

eDNA Malang 2019

by Sapto Andriyono

Submission date: 09-Jan-2023 03:46PM (UTC+0800)

Submission ID: 1990100934

File name: 4225-Article_Text-14753-1-10-20191129.pdf (820.85K)

Word count: 6713

Character count: 37325

Environmental DNA (eDNA) metabarcoding: Diversity study around the Pondok Dadap fish landing station, Malang, Indonesia

SAPTO ANDRIYONO^{1,2,*}, MD. JOB Aidul ALAM¹, HYUN-WOO KIM^{1,3}

¹Interdisciplinary Program of Biomedical, Mechanical and Electrical Engineering, Pukyong National University, Busan, 48513, Republic of Korea
²Department of Marine, Faculty of Fisheries and Marine Science, Universitas Airlangga, C Campus, Jl. Mulyorejo, Surabaya 60115, East Java, Indonesia.

Tel.: +62-31-5911541, *email: sapto.andriyono@fpk.unair.ac.id

³Department of Marine Biology, Pukyong National University, Busan 48513, Republic of Korea

Manuscript received: 27 July 2019. Revision accepted: 27 November 2019.

Abstract. *Andriyono S, Jobaidul Alam Md, Kim HW. 2019. Environmental DNA (eDNA) metabarcoding: Diversity study around the Pondok Dadap fish landing station, Malang, Indonesia. Biodiversitas 20: 3772-3781.* Molecular identification of species is now fast growing and currently widely applied method in the diversity estimation of aquatic biota; even though morphological identification is still carried out. The molecular approach is beneficial complementing on regular surveys e.g. use of nets, traps, fishing rods, and even with poisons. In this study, the eDNA metabarcoding was applied to water samples around the Pondok Dadap fish landing station, Indonesia to determine the diversity of fish around the waters and also to identify marine fish landed in this area. Molecular identification was carried out on fish samples obtained from the fish market improved GenBank database on COI and ITS. While, seawater samples were carried out by using the next-generation sequencing (NGS) platform to obtain the eDNA metabarcoding data for the first time. Molecular identification obtained 34 species (68 sequences of COI and ITS regions) belonging to 28 genera, 18 families, 4 orders, while the eDNA metabarcoding approach identified 53 marine fish species by using the MiFish pipeline representing 38 genera, 27 families, and 7 orders. From the present study, we can able to estimated fish diversity by eDNA metabarcoding, and this finding will be helpful for baseline data preparation for future effective management of resources in this area.

Keywords: Environmental DNA, identification, metabarcoding, molecular

INTRODUCTION

DNA-base identification is very efficient when a comprehensive reference database available. This method is able to prove in the identification of specimens under specific conditions (Meyer and Paulay 2005). Identification based on DNA barcode has been well-accepted globally due to various advantages, it is very simple and uses a universal tool. It could be utilized in all organisms, both in the fresh samples and processed products (Pepe et al. 2007, Giusti et al. 2017). DNA barcoding was launched since 2005 under iBOL (www.boldsystem.org) even though it was introduced in 2003, which used mtDNA segment on Cytochrome C Oxidase subunit I (COI) as the common region for barcode (Hebert et al. 2003).

This barcoding system uses sequences that have a diversity in the single region of mitochondrial DNA Cytochrome C Subunit I gene (COI) and deposited to the Genbank database as central bioinformatics. Scientists have demonstrated their effectiveness in conducting DNA barcoding in freshwater fish and deep-sea fish (Ward et al. 2005, Lakra et al. 2011).

At present, estimating the presence of species in the waters can be carried out using environmental DNA approaches called eDNA. The extraction and analysis of genetic material are obtained directly from the environment by collecting these living particles as an alternative survey approach to monitoring marine fish (Taberlet et al. 2012). This approach is first carried out on terrestrial sediment

samples that can reveal marine mammalian (Foote et al. 2012), bird and plant ecosystems (Willerslev et al. 2003) which are extinct and still exist today. Furthermore, this eDNA metabarcoding approach successfully revealed information on various taxa, various habitats and various weather conditions (Willerslev et al. 2004; Anderson-Carpenter et al. 2011; Taberlet et al. 2012). Several reports stated that metabarcoding through eDNA can also be done in biodiversity studies (Karp et al. 1997; de Vargas et al. 2002; Douglas et al. 2012; Thomsen et al. 2012) and suspect invasive biota (Dejean et al. 2012; Takahara et al. 2013), while observing rare biota (Jerde et al. 2011; Wilcox et al. 2013) which is difficult to collect through traditional survey methods. In this study, we conducted a research to estimate the diversity of marine fish and fish caught in Pondok Dadap Malang with the eDNA approach as well as the initial data for further research related to the biodiversity of aquatic biota around the Sempu Island Nature Reserve which located in front of Pondok Dadap fish landing station.

One of the important fisheries commodities in Indonesia is the tuna pelagic fish group which has important economic value and is the mainstay of Indonesian exports. There are at least four species of tuna in Indonesia and one of the landing sites for tuna in East Java is the Pondok Dadap Port, Malang, Indonesia. This southern region of East Java is one of the suppliers of tuna products in East Java and other types of pelagic and non-pelagic fish, although in small quantities. The purpose of

this study is: (i) to carry out molecular identification of marine fish landed in Pondok Dadap fish landing station due to inexistent clear data about species identification, and (ii) to estimate fish species diversity around the Pondok Dadap fish landing station, which very close to the Sempu Island nature reserve area, through environmental DNA metabarcoding approach based on water sample analysis.

MATERIALS AND METHODS

Sampling location and sample preparation

In this research, for environmental DNA metabarcoding study, seawater samples have been collected from five points around the Pondok-Dadap port, Malang, Indonesia (8°26'05.65"S 112°40'55.31"E), then for barcoding 34 fish specimens were collected in February 2018 for molecular identification. In addition, no specific permission was required for this study, and the individual photograph has been taken by a digital camera. All samples have been collected from the local traditional fish market and those were dead upon the purchasing time and none of the collected specimens were in the endangered category based on the IUCN Red List database.

All specimens for barcoding have been collected based on the morphological characteristics and after collection directly preserved in 90% ethanol then carried out to the laboratory. The collected samples have been preserved at the Department of Marine, Fisheries and Marine Faculty, Universitas Airlangga, Indonesia following the standard laboratory protocol.

DNA extraction and PCR

The genomic DNA was extracted from fish samples by using an Accuprep® Genomic DNA Extraction Kit (Bioneer) according to the manufacturer's guidelines. The anal fin (1 cm) was dissected and mix with 6x lysis buffer, which was further homogenized by the TissueLysor II (Qiagen). Quantification of purified genomic DNA was performed by nanoDrop (ThermoFisher Scientific D1000), aliquoted and stored at the -70°C for further analysis.

Two sets of universal fish primer targeting the Cytochrome c Oxidase I (COI) region, BCL-BCH (Baldwin et al. 2009, Handy et al. 2011) and internal transcribed spacer (ITS) primer sets (Forward F2 5'-CCM YCT AGA GGA GCC TGT YCT RDA A-3'-Reverse R1 5'-CAT GAT GCA AAA GGT AC-3') were used to obtain the partial sequences of each gene, respectively. The ITS region used to improved GenBank database in this region for further our lab works in eDNA metabarcoding. The COI and ITS primer set targeting around 600bp and 700bp sequence, respectively. Both PCR mixture (20µL) contained 11.2 µL ultra-pure water, 1 µL forward and reverse primer (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 (COI and ITS) 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 final extension at 72°C for 5 min. The PCR products were purified with AccuPrep®Gel purification kit (Bioneer, Korea). All sequences were aligned and submitted to NCBI GenBank database.

