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Molecular identification of marine fish from two fish landing sites in Yogyakarta, Indonesia

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Abstract A total of 16 species were identified, each representing a different genera, spanning 10 families and 8 orders. Most samples exhibited genetic similarity and identity values above 97%, confirming reliable species-level identification. Interspecific genetic distances exceeded 0.1, consistent with standard thresholds for species delineation. Notably, two species were classified as near-threatened and endangered, highlighting conservation concerns. These findings demonstrated the utility of COI barcoding as a genetic tool for monitoring marine biodiversity in Indonesia. This approach is supported sustainable fisheries management and the conservation of threatened marine species in Indonesia.

Keywords: *COI,* DNA barcoding, Marine fish, Yogyakarta

Introduction

Indonesia is recognized as a megabiodiverse country with a rich diversity of marine biodiversity. Yogyakarta, a province on the island of Java, has its southern region included in Fisheries Management Area 573 (FMA 573). The dominant fish species in this area are classified as pelagic fish, including mackerel scad (*Decapterus* spp.), mackerel (*Rastrelliger* sp.), and yellowtail scad (*Selaroides* spp.) (Khatami *et al.*, 2019). Despite the high potential, fishery resource utilization in this area remains suboptimal, and several species have experienced growth overfishing. A species must have a clear and globally agreed taxonomy to make it easier for researchers to discuss a species. This study aims to identify species marine fish that caught on the coast of the Special Region of Yogyakarta using molecular approach. Current monitoring activities rely on traditional methods (such as using nets) which depend on capturing target fish species. However, these methods have a negative impact on marine ecosystems due to fishing activities, necessitating alternative, more sustainable approaches

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to conserve marine biodiversity without threatening ecosystem safety. A more sustainable monitoring approach is genetic conservation.

Traditionally, fish species identification has been based on fish morphology. This identification uses key morphological characteristics such as body shape, color pattern, size, number of scales, number of fins, and various relative measurements of fish body parts (Azrita and Syandri, 2015; Kusumanigrum *et al*., 2021). This method is limited, and less accurate, as morphological characteristics can be influenced by environmental factors. These limitations have encouraged development of new methods, such as DNA-based taxonomy. For formulating effective conservation plans and preserving genetic diversity in sampled fish, DNA barcoding is highly promising (Modeel *et al*., 2024). The application of DNA barcoding to determine the characteristics, distribution patterns, and conservation status of a sample is highly beneficial, and the data obtained tends to be reliable (Antil *et al*., 2023; Fitrian and Madduppa, 2020; Ward *et al*., 2005). A foundational step toward genetic conservation is identifying genetic diversity using molecular markers. One gene commonly utilised in DNA barcoding is the COI gene, particularly in fish taxonomy.

The cytochrome c oxidase subunit I (COI) gene serves as a reliable marker for detecting and distinguishing various fish species. Using the COI gene from DNA enables more accurate species identification compared to morphological methods (Hebert *et al*., 2003). Due to its high AT content, the COI gene has proven effective in fish taxonomy and the detection of marine biodiversity (Arisuryanti *et al*., 2024). It serves as an effective marker for assessing species relationships in waters worldwide (Wang *et al*., 2012). In this study, the species identification and genetic relationships between species were conducted using the COI gene as a genetic marker.

Materials and methods

Sample collection

The present study was conducted from April 2024 to September 2024. A total 46 individuals of fish samples were collected from fish landing sites in Depok Beach and Baron Beach. After collection, the fish samples were thoroughly cleaned. Each fish samples were placed in a ziploc bag before being stored in a coolbox and then transported to the Laboratory of Health Biotechnology at tUniversitas Kristen Duta Wacana. The right muscle pectoral fins of the samples were be cut approximately 1 cm^2, then dissected using sterilized surgical scissors. The tissue sample was placed into a 1.5 mL screw-

cap cryogenic tube and preserved in 95% alcohol. These samples were then used in the molecular analysis process.

