiculatus*, and *Siganus fuscescens*), and migratory fish. Regular surveys to determine the fluctuations and effects of human activities are necessary to monitor marine diversity, both the effect of over-exploitation and potential pollution from the coastal areas around the Pelabuhan Ratu Bay.

Keywords— Environmental; DNA; identification; metabarcoding; molecular.

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I. INTRODUCTION

Pelabuhan Ratu is a fish landing center in the southern part of West Java that supplies the significant demands of fish to fulfill the animal protein requirements for the people of the surrounding area, even to Jakarta, the capital city of Indonesia. The Pelabuhan Ratu region, which has led to fisheries activities and port facilities improvement, will continue to be increased for national fisheries requirement, which main landed fish are tuna, skipjack tuna, and Spanish mackerel [1], [2]. Besides being a pelagic fishery center with outstanding economic value, this region also has a high potential for fish diversity. From several reports mentioned that at least 50 fish species are landing in the Pelabuhan Ratu [3] belong to the group of sharks and rays, which is currently a significant concern of many parties [4], [5].

Studies of fisheries stocks around the Pelabuhan Ratu area have been carried out. Fisheries in the southern part of

western Java are still potential due to under-fished and under-effort categories on fishing activities. However, in contrast, other studies related to water fertility around the Pelabuhan Ratu showed has been a decline in capture fisheries production [6]. Studies on the social character of fisheries communities have also been carried out [7]. However, information is still lacking, especially the molecular-based identification of species and stock assessment. The molecular approach in marine species identification is now prevalent; conventional identification based on morphological characters is sometimes made. The molecular-based species identification has been widely agreed upon in the form of barcoding of species [8], [9]. The molecular information can be accessed by anyone openly. Identification through this approach is cost-effective, fast, and no need for taxonomic skills, and also it minimizes errors with accuracy near to 100% [10]. The advantages of molecular identification are that it has a straightforward

method and can identify fresh samples and even processed product samples [11]. This method can overcome the possibility of species misidentification.

At present, the molecular applications in ecology have become more compatible with the latest technology in DNA sequencing with better capabilities. Metabarcoding is the development of bioinformatics that can analyze the data in a considerable number (big data). One application of this technology is the study of diversity using metabarcoding [12]. This approach allows species to choose at one-time sampling. The efficiency of eDNA has succeeded in rare species supported studies [13], invasive species [14]-[18], and collected biota data biodiversity [19]-[22].

In the current study, the application of eDNA metabarcoding was carried out on seawater samples from Pelabuhan Ratu, Sukabumi, West Java to predict the species that lived in these waters. This study also constructed the phylogenetic tree with the identified marine fish species based on the eDNA metabarcoding approach. This study will provide preliminary information on biodiversity, which will help the sustainable use of marine resources in this area.

II. MATERIALS AND METHOD

A. Sampling Location, Molecular Identification, and eDNA Metabarcoding

This research was carried out in the waters around the Pelabuhan Ratu Bay, Sukabumi, Indonesia (6° 59' 20.92" S 106° 32' 29.91" E). The fish specimen has been collected from the market in the Pelabuhan Ratu. The species identification has been made based on the morphological characters the digital camera has taken the individual specimen picture, and after collection, all specimens are directly preserved in 90% ethanol. The collected samples were taken to the laboratory of the Department of Marine, Fisheries and Marine Faculty, Universitas Airlangga, Surabaya, East Java, Indonesia, and preserved following the standard laboratory protocol. According to the manufacturing guidelines, the genomic DNA from fish samples was extracted by using the Accuprep® Genomic DNA Extraction Kit (Bioneer). The small tissue from the pectoral fin (1 cm) was dissected and mix with 6X lysis buffer, which was further homogenized by the TissueLyser II (Qiagen). Quantification of purified genomic DNA was performed by nanoDrop (ThermoFisher Scientific D1000), aliquoted, and stored at -70°C for further analysis.

Two universal fish primer sets targeting the cytochrome c oxidase I (COI) region, BCL-BCH [23], [24]. The PCR mixture (20µL) contained 11.2 µL ultra-pure water, 1 µL primer forward and reversed (0.5 µM), 0.2 µL Ex Taq DNA polymerase (TaKaRa, Japan), 2 µL 10X ExTag Buffer, 2 µL dNTPs (1 µM, TaKaRa, Japan), and 2 µL genomic DNA as a template. The PCR condition was carried out under the following setting: 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 the extension step, and a final extension at 72°C for 5 min. The AccuPrep®Gel purification kit (Bioneer, Korea) has been used for PCR purification. The pairwise evolutionary distance among the family was determined by Kimura 2-Parameter method. The Maximum Likelihood (ML) tree was constructed, and 1000 bootstrap analysis was carried by Mega v7 [25].