Construction of Library and MiSeq sequencing

Total genomic DNA was extracted from five filter membrane by Accuprep® Genomic DNA Extraction Kit (Bioneer) according to the manufacturer's manual. Extracted genomic DNA was quantified using 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 library for NGS analysis. The first PCR of MIFISH primer (MIFISH F-R) was performed to connect the adapters. The adapter primers were forward adapter sequences (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') respectively. The final PCR used N7xx and S5xx primer including Illumina Nextera XT indexing primers. Finally, libraries sent for sequencing on Miseq 600-cycle Reagent Kit v3 (Illumina, USA).

The MiFish universal primer sets were used to construct the amplicon libraries of partial 12S rRNA markers (Miya et al. 2015). The total PCR mixture volume was 20 µL, which contained 1.0 µL of MiFish primers (5 pmol each), 2.0 µL dNTPs (2.5mM), 2.0 µL of 10X EX Taq buffer, 0.6 µL DMSO (3%), 0.2 µL of EX Taq Hot Start (TaKaRa Bio Inc. Japan) and 9.20 µL of ultra-pure water. Here, we used 4.0 µL template due to the low genomic DNA concentration that less than 50 ng/µL. The PCR setting condition followed the MiFish primer protocol (Miya et al. 2015). The gel electrophoresis (1.5% agarose) was performed and the expected size (250 bp~350 bp) was purified by the AccuPrep® Gel Purification Kit (Bioneer, Republic of Korea). Purified amplicons were pass through the second PCR with the corresponding Nextera XT index (Illumina, San Diego, USA) at the end of each amplicon. The total volume for second PCR mixture was 20 µL which contain 1 µL of a couple of index primers (10 pmol), 0.5 µL dNTPs (10 mM), 4 µL 5X Phusion HF Buffer, 8.3 µL ultrapure water, and 0.2 µL Phusion Hot Start Flex DNA polymerase (New England Biolabs, Hitchin, UK), and including 5 µL amplicons result from the first PCR. The second PCR setting conditions began with 94°C for 5 min for initial denaturation, followed by 15 cycles of 94°C for 30 sec for denaturation, 55°C for 30-sec annealing, and 72°C for 30 sec for extension, and an additional 5 min at 72 °C for the final extension. The gel electrophoresis and purification were performed similar to the first PCR process, then PCR products with the expected sizes were analyzed by qubit dsDNAHS Assay Kit (Invitrogen, Carlsbad, CA, USA) for quantification of amplicons concentration. The next-generation sequencing was applied using MiSeq platform (2 X 300 bp).

Before uploaded NGS raw data to the MiFish pipeline, Phytion27 (an open-source software) was used to make pairing of both reverse and forward sequences with the specific script (Zhang 2015). In MiFish pipeline, the raw

reads by MiSeq sequencing run FASTQC, which will trimming for low-quality tail of reads ($QV \leq 20$), assembled paired-end reads and followed by removed N-containing reads, filtered reads by length (~229 bp), run Usearch (0.99 for clustering of identity, and 10 for minimum read size for filtering), BLASTN based on GenBank database, and then created multi-FASTA files for each samples. The next step is run MAFFT, run Morphy for each sample, run Morphy against merged samples, run BLASTN, and finalization of the last process by BLASTN. The total sequences stipulated to operational taxonomic units (OTUs) by compared to the GenBank database, then the sequences were ascertained as 'species', 'genus', and 'unknown' level if the sequence identity more than or similar to 99%, 97-98%, and less than 97%, respectively. The distribution for each species was confirmed by FishBase (<http://www.fishbase.org/>) then taxonomic nomenclature was confirmed under World Register of Marine Species WORMS through online system (<http://www.marinespecies.org/>).

Data analysis

Tables and figures of data were performed by Excel 2010, similarity and biodiversity analysis using Primer v7 program. The data on the common local name of fish landed in Pondok Dadap fish station was provided by the East Java Province's Office of Marine and Fisheries, and also previous study using Underwater Visual Census (UVC) around Sendang Biru, Malang by (Luthfi et al. 2016). Phylogenetic tree constructed by Mega7 (Kumar et al. 2016) using Neighbor-Joining algorithm both eDNA sequences results.

RESULTS AND DISCUSSION

Species identification

A total of 34 (COI and ITS) sequences generated from 34 fish samples representing 28 genera, 18 families, 4 order (Table 1). The Sanger dideoxy sequencing (direct sequencing) of the partial COI and ITS gene regions produced sequences of more than 600 nucleotide base pairs per taxon 607 bp for COI and 629 bp for ITS). The COI gene region is the universal gene for species barcoding (Hubert and Hanner 2015), the ITS region is new segment for species identification which potential for environmental DNA metabarcoding in our future laboratory experiments. All sequences obtained have been convinced that no stop codons were found, deletion and insertion were observed.

The eDNA Metabarcoding

The eDNA metabarcoding by using the MiFish pipeline (Miya et al. 2015, Sato et al. 2018), we were able to identify 53 marine fish species (sequence identity 99-100%) representing 38 genera, 27 families, 7 order. Total reads from the MiFish pipeline by using eDNA samples were 151,465 (Table 2). Here, the Atlantic Bluefin tuna *Thunnus thynnus* is dominated (71.28%), followed by Bigeye tuna, *Thunnus obesus* (12.12%) and Skipjack tuna

Katsuwonus pelamis (7.08%). Atlantic Bluefin tuna *Thunnus thynnus* not inhabit in Indian Ocean and Pacific ocean but detected by eDNA metabarcoding in this research. There are two reason may happen due to some samples biased (Sato et al. 2017) and weakness of PCR primer during library construction which not suitable for tuna fish species. As explained in the article on MiFish primer that this primer has limitation to distinguishing tuna species (Miya et al. 2015), further research is needed in this regard. Based on phylogenetic reconstruction show that some tuna species in one line with other Scombridae species. The species of *Thunnus obesus*, *Thunnus maccoyii*, and also *Thunnus thynnus* clustered in similar lines by zeno in genetic distance and unable distinguished within this those tuna species. Then another tuna species, *Thunnus alalunga* and *Thunnus orientalis*, have low in genetic distance range 0.007-0.014 (Figure 1).

Discussion

Species identification

Molecular identification provides an effective tool for accurate species identification and is widely applied, although there may be limitations due to incomplete database (Teletchea 2009). At present, the application of metabarcoding is also one of the most efficient methods for estimating species that live in a habitat without having to conduct costly and consuming surveys (Rees et al. 2014, Roussel et al. 2015, Piggott 2016, Foote et al. 2012). The challenges remain for application of metabarcoding needs concern on the sensitivity of this method to non-target DNA contamination, primer biases, sequencing artifacts, species misidentification, and also sampling biases (Sato et al. 2017). This method also requires adequate equipment support and bioinformatic data processing.