DNA extraction, amplification, and COI fragment electrophoresis

Total genomic DNA was isolated from 30 mg pectoral fin muscle tissue from each fish specimen. This extraction step was perfomed using the Genomic DNA Mini Kit (Tissue) (Geneaid), according to the manufacturer's instructions. Using a pair of universal primers for the mitochondrial COI gene fragment, (forward (F1): 5'- TCAACCAACCACAAAGACATTGGCAC-3' and reverse (R1): 5'-TAGACTTCTGGGTGGCCAAAGAATCA-3') (Ward *et al*., 2005).The amplification was conducted using the PeqSTAR thermal cycler (Peqlab) with PCR reaction consisted of a 25 µL total reaction volume, including 12.5 µL MyTaq HS Red Mix (Bioline), 5 µL genomic DNA, 5.5 µL sterile water (ddH_2O), and 1 µL of each primer. The PCR condition consisted of an initial pre-denaturation at 94℃ for 4 min, followed by 28 cycles of denaturation at 94℃ for 45s, annealing at 50℃ for 40s, and extension at 72℃ for 40s, concluding with a final extension at 72℃ for 10 min (Zhang *et al*., 2020). ddH_2O was included as a negative control to detect DNA contaminant. The amplicons (3 µL - 5 µL) were analyzed electrophoresis using 1% gel agarose stained with florosafe (1st BASE Biochemicals). Electrophoresis was run at 100 V for 30 minutes, after which the gel was observed under ultraviolet light using a Geldoc. The experiment was conducted at Research Laboratory, Department of Biology, Biotechnology Faculty, Universitas Kristen Duta Wacana, Yogyakarta.

Sequencing and phylogeny analysis

Positive band amplification samples were sent to PT. Genetika Science Indonesia for sequencing. The DNA sequences obtained from sequencing will be edited to create an example of forward and reverse COI fragments. subsequently, alignment will be performed using BioEdit and MEGA 11 software (Tamura *et al*., 2021). All the obtained sequences of the target region were then analyzed by alignment using data available in GenBank by the BLAST system (https://blast.ncbi.nlm.nih.gov).For the phylogenetic reconstruction, we utilized a total of 46 sequences. The phylogenetic tree was constructed for COI sequence alignments using the Neighbor- Joining (NJ) methods, with the Kimura-2 Parameter (K2P) substitution model and 1000 bootstrap replications on MEGA X software.

Results

COI gene fragments (644 bp) were successfully obtained from 16 marine fish species at two landing sites of Yogyakarta. We used this 644 bp *COI* gene fragment in conducting similarity analysis using BLAST-N (https://blast.ncbi.nlm.nih.gov). All the sample sequences were showed high identity percentage value above 98% except for *Cynoglossus cynoglossus* and *Hilsa kelee* (Table 1). The phylogenetic tree showed that each sample sequence was grouped with the respective reference sequence. The 16 fish species belonged to eight families, 10 orders, and 16 Genera. The common name, taxonomic status, fish group name, habitat, IUCN conservational status, and the Genbank accession number for all the samples are shown in Table 2.

Table 1. Similiarity result based on BLAST-N of marine fish species found in two landing sites in Yogyakarta

ID	Organism species of sample sequence	Query cover	E-value	Identity (%)	Accession number
YK1	*Auxis thazard**	0.99	0	99.29	MK801690
YK2	*Megalaspis cordyla**	0.94	0	99.56	KU535567
YK3	*Eleutheronema rhadinum*	0.99	0	99.72	MW845829
YK4	*Plicofollis dussumieri*	0.94	0	99.85	JN312820
YK5	*Argyrosomus japonicus*	0.99	0	99.15	KT184692
YK6	*Pennahia macrocephalus*	0.99	0	98.15	NC_031409
YK7	*Cynoglossus cynoglossus*	0.95	0	89.28	MK572144
YK8	*Protonibea diacanthus*	0.92	0	100	JN312910
YK9	*Trichiurus lepturus nanhaiensis*	0.99	0	100	KJ202212
YK10	*Hilsa kelee**	0.99	0	89.60	AP011613

YK11	*Upeneus quadrilineatus*	0.92	0	99.85	HQ564510
YK12	*Gazza minuta*	1	0	98.00	NC_026232
YK13	*Caranx tille*	0.93	0	99.56	KU535570
YK14	*Scomberomorus guttatus*	0.96	0	99.56	OM462843
YK15	*Selar crumenophthalmus*	1	0	99.85	KY894985
YK16	*Scomber australasicus*	1	0	99.71	AB102725

*Small pelagic fish

The lowest interspecies distance was found between *Megalaspis cordyla* and *Caranx tille* (0,103) while the highest distance was found between *Gazza minuta* and *Eleutheronema tetradactylum* (0,300 (Table 3). All the species were clearly seen the cluster into different group in the Neighbour-joining tree (Figure 1).