In addition to the collected fish samples, 5 liters of seawater samples have been collected from the 5 points around the port (Figure 1) and filtered by the 0.45 µm pore-sized GN-6 membrane filter (PALL Life sciences, Mexico). According to the manufacture manual, the total genomic DNA was extracted from five membrane filters by the DNeasy® Blood and Tissue Kit (Qiagen, Germany). The extracted genomic DNA was quantified by the Nanodrop spectrophotometer ND1000 (Thermo Scientific, Waltham, MA, USA) and stored at -80°C for further analysis. The Nextera XT index kit (Illumina, USA) was used to construct the NGS analysis library. The first PCR of MiFish primer (MiFish F-R) was performed to connect the adapters (forward adapter primer 5'-TCG TCG GCA GCG TCA GAT GTG TAT AAG AGA CAG -3' and reverse adapter sequences 5'- GTC TCG TGG GCT CGG AGA TGT GTA TAA GAG ACA G-3'). The final PCR used N7xx and S5xx primers, including Illumina Nextera XT indexing primers. Finally, the libraries were sequenced on the Miseq 600-cycle Reagent Kit v3 (Illumina, USA).

B. Data Analysis

The paired-end sequences were filtered from the obtained raw reads and pairing using the Python27 program before uploading to the MiFish Pipeline. The taxonomic assignment was classified into three groups, i.e., species level (identity ≥99%), genus level (identity ≥97-98%), and the sequences with less than 95% identity were as unknown. Furthermore, taxonomic confirmation for scientific species was obtained from the FishBase (<http://www.fishbase.org>) and WoRMS (<http://www.marinespecies.org/>). The Phylogenetic tree reconstruction with the maximum likelihood was done by the Mega7 program [25].

III. RESULTS AND DISCUSSION

A. Molecular Identification (Barcoding and Metabarcoding) of Marine Fish Species

A total of 23 COI sequences was generated from 23 fish samples representing 20 genera, 15 families, and three orders (Table 1). The direct sequencing (Sanger sequencing) of the COI gene region produced more than 600 base pairs of nucleotides per taxon. The un-ambiguity and simplicity were observed among all the sequences. Besides, no stop codon, deletion, and insertion were observed in all the sequences.

TABLE I
MARINE FISH IDENTIFICATION BASED ON THE MOLECULAR APPROACH BY THE PARTIAL COI GENE REGION

No.	Sample ID	Species Name	Common Name	BLASTN result for partial region of COI genes	
				Query Cover (%)	Identity (%)
1	pr-1	<i>Platax teira</i>	Longfin batfish	100	99
2	pr-2	<i>Drepane punctata</i>	Spotted sicklefish	98	99
3	pr-3	<i>Leiognathus robustus</i>	Smooth jawfish	96	100
4	pr-6	<i>Parastromateus niger</i>	Black pomfret	100	100
5	pr-9	<i>Atule mate</i>	Yellowtail scad	99	98
6	pr-12	<i>Caranx sexfasciatus</i>	Bigeye trevally	100	100
7	pr-16	<i>Sardinella jussieu</i>	Mauritian sardinella	100	100
8	pr-18	<i>Sardinella jussieu</i>	Mauritian sardinella	98	99
9	pr-20	<i>Lactarius lactarius</i>	False trevally	100	100
10	pr-23	<i>Nemipterus marginatus</i>	Red filament treadfin bream	100	100
11	pr-25	<i>Upeneus margarethae</i>	Margaretha's goatfish	100	100
12	pr-28	<i>Trichiurus lepturus</i>	Largehead hairtail	100	100
13	pr-32	<i>Caesio cuning</i>	Redbelly yellowtail fusilier	100	100
14	pr-34	<i>Lutjanus erythropterus</i>	Crimson snapper	100	100
15	pr-35	<i>Lethrinus mahsena</i>	Sky emperor	100	100
16	pr-36	<i>Scomberomorus guttatus</i>	Indo-Pacific king mackerel	100	100
17	pr-40	<i>Cynoglossus lida</i>	Roughscale tonguesole	100	100
18	pr-42	<i>Thunnus albacares</i>	Yellowfin tuna	100	100
19	pr-43	<i>Selaroides leptolepis</i>	Yellowstripe scad	100	100
20	pr-44	<i>Sardinella jussieu</i>	Mauritian sardinella	100	100
21	pr-45	<i>Sardinella jussieu</i>	Mauritian sardinella	100	100
22	pr-50	<i>Photopectoralis aureus</i>	Golden ponyfish	100	99
23	pr-51	<i>Istiophorus platypterus</i>	Indo-Pacific sailfish	100	100

The accuracy of fish identification at the species levels still has limitations in the developing countries, including in Indonesia. The statistical data on fish catches in several regions made grouping fish species into the family level, and even many non-popular fish are categorized as other fish groups [26].