In this report, we have collected fish sold at the Pondok Dadap port and at the same time deposit the molecular data of Indonesian fish in the GenBank database as a new sequence. The molecular identification method in the COI region has become very common (Matzen da Silva et al. 2011; Aziz et al. 2016; Udayasuriyan and Kalpana 2018). Here, we also used the ITS region beside the COI region. ITS region has not been widely used in molecular identification, since COI is most popular gene marker (Avisé 2012). ITS region generally has been used to identify types of fungi (Das and Deb, 2015; Badotti et al. 2017), but this time, the accuracy of identification in vertebrates also produced quite good results. Out of the 34 species that were successfully identified, 13 species did not immediately get results at the species level due to the limitations of the information in the NCBI GenBank database. Unlike the COI region, which is more than enough related to the vertebrate database, so that in this study, the results of identification with the COI region became confirmation data in the ITS region. In this study, the 13 sequences generated were new entries in the GenBank database in the ITS region. Furthermore, in further research, the ITS region can be used in identification at the species level.

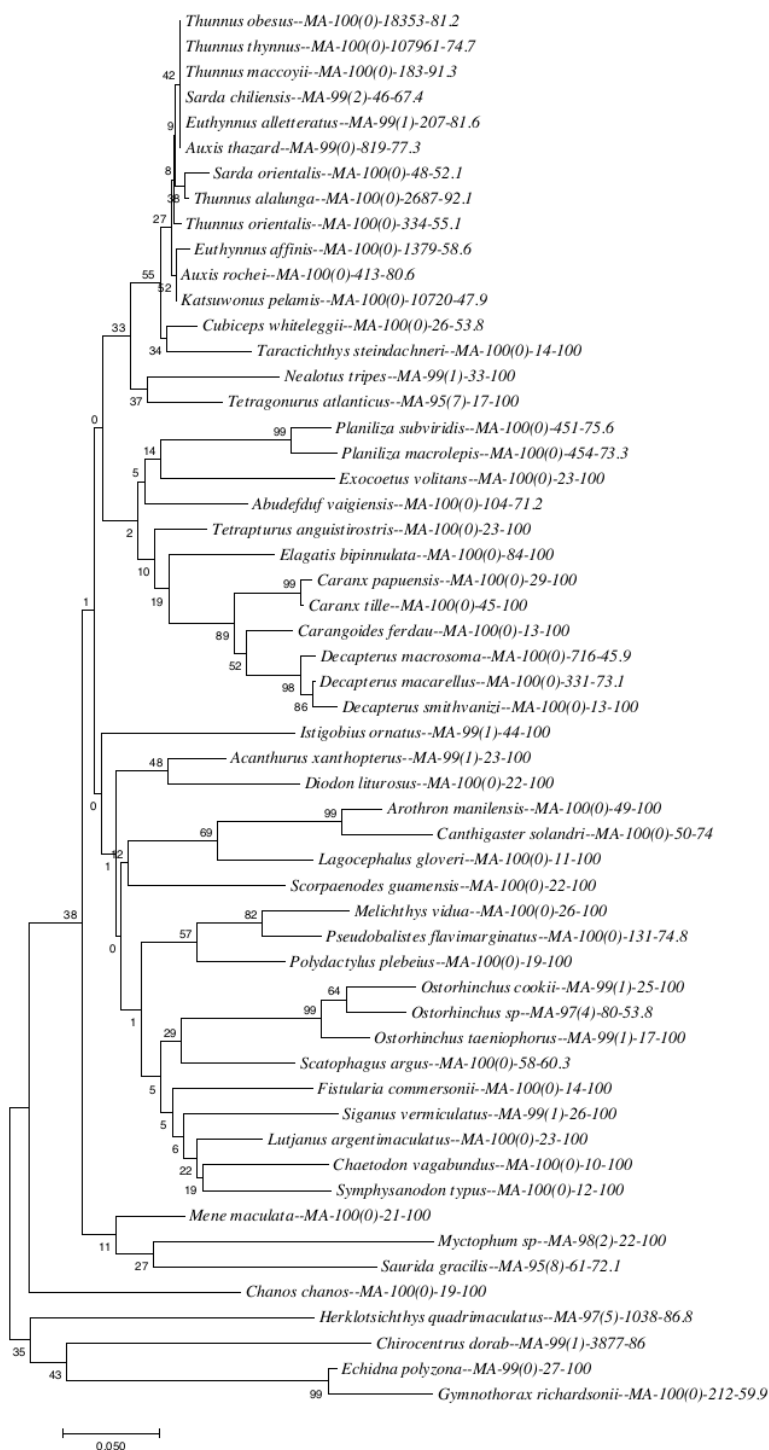


Figure 1. Neighbor-Joining method of phylogenetic tree analysis for sequences generated by eDNA metabarcoding

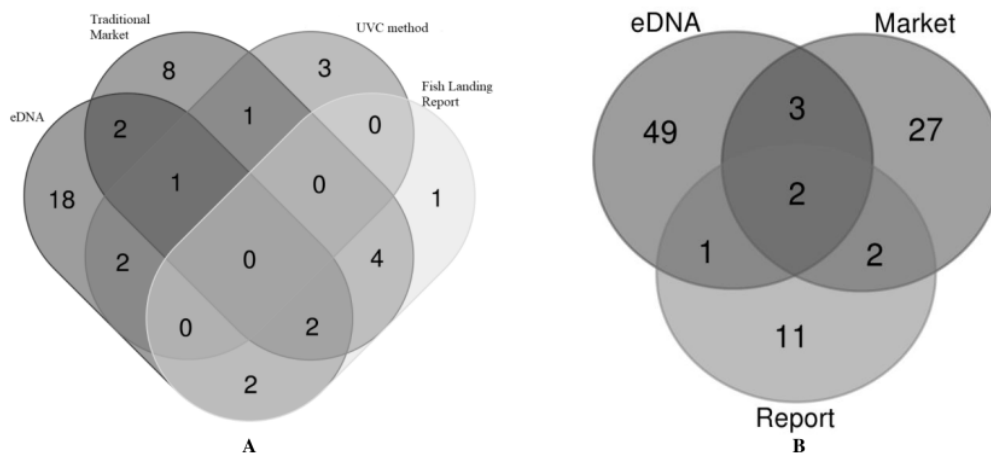


Figure 2. Venn diagrams on fish species data collection methods at the Family level (A: 4 methods) and at species level (B: 3 methods).

The eDNA metabarcoding analysis

In this study, we demonstrated the efficiency of eDNA applications around the fish landing station in Pondok Dadap Malang and at the same time carried out molecular identification of marine fish species sold by traditional fishermen in locations not far from the port. Overlay results from species identified between eDNA metabarcoding and fish sold in conventional markets show only five families were found through both approaches.

The effectiveness of eDNA metabarcoding has been successfully proven and supported in studies on numerous aquatic biota (Pilliod et al. 2013, Rees et al. 2014) that are difficult to collect and endangered (Thomsen et al. 2012, Laramie et al. 2015, Ikeda et al. 2016), as well as endemic (Jerde et al. 2011), and invasive species (Dejean et al. 2012, Takahara et al. 2013). Furthermore, eDNA can provide an overview of biodiversity (Thomsen et al. 2012) in the region that is related to periodic studies and compare it with diversity in the other areas. This method is considered entirely environment-friendly, reduces the survey costs which could be quite high, requiring a considerable amount of equipment, or in other words, this method is very cost-effective (Smart et al. 2016).