In this study, small pelagic fish were identified from five species under three families: Carangidae, Scombridae, and Clupeidae (Figure 2). Among that species, it found that *Hilsa kelee* (YK10) was distinct nucleotide differences from several reference sequences found in Genbank. Thus, the sequence was not clustered into monophyletic clade with the references.

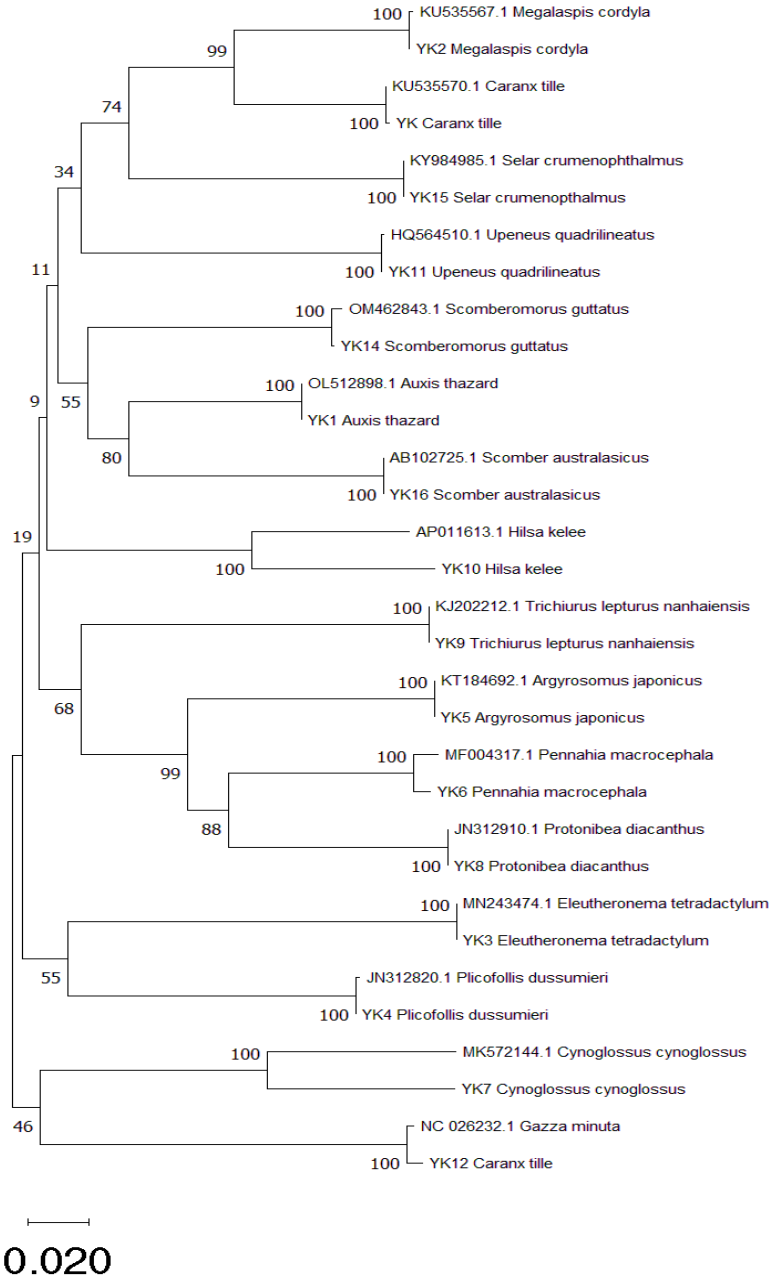

Figure 1. Neighbour-joining tree of *COI* gene fragment from 16 species of marine fish compared to reference sequences obtained from Genbank