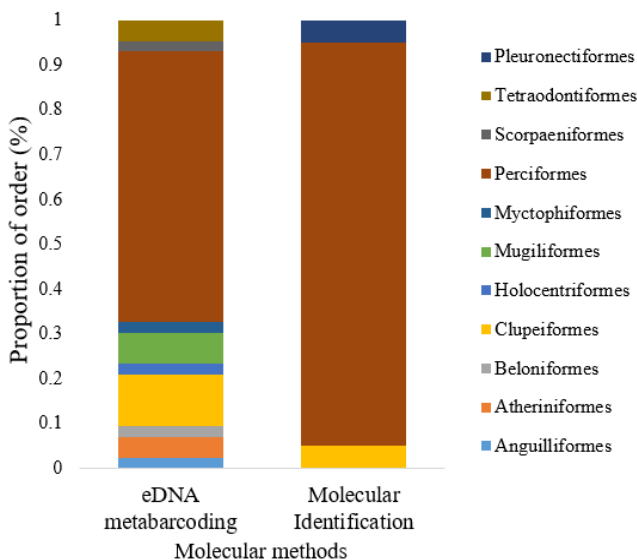


Fig. 1 Proportion eDNA metabarcoding and fish barcoding

In the current research, the eDNA metabarcoding successfully identified 44 marine fish species (identity 99-100%) from the MiFish pipeline representing 36 genera, 24

families, ten orders. Total reads from the eDNA sample are 133,794, and 3,386 reads (2.535%) were removed from the further analysis (under 95% sequence identity). The proportion of the two molecular techniques was performed in Figure 1. The 130,408 merged reads were able to assign at the species level (99.8%) and genus level (0.2%). The eDNA result identified the estuarine fish *Liza macrolepis* (38.33%), followed by *Acanthurus xanthopterus* (16.63%) and *Selar boops* (9.69%) (Table 2).

B. Perciformes

The fish species under the order Perciformes dominated in the identification of the eDNA metabarcoding method (Table 2). This order is also a group of essential pelagic and economical fish such as *Thunnus obesus*, *Katsuwonus pelamis*, *Lutjanus argentimaculatus*, *Auxis thazard*, and *Tetrapturus angustirostris*. The Tuna capture fisheries have experienced a reasonably high development with marked utilisation rates of 82.43% with the level of exploitation to catch it as large as (104.21%) [27]. This condition shows the high exploitation of tuna fisheries in this region due to tuna's potential, which is still the leading export product. In this study, identified Scombridae families (*Thunnus obesus*, *Katsuwonus pelamis*, *Auxis thazard*, and *Auxis* sp.), which made possible from the fisher catch landed at Pelabuhan Ratu fish port [3], [26], [28], [29]. The *Thunnus obesus* and *Katsuwonus pelamis* fish are the main target catch, while *Auxis thazard* types are included in the bycatch category [30].