Here, we also overlaid the results with previous studies that carried out underwater surveys in the waters around the port and Sempu Island (Luthfi et al. 2016). This reported that only gathered three families marine fish which included in this eDNA metabarcoding list result. The advantages of eDNA metabarcoding complement to other methods, most species detected and had similarity values ranging from 95-100%, with a significant proportion of 72.41% and 1.72% having 100% and 99% identities with

the GenBank voucher sequences. This result is in line with previous studies, comparing traditional survey methods (underwater visual census and trawling) with eDNA sampling, which showed that latter superiority in detecting higher number of species in water (Yamamoto et al. 2017).

A previous underwater visual census (UVC) method only found seven families of reef fish which did not coincide with the capture fisheries results reported by the Pondok Dadap fish landing station office Malang (Luthfi et al. 2016). The Pondok Dadap fish landing office only reported the specific on pelagic fish groups especially reporting catches of tuna fish (Hermawan 2006, Firdaus and Witomo 2014). Current study, we overlaid eDNA metabarcoding result, barcoding species from fish market, fish list from Pondok Dadap fish landing station, and previous scientific report using UVC method. The Venn diagram only able overlaid until family level (Figure 2A), and remaining is overlaid between three data until species level (Figure 2B). This report complements each other as baseline data of marine fish diversity around Pondok Dadap fish landing station. The eDNA metabarcoding results show that they were unable to detect all fish which was reported by Pondok Dadap fish landing station office due to two possibilities. First, the official report from fish landing station only focused on certain commodities (Scombridae) and they use the local name without confirmation of scientific name of each species. Here, we try to convert the local name to scientific name base on the other scientific reports (Faizah and Aisayah 2017). Secondly, almost all small-size tuna fish species at fish landing station reports were categorized as baby tuna and bring possibility of mixed within tuna species.

Table 1. List of marine fish species identified by COI and ITS gene region. Grey color at ITS region shown that new entry for GenBank database

Order	Family	Species	Common name	Habitat distribution	GenBank accession no. for confirmation	COI gene	GenBank accession no. ITS gene
Perciformes	Serranidae	<i>Cephalopholis sommerati</i>	Tomato hind	Indo Pacific	KU668634	MH085806	MH190804
	Drepanidae	<i>Drepane punctata</i>	Spotted sickle fish	Indo-West Pacific	KM273123	MH085841	MH085677
	Acanthuridae	<i>Acanthurus bariene</i>	Black-spot surgeonfish	Indo-West Pacific	KF009560	MH085850	MH085682
		<i>Pomacanthus annularis</i>	Bluering angelfish	Indo-West Pacific	FJ583876	MH085785	MH085679
	Pomacanthidae	<i>Pomacanthus semicirculatus</i>	Semicircle angelfish	Indo-West Pacific	FJ583886	MH085786	MH085680
		<i>Sphyræna pacifica</i>	Sawtooth barracuda	Indo-West Pacific	KC970510	MH085781	MH085673
	Balistidae	<i>Stiffliæna chrysoptera</i>	Halfmoon triggerfish	Indo-West Pacific	FJ584131	MH085791	MH190805
		<i>Canthidermis maculata</i>	Rough triggerfish	Western Pacific	AP009206	MH085790	MH085689
	Scombridae	<i>Scomber australasicus</i>	Blue mackerel	Indo-West Pacific	KX781882	MH085913	MH085694
		<i>Sarda orientalis</i>	Striped bonito	Indo Pacific	KX781833	MH085916	MH085692
		<i>Euthynnus affinis</i>	Kawakawa	Indo-West Pacific	KX768124	MH085691	MH085691
		<i>Katsuwonus pelamis</i>	Skipjack tuna	Worldwide	KF597042	MH085920	MH085683
		<i>Auxis thazard</i>	Frigate tuna	Atlantic, Indian and Pacific (Western central)	KM055419	MH190813	MH190806
	Priacanthidae	<i>Priacanthus tayenus</i>	Purple-spotted bigeye	Indo-West Pacific	KT985639	MH085759	MH085676
		<i>Lutjanus erythropterus</i>	Crimson snapper	Indo-West Pacific	KP939271	MH085859	MH085669
	Lutjanidae	<i>Lutjanus gibbus</i>	Humpback red snapper	Indo Pacific	MF409615	MH190812	MH085686
		<i>Lutjanus bengalensis</i>	Bengal snapper	Indo-West Pacific	FJ171339	MH085862	MH085668
		<i>Lutjanus notus</i>	Blue striped snapper	Western Indian Ocean	JF483844	MH190812	MH085688
		<i>Scolopsis ciliata</i>	Saw-jawed monocle bream	Indo-West Pacific	KY362946	MH085856	MH085685
<i>Ariete mate</i>		Yellowtail scad	Indo Pacific	KU170601	MH085895	MH085672	
Carangidae		<i>Decapterus macarellus</i>	Mackerel scad	Western Atlantic, Global	KY371379	MH085882	MH085695
		<i>Decapterus maruadsi</i>	Japanese scad	Indo-West Pacific	KX610924	MH085880	MH085675
		<i>Alecis indicus</i>	Indian threadfish	Indo Pacific	NC037050	MH085892	MH085678
		<i>Megalaspis cordyla</i>	Torpedo scad	Indo-West Pacific	KM522836	-	-
Scaridae		<i>Scarus niger</i>	Dusky parrotfish	Indo Pacific	KP194654	MH085810	MH085681
	<i>Cubiceps pauciradiatus</i>	Bigeye cigarfish	Atlantic, Indian, and Pacific	MP956610	MH190814	MH085697	
Nomeidae	<i>Coryphaena hippurus</i>	Common dolphinfish	Atlantic, Indian, and Pacific	AP009206	MH085771	MH085696	
	<i>Parapercis hexophthalma</i>	Speckled sand perch	Indo Pacific	MF123971	MH085798	MH085687	
Beryciformes	<i>Photoproctalis bindus</i>	Orangefin ponyfish	Indo-West Pacific	KY849543	MH085768	MH085674	
	<i>Sargocentron diadema</i>	Crown squirrelfish	Indo Pacific	JF494418	MH085901	MH085645	
	<i>Myripristis bernardi</i>	Bloch eye soldierfish	Indo-Pacific and Eastern Pacific	AP002940	MH085854	MH085670	
	<i>Myripristis adusta</i>	Shadownfin soldierfish	Indo-Pacific	KU943296	MH190811	MH085671	
Siluriformes	Ariidae	<i>Netuma thalassina</i>	Indo-West Pacific	KC569771	MH085824	MH085690	
Belontiiformes	Belontiidae	<i>Tylosurus acus</i>	Agujon needlefish	Western Atlantic	KC970513	MH085783	MH085684

Table 2. List of marine fish species detected by eDNA metabarcoding approach including read number and read proportion

Order	Family	Species name	Distribution	Identity (%)	Total read	Read proportion (%)
Anguilliformes	Muraenidae	<i>Echidna polyzona</i>	Indo-Pacific	99	27	100
		<i>Gymnothorax richardsonii</i>	Indo-Pacific	100	212	59.9
Aulopiformes	Synodontidae	<i>Saurida gracilis (unknown)</i>	Indo-Pacific	95	61	72.1
Belontiiformes	Exocoetidae	<i>Exocoetus volitans</i>	Widespread in tropical and subtropical	100	23	100
Clupeiformes	Clupeidae	<i>Herkloticichthys sp.</i>	Indo-Pacific	97	1038	86.8
	Chirocentridae	<i>Chirocentrus dorab</i>	Indo-Pacific	99	3877	86
Gonorynchiiformes	Channidae	<i>Chanos chanos</i>	Indo-Pacific	100	19	100
Mugiliformes	Mugilidae	<i>Planiliza macrolepis</i>	Indo-Pacific	100	454	73.3
		<i>Planiliza subviridis</i>	Indo-Pacific	100	451	75.6
Myctophiformes	Myctophidae	<i>Myctophum sp.</i>	Atlantic, Indian and Pacific	98	22	100
Perciformes	Tetraodontidae	<i>Tetraodonurus atlanticus (unknown)</i>	Atlantic, Indian and Pacific	95	17	100
	Apogonidae	<i>Ostorhinchus sp.</i>	Indo-Pacific	97	80	53.8
		<i>Acanthurus xanthopterus</i>	Indo-Pacific	99	23	100
	Gobiidae	<i>Istigobius ornatus</i>	Indo-Pacific	99	44	100
	Gempylidae	<i>Nealotus tripes</i>	Atlantic, Indian and Pacific	99	33	100
	Apogonidae	<i>Ostorhinchus cookii</i>	Indo-Pacific	99	25	100
		<i>Ostorhinchus taeniophorus</i>	Indo-Pacific	99	17	100
	Siganidae	<i>Siganus vermiculatus</i>	Indo-West Pacific	99	26	100
	Pomacentridae	<i>Abudefduf vaigtensis</i>	Indo-Pacific	100	104	71.2
	Chaetodontidae	<i>Chaetodon vaigabundus</i>	Indo-Pacific	100	10	100
	Nomeidae	<i>Cubiceps whiteleggii</i>	Indo-West Pacific	100	26	53.8
	Carangidae	<i>Carangoides ferdau</i>	Indo-Pacific	100	13	100
		<i>Caranx papuensis</i>	Indo-Pacific	100	29	100
		<i>Caranx tille</i>	Indo-West Pacific	100	45	100
		<i>Decapterus macarellus</i>	Circumglobal	100	331	73.1
		<i>Decapterus macrostoma</i>	Indo-Pacific and Southeast Atlantic	100	716	45.9
		<i>Decapterus smithvanizi</i>	-	100	13	100
		<i>Elagatis bipinnulata</i>	Indo-Pacific	100	84	100
Lutjanidae		<i>Lutjanus argentimaculatus</i>	Indo-Pacific	100	84	100
Menidae		<i>Mene maculata</i>	Indo-West Pacific	100	23	100
Polynemidae		<i>Polydactylus plebeius</i>	Indo-West Pacific	100	21	100
Scatophagidae		<i>Scatophagus argus</i>	Indo-Pacific	100	19	100
Symphysanodontidae		<i>Symphysanodon typus</i>	Indo-Pacific	100	58	60.3
Bramidae		<i>Taracichthys steindachneri</i>	Pacific Ocean	100	12	100
Istiophoridae		<i>Tetrapturus angustirostris</i>	Indo-Pacific and Eastern Central Pacific	100	14	100
		<i>Tetrapturus angustirostris</i>	Indian and Pacific	100	23	100

The Pondok Dadap fish landing station reported only noted for the pelagic fish caught from the Indian Ocean waters (includes the South Nusa Tenggara, the Sawu Sea, and the Western Timor Sea (Firdaus and Witomo 2014), and remaining as non-pelagic species from around Sempu Island. Here, the eDNA metabarcoding method was able to detect five tuna species (*Thunnus alalunga*, *T. obesus*, *T. maccoyii*, *T. orientalis*, and *T. thynnus*), two species bonito (*Sarda chiliensis* and *Sarda orientalis*), and two species little tunny (*Euthynnus alletteratus* and *Euthynnus affinis*), which not reported clearly in fish landing report (Table 2). Almost all Scombridae species which have been identified on the eDNA metabarcoding by DNA contamination from those fish during landing process at Pondok Dadap fish landing station. The Atlantic bluefin tuna was identified by eDNA metabarcoding which biased of this method due to the MiFish primer which developed can not able distinguished the tuna species (Miya et al. 2015, Sato et al. 2018).

The results of these studies of fish samples from traditional markets and eDNA metabarcoding, the most fish groups obtained are the Perciformes order (Figure 3). In traditional markets, the proportion of Perciformes group was 85.29%, which was higher than result from eDNA metabarcoding which was 67.27%. These fish belong to the Scombridae, Lutjanidae and Carangidae families. Perciformes group is an economically important fish such as *Thunnus albacores*, *Katsuwonus pelamis*, *Euthynnus* sp, *Istiophorus* sp., and *Scomberomerus* sp. (Firdaus and Witomo 2014). However, the fish group in the Scombridae fish family is at the top of the list of fish caught reported by the Pondok Dadap fish landing station with the highest economic value, especially for tuna fish species (Abdullah and Rehbein 2014).

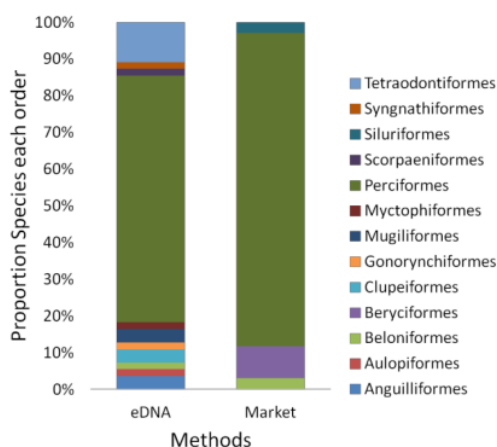


Figure 3. Comparison of species proportion each order between eDNA metabarcoding around Pondok Dadap fish landing station and fishes species sold at Sendang Biru fish market Malang, Indonesia

In the list of all species of marine fish, Perciformes are the most consumed fish and are abundant in shallow waters and pelagic fish that have high economic value (Jaafar et al. 2012). This catch is a source of food and protein for people in coastal areas of developing countries such as in Indonesia which is still very dependent on the availability of natural resources (Ferrol-Schulte et al. 2015). By understanding the potential and diversity of fish in the Pondok Dadap area of Malang, these natural resources must be appropriately managed and adequately for a better future.

In conclusion, the eDNA metabarcoding has successfully identified tropical fish in Malang Indonesia with the MiFish pipeline. This approach with eDNA provides a better picture of the types of fish that are likely to have habitats around the Pondok Dadap fish landing station. This information also complements data on reef fish species using UVC. In addition, this study also succeeded in documenting genetic information from 34 marine fish species in the COI and ITS gene regions, and 13 of them were the first entries for the ITS segment in the NCBI GenBank database. The eDNA approach is carried out quite efficiently in gathering diversity data and becoming a method that complements the results of traditional survey methods. Periodic monitoring and investigation are needed to manage fisheries resources around Pondok Dadap fish landing station. This management can be done by conducting regular surveys and collaborating between all stakeholders to preserve fisheries resources for a better future.

ACKNOWLEDGEMENTS

This work was supported by an educational grant from the LPDP BUDI-LN batch I 2016 and molecular physiology, Pukyong National University, Korea.