TABLE II
LIST OF MARINE FISH SPECIES IDENTIFIED BY THE EDNA METABARCODING

No.	Species name	Family	Order	Identity (%)	Total Reads
1	<i>Gymnothorax chilospilus</i>	Muraenidae	Anguilliformes	100	1018
2	<i>Atherinomorus lacunosus</i>	Atherinidae	Atheriniformes	100	402
3	<i>Hypoatherina lunata</i>	Atherinidae	Atheriniformes	100	127
4	<i>Tylosurus crocodilus</i>	Belonidae	Beloniformes	99.41	630
5	<i>Amblygaster sirm</i>	Clupeidae	Clupeiformes	100	59
6	<i>Encrasicholina devisi</i>	Engraulidae	Clupeiformes	100	241
7	<i>Herklotsichthys quadrimaculatus</i>	Clupeidae	Clupeiformes	100	1747
8	<i>Konosirus punctatus</i>	Clupeidae	Clupeiformes	100	10
9	<i>Nematalosa come</i>	Clupeidae	Clupeiformes	98.83	16
10	<i>Myripristis berndti</i>	Holocentridae	Holocentriiformes	100	124
11	<i>Crenimugil seheli</i>	Mugilidae	Mugiliformes	98.84	682
12	<i>Liza macrolepis</i>	Mugilidae	Mugiliformes	100	51292
13	<i>Plicomugil labiosus</i>	Mugilidae	Mugiliformes	100	3999
14	<i>Lampadena luminosa</i>	Myctophidae	Myctophiformes	100	490
15	<i>Abudefduf sordidus</i>	Pomacentridae	Perciformes	100	741
16	<i>Abudefduf vaigiensis</i>	Pomacentridae	Perciformes	100	821
17	<i>Acanthurus lineatus</i>	Acanthuridae	Perciformes	100	495
18	<i>Acanthurus xanthopterus</i>	Acanthuridae	Perciformes	100	22260
19	<i>Auxis thazard</i>	Scombridae	Perciformes	99.41	8483
20	<i>Bathygobius hongkongensis</i>	Gobiidae	Perciformes	99.41	264
21	<i>Bathygobius</i> sp.	Gobiidae	Perciformes	98.2	261
22	<i>Caesio caeruleaurea</i>	Caesionidae	Perciformes	100	1380
23	<i>Caesio cuning</i>	Caesionidae	Perciformes	100	375
24	<i>Decapterus macrosoma</i>	Carangidae	Perciformes	100	47
25	<i>Decapterus russelli</i>	Carangidae	Perciformes	100	75
26	<i>Epinephelus septemfasciatus</i>	Serranidae	Perciformes	100	54
27	<i>Katsuwonus pelamis</i>	Scombridae	Perciformes	100	2738
28	<i>Kyphosus cinerascens</i>	Kyphosidae	Perciformes	100	735
29	<i>Lutjanus argentimaculatus</i>	Lutjanidae	Perciformes	100	3384
30	<i>Ostorhinchus cookii</i>	Apogonidae	Perciformes	99.4	2129
31	<i>Plectorhinchus chaetodonoides</i>	Haemulidae	Perciformes	100	19
32	<i>Pomacentrus moluccensis</i>	Pomacentridae	Perciformes	99.41	559
33	<i>Pomacentrus tripunctatus</i>	Pomacentridae	Perciformes	100	457
34	<i>Pterocaesio digramma</i>	Caesionidae	Perciformes	100	627
35	<i>Selar boops</i>	Carangidae	Perciformes	100	12972
36	<i>Siganus vermiculatus</i>	Siganidae	Perciformes	99.41	596
37	<i>Siganus vermiculatus</i>	Siganidae	Perciformes	100	596
38	<i>Tetrapturus angustirostris</i>	Istiophoridae	Perciformes	100	687
39	<i>Thunnus obesus</i>	Scombridae	Perciformes	100	259
40	<i>Upeneus sulphureus</i>	Mullidae	Perciformes	100	4548
41	<i>Upeneus vittatus</i>	Mullidae	Perciformes	98.83	774
42	<i>Parapterois heterura</i>	Scorpaenidae	Scorpaeniformes	100	861
43	<i>Odonus niger</i>	Balistidae	Tetraodontiformes	100	993
44	<i>Pseudobalistes flavimarginatus</i>	Balistidae	Tetraodontiformes	100	1381

Moreover, the fish species under the family Istiophoridae (*Tetrapturus angustirostris*) also identified and are fishermen's catch. Some publications mention other species such as *Istiophorus orientalis* [3] and *Istiophorus platypterus* [28] under the family Istiophoridae, other species which are classified as the bycatch are *Decapterus macrosoma* and *Decapterus russelli* [30]. The eDNA approach in this study proves the effectiveness of biodiversity studies by the molecular method. This method can obtain relatively faster data on species diversity around the shallow waters in Pelabuhan Ratu Bay.

The family Pomacentridae is a general constituent of reef fish in Indonesia. Besides this family, Acanthuridae, Gobiidae, Serranidae, Siganidae, and Haemulidae are also groups of the coral-reef associated fish. These fish sometimes become the catch of the fishermen bought and sold at the Pelabuhan Ratu fish market. Other studies that

support the existence of these fish in the vicinity of Pelabuhan Ratu Bay are not widely used. Other areas in the southern part of Java Island mentioned that the group of reef fish identified also had a composition similar to the results of eDNA metabarcoding at the Pelabuhan Ratu. Nine families from marine fish, including Serranidae, Caesionidae, Acanthuridae, Lutjanidae, Mullidae, Nemipteridae, Scaridae, Haemulidae, and Carangidae were identified in the Prigi Bay, Trenggalek [31]. Chaetodontidae fish is the bioindicator of the healthy condition of coral reefs [32]. Here, we did not find this group of fish in the eDNA metabarcoding method, which indicated that coral reefs in this area are not a dominant ecosystem. The phylogenetic tree from the eDNA sequences figure-out the fish in separate families (Figure 2), although this tree is generated from partial segment of 12S ribosomal RNA with a length of ± 160 bp.