REFERENCES

- Abdullah A, Rehbein H. 2014. Authentication of raw and processed tuna from Indonesian markets using DNA barcoding, nuclear gene, and character-based approach. *J Euro Food Res Tech* 239: 695-706.
- Anderson-Carpenter LL, McLachlan JS, Jacson ST, Kuch M., Lumibao, CY, Poinar HN. 2011. Ancient DNA from lake sediments: bridging the gap between paleoecology and genetics. *BMC Evol Biol* 11: 30-45.
- Avisé JC. 2012. *Molecular Markers, Natural History and Evolution*, Springer Science & Business Media, New York.
- Aziz NMA, Esa Y, Arshad A. 2016. DNA barcoding and phylogenetic analysis of Malaysian groupers (Subfamily: Epinephelinae) using mitochondrial Cytochrome c oxidase I (COI) gene. *J Environ Biol* 37: 725-733.
- Badotti F, De Oliveira FS, Garcia CF, Vaz ABM, Fonseca PLC, Nahum, LA, Oliveira G, Goes-Neto A. 2017. Effectiveness of ITS and sub-regions as DNA barcode markers for the identification of Basidiomycota (Fungi). *BMC Microbiol* 17: 42.
- Bakwin CC, Mounts JH, Smith DG, Weight LA. 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.
- Das S, Deb B. 2015. DNA barcoding of fungi using Ribosomal ITS Marker for genetic diversity analysis: a review. *Intl J Pure Appl Biosci* 3: 160-167.

- De Vargas C, Bonzon M, Rees NW, Pawlowski J, Zaninetti L. 2002. A molecular approach to biodiversity and biogeography in the planktonic foraminifer *Globigerinella siphonifera* (d'Orbigny). *Mar Micropaleontol* 45: 101-116.
- Dejean T, Valentini A, Miquel C, Taberlet P, Bellemain E, Miaud C. 2012. Improved detection of an alien invasive species through environmental DNA barcoding: the example of the American bullfrog *Lithobates catesbeianus*. *J App Ecol* 49: 953-959.
- Douglas WY, Ji Y, Emerson BC, Wang X, Ye C, Yang C, Ding Z. 2012. Biodiversity soup: metabarcoding of arthropods for rapid biodiversity assessment and biomonitoring. *Methods Ecol Evol* 3: 613-623.
- Faizah R, Aisayah A. 2017. Komposisi Jenis dan Distribusi Ukuran Ikan Pelagis Besar Hasil Tangkapan Pancing Ulur di Sendang Biru, Jawa Timur. *J Bawal WRPT* 3: 377-385. [Indonesian]
- Ferrol-Schulte D, Gorris P, Baitoningsih W, Adhuri DS, Ferse SC. 2015. Coastal livelihood vulnerability to marine resource degradation: A review of the Indonesian national coastal and marine policy framework. *Mar Pol* 52: 163-171.
- Firdaus M, Witomo CM. 2014. Analisis tingkat kesejahteraan dan ketimpangan pendapatan rumah tangga nelayan pelagis besar di Sendang Biru, Kabupaten Malang, Jawa Timur. *J Sosek Kelautan dan Perikanan* 9: 155-168. [Indonesian]
- Footo AD, Thomsen PF, Sveegaard S, Wahlberg M, Kielgast J, Kyhn LA, Salling AB, Galatius A, Orlando L, Gilbert MTP. 2012. Investigating the potential use of environmental DNA (eDNA) for genetic monitoring of marine mammals. *PLoS One* 7: e41781.
- Giusti A, Armani A, Sotelo CG. 2017. Advances in the analysis of complex food matrices: Species identification in surimi-based products using Next Generation Sequencing technologies. *PLoS One* 12: 1-18.
- 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. *J AOAC Intl* 94: 201-210.
- Hebert PD, Cywinska A, Ball SL, Dewaard JR. 2003. Biological identifications through DNA barcodes. *Proc R Soc London B Biol Sci* 270: 313-321.
- Hermawan D. 2006. The prospective of Sendang Biru coastal zone development for integrated fisheries industry. *J. Prot.* 13 (2): 203-210.
- Hubert N, Hanner R. 2015. DNA barcoding, species delineation and taxonomy: a historical perspective. *DNA barcodes*, 3, 44-58.
- Ikeda K, Doi H, Tanaka K, Kawai T, Negishi JN. 2016. Using environmental DNA to detect an endangered crayfish *Cambaroides japonicus* in streams. *Con Gen Res* 8: 231-234.
- Jaafar TNAM, Taylor MI, Nor SAM, De Bruyn M, Carvalho GR. 2012. DNA barcoding reveals cryptic diversity within commercially exploited Indo-Malay Carangidae (Teleostei: Perciformes). *PLoS One* 7: e49623.
- Jerde CL, Mahon AR, Chadderton WL, Lodge DM. 2011. "Sight-unseen" detection of rare aquatic species using environmental DNA. *Con Lett* 4: 150-157.
- Karp A, Edwards KJ, Bruford M, Funk S, Vosman B, Morgante M, Seberg O, Kremer A, Boursot P, Arcander P. 1997. Molecular technologies for biodiversity evaluation: opportunities and challenges. *Nature biotech*, 15: 625.
- Kumar S, Stecher G, Tamura K. 2016. MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Mol Biol Evol* 33: 1870-1874.
- Lakra W, Verma M, Goswami M, Lal KK, Mohindra V, Punia P, Gopalakrishnan A, Singh K, Ward RD, Hebert P. 2011. DNA barcoding Indian marine fishes. *Mol Ecol Res* 11: 60-71.
- Laramie MB, Pilliod DS, Goldberg CS. 2015. Characterizing the distribution of an endangered salmonid using environmental DNA analysis. *Biol Con* 183: 29-37.
- Luthfi OM, Pujarahayu P, Wahyudiarto A, Fakri SR, Sofyan M, Ramadhan F, Murian S, Tovani I, Mahmud M, Adi D. 2016. Biodiversitas dan populasi ikan karang di perairan Selat Sempu Sendang Biru Kabupaten Malang Jawa Timur. *J Kelautan: Indon J Mar Sci Tech* 9: 43-49.
- Matzen Da Silva J, Creer S, Dos Santos A, Costa A, Cunha M. 2011. Systematic and evolutionary insights derived from mtDNA COI Barcode. *PLoS One* 6 (5): e19449.
- Meyer CP, Paulay G. 2005. DNA barcoding: error rates based on comprehensive sampling. *PLoS Biol* 3: e422.
- Miya M, Sato Y, Fukunaga T, Sado T, Poulsen JY, Sato K, Minamoto T, Yamamoto S, Yamanaka H, Araki H. 2015. MiFish, a set of universal PCR primers for metabarcoding environmental DNA from fishes: detection of more than 230 subtropical marine species. *Royal Soc Open Sci* 2: 150088.
- Pepe T, Trotta M, Di Marco I, Anastasio A, Bautista JM, Cortesi ML. 2007. Fish species identification in surimi-based products. *J Agric Food Chem* 55: 3681-3685.
- Piggott MP. 2016. Evaluating the effects of laboratory protocols on eDNA detection probability for an endangered freshwater fish. *Ecol Evol* 6: 2739-2750.
- Pilliod DS, Goldberg CS, Laramie MB, Waits LP. 2013. Application of environmental DNA for inventory and monitoring of aquatic species. US Department of the Interior, US Geological Survey, Washington, DC.
- Rees HC, Maddison BC, Middleditch DJ, Patmore JR, Gough KC. 2014. The detection of aquatic animal species using environmental DNA—a review of eDNA as a survey tool in ecology. *J Appl Ecol* 51: 1450-1459.
- Roussel JM, Paillisson JM, Treguier A, Petit E. 2015. The downside of eDNA as a survey tool in water bodies. *J App Ecol* 52: 823-826.
- Sato H, Sogo Y, Doi H, Yamanaka H. 2017. Usefulness and limitations of sample pooling for environmental DNA metabarcoding of freshwater fish communities. *Sci Rep* 7: 14860.
- Sato Y, Miya M, Fukunaga T, Sado T, Iwasaki W. 2018. MitoFish and MiFish pipeline: a mitochondrial genome database of fish with an analysis pipeline for environmental DNA metabarcoding. *Mol Bio&Evo*, 35: 1553-1555.
- Smart AS, Weeks AR, Rooyen AR, Moore A, Mccarthy MA, Tingley R. 2016. Assessing the cost-efficiency of environmental DNA sampling. *Methods Ecol Evol* 7: 1291-1298.
- Taberlet P, Coissac E, Hajibabaei M, Rieseberg LH. 2012. Environmental DNA. *Mol Ecol* 21: 1789-1793.
- Takahara T, Minamoto T, Doi H. 2013. Using environmental DNA to estimate the distribution of an invasive fish species in ponds. *PLoS One* 8: e56584.
- Teletchea F. 2009. Molecular identification methods of fish species: reassessment and possible applications. *Rev Fish Biol Fish* 19: 265.
- Thomsen PF, Kielgast J, Iversen LL, Wiuf C, Rasmussen M, Gilbert MTP, Orlando L, Willerslev E. 2012. Monitoring endangered freshwater biodiversity using environmental DNA. *Mol Ecol* 21: 2565-2573.
- Udayasuriyan R, Kalpana R. 2018. DNA Barcoding of Freshwater Prawn Species of Two Genera Macrobrachium and Caridina Using mt-COI Gene. *J Gen Prot* 2017.
- Ward RD, Zemlak TS, Innes BH, Last PR, Hebert PD. 2005. DNA barcoding Australia's fish species. *Philo Trans R Soc London B Biol Sci* 360: 1847-1857.
- Wilcox TM, Mckelvey KS, Young MK, Jane SF, Lowe WH, Whiteley AR, Schwartz MK. 2013. Robust detection of rare species using environmental DNA: the importance of primer specificity. *PLoS One* 8: e59520.
- Willerslev E, Hansen AJ, Binladen J, Brand TB, Gilbert MTP, Shapiro B, Bunce M, Wiuf C, Gilchinsky DA, Cooper A. 2003. Diverse plant and animal genetic records from Holocene and Pleistocene sediments. *Science* 300: 791-795.
- Willerslev E, Hansen AJ, Poinar HN. 2004. Isolation of nucleic acids and cultures from fossil ice and permafrost. *Trends Ecol Evol* 19: 141-147.
- Yamamoto S, Masuda R, Sato Y, Sado T, Araki H, Kondoh M, Minamoto T, Miya M. 2017. Environmental DNA metabarcoding reveals local fish communities in a species-rich coastal sea. *Sci Rep* 7: 40368.
- Zhang Y. 2015. An Introduction to Python and computer programming. An Introduction to Python and Computer Programming. Springer, Dordrecht.