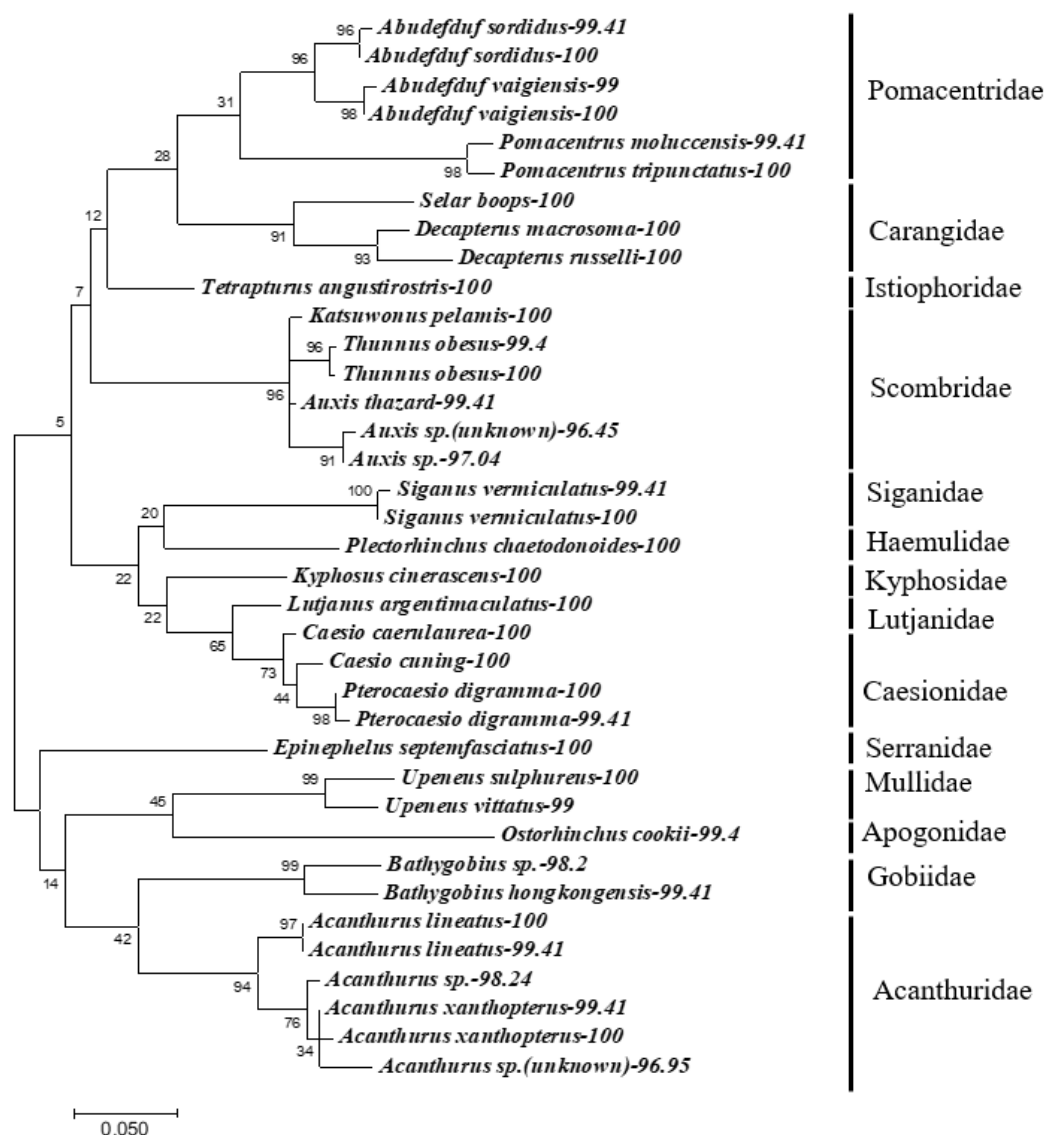


Fig. 2 Phylogenetic reconstruction oh haplotype order Perciformes generated by eDNA metabarcoding

The fish species detection rate from the eDNA metabarcoding was more than the DNA barcoding method, which is only limited to the collected fish samples. At certain times, the availability of fish in the Port Ratu fish

market is meager. This condition happened because several economically important fish are not traded locally but directly sent to the processing industry or sent straight to Jakarta. However, identification with the metabarcoding

approach can provide an overview of many types of fish that have the potential to be collected from around the Teluk Pelabuhan Ratu. The eDNA metabarcoding approach by MiFish pipeline using the short sequences of the mitochondrial partial 12S ribosomal RNA [33], [34], which can not distinguish some closely related marine fish species. For instance, only *Thunnus obesus* can be found in eDNA and barcoding (Figure 3).

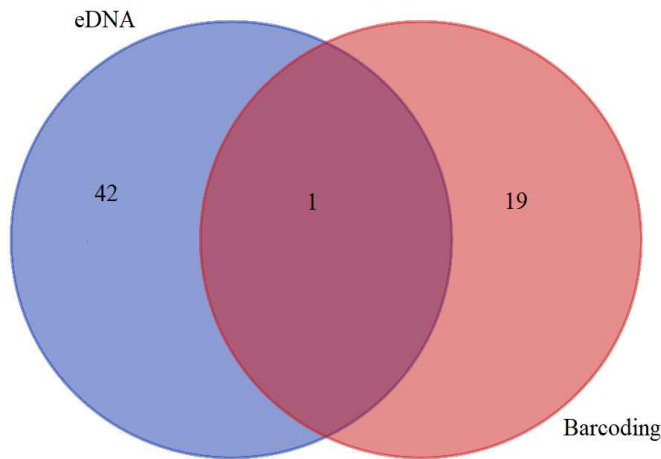


Fig. 3 Venn diagram fish detected by eDNA and fish barcoding

In the Istiophoridae family, it was identified that there were different types of *Istiophorus platypterus* (barcoding) and *Tetrapturus angustirostris* (eDNA metabarcoding). This sequences of eDNA metabarcoding are only about ~170 bp, whereas the DNA barcoding can produce sequences of more than 500 bp in the COI region. The COI region was adopted

as barcoding for determining molecular identification globally [9].