eDNA Malang 2019

ORIGINALITY REPORT

7%

SIMILARITY INDEX

1%

INTERNET SOURCES

6%

PUBLICATIONS

0%

STUDENT PAPERS

PRIMARY SOURCES

- 1** M. Otani, T. Oumi, S. Uwai, T. Hanyuda, R. E. Prabowo, T. Yamaguchi, H. Kawai. <1%
"Occurrence and diversity of barnacles on international ships visiting Osaka Bay, Japan, and the risk of their introduction", Biofouling, 2007
Publication
 - 2** David Osca, Valentina Tanduo, Francesco Tiralongo, Ioannis Giovos, Sara A. A. Almabruk, Fabio Crocetta, Jamila Rizgalla. <1%
"The Indo-Pacific Sergeant Abudefduf vaigiensis (Quoy & Gaimard, 1825) (Perciformes: Pomacentridae) in Libya, South-Central Mediterranean Sea", Journal of Marine Science and Engineering, 2019
Publication
 - 3** Masayuki K. Sakata, Nobutaka Maki, Hideki Sugiyama, Toshifumi Minamoto. <1%
"Identifying a breeding habitat of a critically endangered fish, *Acheilognathus typus*, in a natural river in Japan", The Science of Nature, 2017
Publication
-

4

Chen, Fenfang, Hongyu Ma, Chunyan Ma, Heng Zhang, Mengdi Zhao, Yongyong Meng, Hongqing Wei, and Lingbo Ma. "Sequencing and characterization of mitochondrial DNA genome for *Brama japonica* (Perciformes: Bramidae) with phylogenetic consideration", *Biochemical Systematics and Ecology*, 2016.

Publication

<1 %

5

M Nisa, G Mahasri, L Sulmartiwi. "Gill and skin pathology of hybrid grouper (*E. fuscoguttatus* x *E. lanceolatus*) infested *Zeylanicobdella arugamensis* worms in different infestations degree", *IOP Conference Series: Earth and Environmental Science*, 2021

Publication

<1 %

6

Thomsen, Philip Francis, Jos Kielgast, Lars Lønsmann Iversen, Peter Rask Møller, Morten Rasmussen, and Eske Willerslev. "Detection of a Diverse Marine Fish Fauna Using Environmental DNA from Seawater Samples", *PLoS ONE*, 2012.

Publication

<1 %

7

aolp.osa.org

Internet Source

<1 %

8

core.ac.uk

Internet Source

<1 %

9

Asvin P. Torres, Ferran Palero, Antonina Dos Santos, Pere Abelló, Edurne Blanco, Alexandra Boné, Guillermo Guerao. "Larval stages of the deep-sea lobster *Polycheltes typhlops* (Decapoda, Polychelida) identified by DNA analysis: morphology, systematic, distribution and ecology", Helgoland Marine Research, 2014

Publication

<1 %

10

Delphine Melayah, Zélia Bontemps, Maxime Bruto, Agnès Nguyen, Philippe Oger, Mylène Hugoni. "Chapter 2 Metabarcoding of the Three Domains of Life in Aquatic Saline Ecosystems", Springer Science and Business Media LLC, 2023

Publication

<1 %

11

Lori Lawson Handley, Daniel S. Read, Ian J. Winfield, Helen Kimbell et al. "Temporal and spatial variation in distribution of fish environmental DNA in England's largest lake", Environmental DNA, 2019

Publication

<1 %

12

Mark Y. Stoeckle, Mithun Das Mishu, Zachary Charlop-Powers. "Improved Environmental DNA Reference Library Detects Overlooked Marine Fishes in New Jersey, United States", Frontiers in Marine Science, 2020

Publication

<1 %

13

Pääbo, Svante, Hendrik Poinar, David Serre, Viviane Jaenicke-Després, Juliane Hebler, Nadin Rohland, Melanie Kuch, Johannes Krause, Linda Vigilant, and Michael Hofreiter. "Genetic Analyses from Ancient DNA", Annual Review of Genetics, 2004.

Publication

<1 %

14

Sara M. Francisco, Cristina S. Lima, Inês Moreira, Adel A. Basyouny Shahin, Abderraouf Ben Faleh. "DNA barcoding of commercially relevant marine fish species in Tunisian waters", Journal of the Marine Biological Association of the United Kingdom, 2022

Publication

<1 %

15

A. Armani, A. Giusti, L. Guardone, L. Castigliero, D. Gianfaldoni, A. Guidi. "Universal Primers Used for Species Identification of Foodstuff of Animal Origin: Effects of Oligonucleotide Tails on PCR Amplification and Sequencing Performance", Food Analytical Methods, 2015

Publication

<1 %

16

Asma Karim, Asad Iqbal, Rehan Akhtar, Muhammad Rizwan, Ali Amar, Usman Qamar, Shah Jahan. "Barcoding of fresh water fishes from Pakistan", Mitochondrial DNA, 2015

Publication

<1 %

17

Clisten Fátima Staffen, Mari Dalva Staffen, Mariana Londero Becker, Sara Emelie Löfgren et al. "DNA barcoding reveals the mislabeling of fish in a popular tourist destination in Brazil", PeerJ, 2017

Publication

<1 %

18

Jong-Oh Kim, Yong Bae Seo, Jiyoung Shin, Ji-Young Yang, Gun-Do Kim. " The complete mitochondrial genome of bobo croaker (Perciformes: Sciaenidae) ", Mitochondrial DNA Part B, 2019

Publication

<1 %

19

Nur Fadli, Zainal A. Muchlisin, Mohd N. Siti-Azizah. "DNA barcoding of commercially important groupers (Epinephelidae) in Aceh, Indonesia", Fisheries Research, 2021

Publication

<1 %

20

Renxie Wu, Haoran Zhang, Jing Liu, Sufang Niu, Yao Xiao, Yongxia Chen. "DNA barcoding of the family Sparidae along the coast of China and revelation of potential cryptic diversity in the Indo- West Pacific oceans based on COI and 16S rRNA genes", Journal of Oceanology and Limnology, 2018

Publication

<1 %

21

S. Ayyappan, J. K. Jena, A. Gopalakrishnan. "Molecular Tools for Sustainable Management

<1 %

of Aquatic Germplasm Resources of India",
Agricultural Research, 2014

Publication

22

Subhash Singh Solanki, Poonam Kashyap,
Ashutosh Vats, Vijay Kumar et al.

"Amplification, Copy Number Variation,
Expression and Association of Non-
synonymous SNP of Bovine Beta-defensin 129
Gene With Distinct Fertility of Cattle Bull",
Research Square Platform LLC, 2021

Publication

<1 %

23

A Romdon, A Sembiring, N P D Pertiwi, M D A
Malik, L Pay, Y Hehuwat, W N Satrioajie.

"Description and identification of tuna larvae
based on genetic and morphological analysis",
IOP Conference Series: Earth and
Environmental Science, 2020

Publication

<1 %

24

Céline Lavire. "Presence of *Hydrogenophilus*
thermoluteolus DNA in accretion ice in the
subglacial Lake Vostok, Antarctica, assessed
using *rrs*, *cbb* and *hox*", *Environmental*
Microbiology, 12/2006

Publication

<1 %

25

Elena Valsecchi, Jonas Bylemans, Simon J.
Goodman, Roberto Lombardi, Ian Carr, Laura
Castellano, Andrea Galimberti, Paolo Galli.
"Novel universal primers for metabarcoding

<1 %

environmental DNA surveys of marine mammals and other marine vertebrates",
Environmental DNA, 2020

Publication

26

Hideyuki Doi, Keiichi Fukaya, Shin-ichiro Oka, Keiichi Sato, Michio Kondoh, Masaki Miya. "Evaluation of detection probabilities at the water-filtering and initial PCR steps in environmental DNA metabarcoding using a multispecies site occupancy model", Scientific Reports, 2019

Publication

<1 %

27

Jianlong Li, Lori J. Lawson Handley, Lynsey R. Harper, Rein Brys et al. "Limited dispersion and quick degradation of environmental DNA in fish ponds inferred by metabarcoding", Environmental DNA, 2019

Publication

<1 %

28

McKelvey, K. S., M. K. Young, W. L. Knotek, K. J. Carim, T. M. Wilcox, T. M. Padgett-Stewart, and M. K. Schwartz. "Sampling large geographic areas for rare species using environmental DNA: a study of bull trout *Salvelinus confluentus* occupancy in western Montana : edna sampling delineates *salvelinus confluentus* range", Journal of Fish Biology, 2016.

Publication

<1 %

29

Salvador Barahona, Yassef Yuivar, Gabriel Socias, Jennifer Alcaíno, Víctor Cifuentes, Marcelo Baeza. "Identification and characterization of yeasts isolated from sedimentary rocks of Union Glacier at the Antarctica", *Extremophiles*, 2016

Publication

<1 %

30

Shaohua Gong, Yanfei Ding, Yi Wang, Guangze Jiang, Cheng Zhu. "Advances in DNA Barcoding of Toxic Marine Organisms", *International Journal of Molecular Sciences*, 2018

Publication

<1 %

31

Tapan K. Barik, Surya N. Swain, Bijayalaxmi Sahu, Bibarani Tripathy, Usha R. Acharya. "Morphological and genetic analyses of the first record of longrakered trevally, (Perciformes: Carangidae) and of the pinjalo snapper, (Perciformes: Lutjanidae) in the Odisha coast, Bay of Bengal ", *Mitochondrial DNA Part A*, 2017

Publication

<1 %

32

Thierry Comtet, Anna Sandionigi, Frédérique Viard, Maurizio Casiraghi. "DNA (meta)barcoding of biological invasions: a powerful tool to elucidate invasion processes and help managing aliens", *Biological Invasions*, 2015

<1 %

33

Tony Colin, Sertaç Kaya, Robert T. Schwarz, Marcus T. Knopp, Andreas Knopp. "Unlocking higher data volumes from space to Earth: A boost to scientific experiments on board space stations", *Acta Astronautica*, 2022

Publication

<1 %

34

Cameron, Simon J. S., Sharon Huws, Matthew J. Hegarty, Daniel P. M. Smith, and Luis A. J. Mur. "The human salivary microbiome exhibits temporal stability in bacterial diversity", *FEMS Microbiology Ecology*, 2015.

Publication

<1 %

35

Ikuo Tsushima, Yoshiyuki Shibayama, Koya Komori, Seiichiro Okamoto. "Dissolution Tests and Microbial Community Analysis Using the Bottom Sediment before and after a Heavy Storm", *Journal of Water and Environment Technology*, 2016

Publication

<1 %

36

Paula Suarez-Bregua, Miguel Álvarez-González, Kim M. Parsons, Josep Rotllant, Graham J. Pierce, Camilo Saavedra. "Environmental DNA (eDNA) for monitoring marine mammals: Challenges and opportunities", *Frontiers in Marine Science*, 2022

Publication

<1 %

Exclude quotes On

Exclude matches Off

Exclude bibliography On

eDNA Malang 2019

GRADEMARK REPORT

FINAL GRADE

/100

GENERAL COMMENTS

Instructor

PAGE 1

PAGE 2

PAGE 3

PAGE 4

PAGE 5

PAGE 6

PAGE 7

PAGE 8

PAGE 9

PAGE 10