C. Clupeiformes and Mugiliformes

Other economically fish groups are the order Clupeiformes [26]. Which generally becomes a consequence of the local community because of the relatively lower price. This type of fish found in the Pelabuhan Ratu fish market included the sardine and anchovy. In this study, the results of eDNA metabarcoding were able to identify five species in the Clupeidae family, namely *Herklotsichthys quadrimaculatus*, *Amblygaster sirm*, *Konosirus punctatus*, *Nematalosa come*, and *Encrasicholina devisi*. Several studies stated that the anchovy species caught around the Port Ratu range are *Stolephorus* sp., which is reported without identification in the molecular or morphology identification methods [35]. At present, the FishBase database has revised the taxonomy of *Stolephorus devisi* [36] into the *Encrasicholina devisi* [37] as identified in this eDNA metabarcoding result. We also found the anchovy on the fish market at Pelabuhan Ratu.

The family Mugilidae is an estuarine fish that is widely used by the community for domestic consumption. Fish species from the Mugilidae group are generally obtained from waters that are almost adjacent to estuaries with somewhat varying wide range of salinity. Other types of fish species reported in Java's southern region (Ujung Kulon, Pandeglang) is *Liza subviridis* [38]. In this study, we were able to identify successfully three species from the family Mugilidae (*Liza macrolepis*, *Crenimugil seheli*, and two haplotypes of *Plicomugil labiosus*), and the phylogenetic tree also able to distinguished two families of the order Clupeiformes (Figure 4).

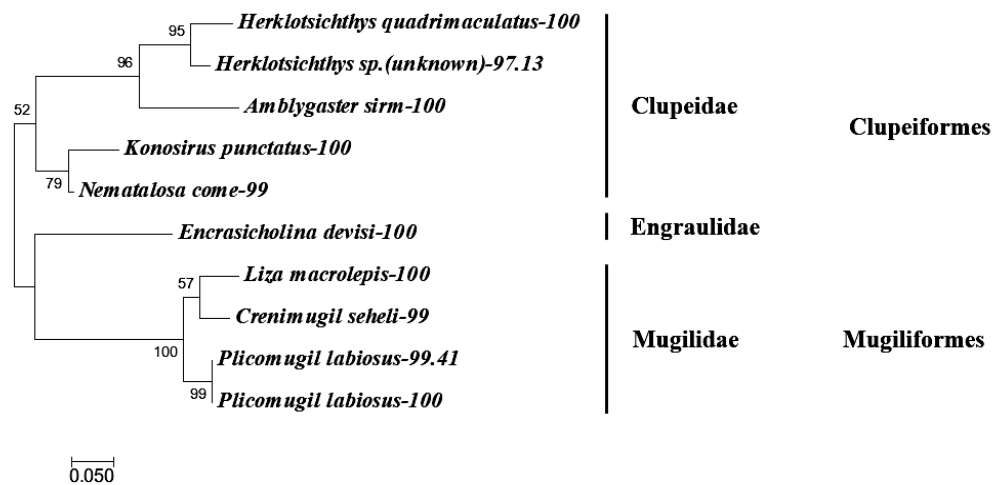


Fig. 4 Phylogenetic reconstruction oh haplotype order Clupeiformes and Mugiliformes generated by eDNA metabarcoding

D. The Other Order

Here, the reconstructed phylogenetic tree results can separate all species from each family (Figure 5). This result is fascinating and shows that the Pelabuhan Ratu Bay has a high diversity of marine fish species. The Muraenidae group from order Anguilliformes is often found to be the bycatch from the several fishers. The species that has received the

most attention today as a cultivation commodity is *Anguilla* spp. [39]. Unfortunately, the eDNA metabarcoding did not find this species. This condition is possible because of the limited number of samples and is carried out in just one-time sampling in that area. The periodic monitoring will help determine the pattern of changes in the composition of marine fish in this region.

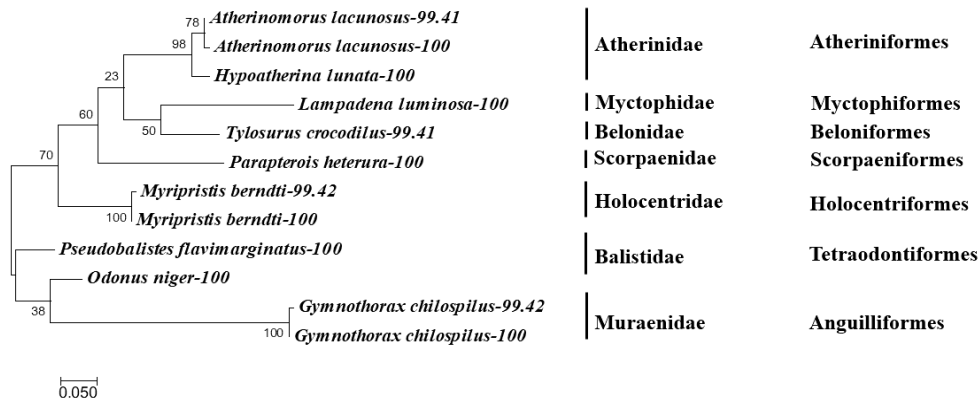


Fig. 5 Phylogenetic reconstruction of haplotype small number of diverse of piscine order generated by eDNA metabarcoding

E. IUCN, CITES List and Venomous Fish

Almost all the fish identified in this region are in the Least Concern (LC) and Not Evaluated categories (Table 3) based on the FishBase (www.fishbase.org) and IUCN Redlist database (www.iucnredlist.org). Out of the entire list of

species identified, only the *Thunnus obesus* is included in the Vulnerable (VU) category [40]. This condition signals that the management of tuna fisheries in Indonesia must be done wisely so that the tuna fish population will not be over-exploited or not extinct from this area.

TABLE III
THE IUCN AND CITES STATUS OF MARINE FISH SPECIES LIST IDENTIFIED BY EDNA METABARCODING

No.	Species name	Common Name	Distribution	IUCN status	CITES	Threat to humans
1	<i>Abudefduf sordidus</i>	Blackspot sergeant	Indo-Pacific	Least Concern	Not Evaluated	Harmless
2	<i>Abudefduf vaigiensis</i>	Indo-Pacific sergeant	Indo-Pacific	Least Concern	Not Evaluated	poisoning
3	<i>Acanthurus lineatus</i>	Lined surgeonfish	Indo-Pacific	Least Concern	Not Evaluated	Venomous
4	<i>Acanthurus xanthopterus</i>	Yellowfin surgeonfish	Indo-Pacific	Least Concern	Not Evaluated	Venomous
5	<i>Amblygaster sirm</i>	Spotted sardinella	Indo-West Pacific	Least Concern	Not Evaluated	Harmless
6	<i>Atherinomorus lacunosus</i>	Wide-banded hardyhead silverside	Indo-Pacific	Not Evaluated	Not Evaluated	Harmless
7	<i>Caesio caerulea</i>	Blue and gold fusilier	Indo-West Pacific	Least Concern	Not Evaluated	Harmless
8	<i>Caesio cuning</i>	Redbelly yellowtail fusilier	Indo-West Pacific	Least Concern	Not Evaluated	Harmless
9	<i>Decapterus macrosoma</i>	Shortfin scad	Indo-Pacific and Southeast Atlantic	Least Concern	Not Evaluated	Harmless
10	<i>Decapterus russelli</i>	Indian scad	Indo-West Pacific	Least Concern	Not Evaluated	Harmless
11	<i>Encrasicholina devisi</i>	Devis' anchovy	Indo-Pacific	Not Evaluated	Not Evaluated	Harmless
12	<i>Epinephelus septemfasciatus</i>	Convict grouper	Northwest Pacific	Data Deficient	Not Evaluated	Harmless
13	<i>Gymnothorax chilospilus</i>	Lipspot moray	Indo-Pacific	Not Evaluated	Not Evaluated	Harmless
14	<i>Herklotsichthys quadrimaculatus</i>	Bluestripe herring	Indo-Pacific	Least Concern	Not Evaluated	Potential pest
15	<i>Hypoatherina lunata</i>	Okinawan silverside	Western Pacific	Not Evaluated	Not Evaluated	Harmless
16	<i>Katsuwonus pelamis</i>	Skipjack tuna	Cosmopolitan in tropical and warm-temperate waters	Least Concern	Not Evaluated	poisoning
17	<i>Konosirus punctatus</i>	Dotted gizzard shad	Indo-West Pacific	Least Concern	Not Evaluated	Harmless
18	<i>Kyphosus cinerascens</i>	Blue sea chub	Indo-Pacific	Least Concern	Not Evaluated	poisoning
19	<i>Lampadena luminosa</i>	Luminous lanternfish	Atlantic, Indian, and Pacific	Least Concern	Not Evaluated	Harmless
20	<i>Liza macrolepis</i>	Largescale mullet	Indo-Pacific	Least Concern	Not Evaluated	Harmless
21	<i>Lutjanus argentimaculatus</i>	Mangrove red snapper	Indo-West Pacific	Least Concern	Not Evaluated	poisoning
22	<i>Myripristis berndti</i>	Blotcheye soldierfish	Indo-Pacific and Eastern Pacific	Least Concern	Not Evaluated	Harmless
23	<i>Odonus niger</i>	Red-toothed triggerfish	Indo-Pacific	Not Evaluated	Not Evaluated	Harmless
24	<i>Plicomugil labiosus</i>	Hornlip mullet	Indo-Pacific	Not Evaluated	Not Evaluated	Harmless
25	<i>Parapterois heterura</i>	Blackfoot firefish	Indo-West Pacific	Least Concern	Not Evaluated	Venomous
26	<i>Plectorhinchus</i>	Harlequin sweetlips	Indo-West Pacific	Not Evaluated	Not Evaluated	Harmless

No.	Species name	Common Name	Distribution	IUCN status	CITES	Threat to humans
	<i>chaetodonoides</i>					
27	<i>Pomacentrus tripunctatus</i>	Threespot damsel	Indo-West Pacific	Not Evaluated	Not Evaluated	Harmless
28	<i>Pseudobalistes flavimarginatus</i>	Yellowmargin triggerfish	Indo-Pacific	Not Evaluated	Not Evaluated	poisoning
29	<i>Pterocaesio digramma</i>	Double-lined fusilier	Western Pacific	Least Concern	Not Evaluated	Harmless
30	<i>Selar boops</i>	Oxeye scad	Pacific Ocean	Least Concern	Not Evaluated	Harmless
31	<i>Siganus vermiculatus</i>	Vermiculated spinefoot	Indo-West Pacific	Least Concern	Not Evaluated	Venomous
32	<i>Tetrapturus angustirostris</i>	Shortbill spearfish	Indian and Pacific	Data deficient	Not Evaluated	Harmless
33	<i>Thunnus obesus</i>	Bigeye tuna	Atlantic, Indian, and Pacific	Vulnerable	Not Evaluated	Harmless
34	<i>Upeneus sulphureus</i>	Sulphur goatfish	Indo-West Pacific	Least Concern	Not Evaluated	Harmless
35	<i>Auxis thazard</i>	Frigate tuna	Atlantic, Indian, and Pacific (Western Central)	Least Concern	Not Evaluated	Harmless
36	<i>Bathygobius hongkongensis</i>	Gobi	Northwest Pacific	Not Evaluated	Not Evaluated	Harmless
37	<i>Pomacentrus moluccensis</i>	Lemon damsel	Western Pacific	Not Evaluated	Not Evaluated	Harmless
38	<i>Siganus vermiculatus</i>	Vermiculated spinefoot	Indo-West Pacific	Least Concern	Not Evaluated	Venomous
39	<i>Tylosurus crocodilus</i>	Hound needlefish	Indo-West Pacific	Least Concern	Not Evaluated	Traumatogenic
40	<i>Ostorhinchus cookii</i>	Cook's cardinalfish	Indo-Pacific	Not Evaluated	Not Evaluated	Harmless
41	<i>Crenimugil seheli</i>	Bluespot mullet	Indo-Pacific	Not Evaluated	Not Evaluated	Harmless
42	<i>Nematalosa come</i>	Western Pacific gizzard shad	Western Pacific	Least Concern	Not Evaluated	Harmless
43	<i>Upeneus vittatus</i>	Yellowstriped goatfish	Indo-Pacific	Least Concern	Not Evaluated	Harmless
44	<i>Bathygobius sp.</i>	Brownboy goby	Indo-West Pacific and Southeast Atlantic	Not Evaluated	Not Evaluated	Harmless

Several fishes also have poisons in their body that need to be watched out. Two species, including *Acanthurus lineatus* and *Acanthurus xanthophores*, need to be aware. Those species have poison glands in their body [41]. However, some fish species have quite large venom glands, namely *Parapterois heterura*, which is included in the Scorpaenidae family. The fish in this family group has been known as fish that have poisons [42].

The *Siganus vermiculatus*, which is classified as an herbivore fish, also needs to be wary because of venom's potential in the dorsal fin spines. This fish is common in the coastal areas, feeds on the algae growing on seagrasses, and is sometimes treated as a harmful organism for the seaweed cultivation. Another type of fish in the genus *Siganus* is reported to have a potential toxin; namely, *Siganus fuscescens* also need to be considered for human consumption safely [43].

IV. CONCLUSION

We conclude that the diversity of marine fish on the Port of Ratu Bay's surrounding waters is high. In this study, 44 fish species were detected from the ten orders and 24 families. Almost all belong to the economic fish groups and reef fish that can be utilized as fisheries commodities. The eDNA metabarcoding approach is expected to be the initial database for diversity that can be followed up to maintain the supply of fishery products in the West Java region. Of the types of fish identified, fish fall into the category of vulnerable fish, venomous fish, and migratory fish. This study is expected to supplement periodic surveys to determine the fluctuations and effects of human activities,

including over-exploitation and potential pollution from the coastal areas around the Pelabuhan Ratu Bay.

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