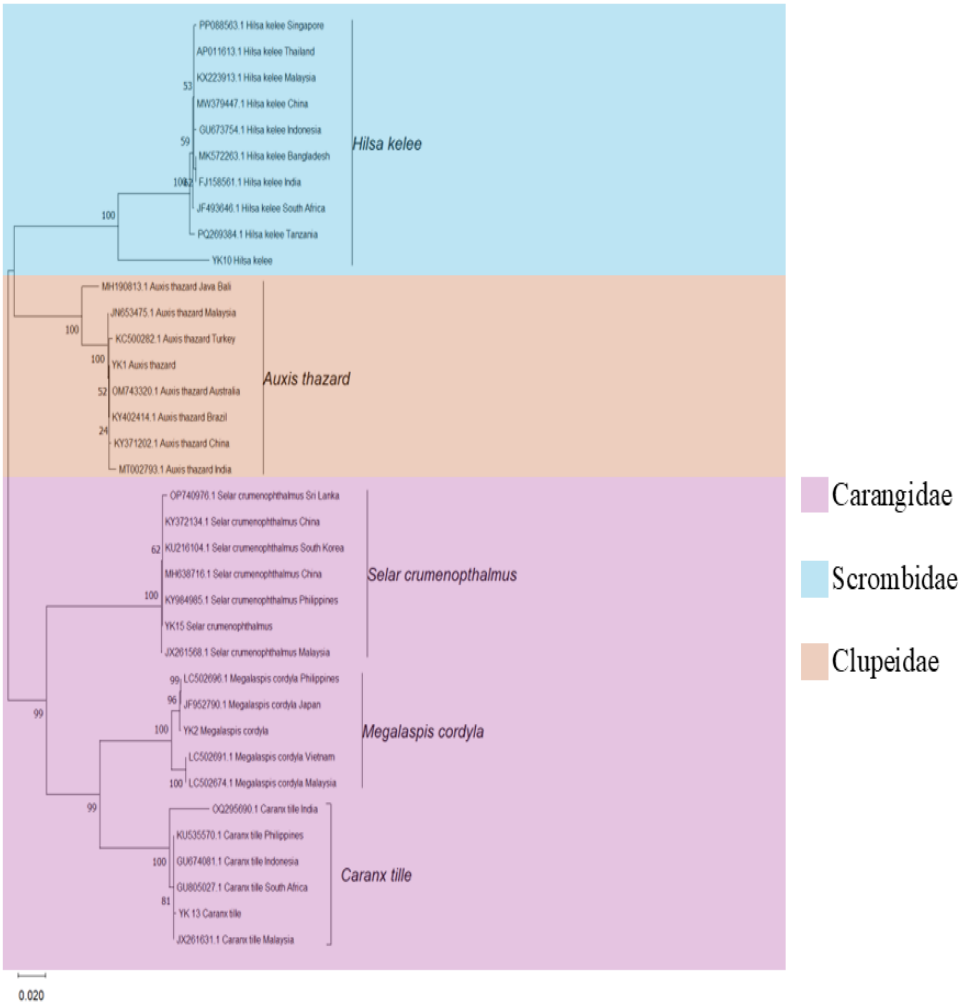

Figure 2. Phylogenetic tree of small pelagic fish consisted of three Order originated from two landing sites based on *COI* gene fragment. The tree was constructed using Neighbour-joining algorithm in MEGA X

Table 2. The list of fish species identified by COI region from fish landing sites in Depok Beach and Baron Beach, Yogyakarta

ID	Species name	Family	Order	Common name	Fish group	Habitat	IUCN status
YK1	*Auxis thazard**	Scrombridae	Scrombiformes	Frigate tuna	Small pelagic	Atlantic, Mediterranean, Indian and Pacific (Western Central)	LC
YK2	*Megalaspis cordyla**	Carangidae	Carangiformes	Torpedo scad	Small pelagic	Indo-West Pacific	LC
YK3	*Eleutheronema tetradactylum*	Polynemidae	Perciformes	East Asian four-finger threadfin	Demersal	Northwest Pacific	NE
YK4	*Plicofollis dussumieri*	Ariidae	Siluriformes	Blacktip sea catfish	Demersal	Indo-west Pacific	LC
YK5	*Argyrosomus japonicus*	Sciaenidae	Perciformes	Japanese meagre	Demersal	Indo-west Pacific	EN
YK6	*Pennahia macrocephalus*	Sciaenidae	Perciformes	Big-head pennah croaker	Demersal	Indo-west Pacific	LC
YK7	*Cynoglossus cynoglossus*	Cynoglossidae	Pleuronectiformes	Bengal tongue sole	Demersal	Indo-West Pacific	LC
YK8	*Protonibea diacanthus*	Sciaenidae	Perciformes	Blackspotted croaker	Demersal	Indo-West Pacific	NT

ID	Species name	Family	Order	Common name	Fish group	Habitat	IUCN status
YK9	*Trichiurus lepturus nanhaiensis*	Trichiuridae	Scrombriformes	Largehead hairtail	Demersal	Circumtropical and warm temperate seas	LC
YK10	*Hilsa kelee**	Clupeidae	Clupeiformes	Kelee shad	Small pelagic	Indo-West Pacific	LC
YK11	*Upeneus quadrilineatus*	Mullidae	Syngnathiformes	Four-stripe goatfish	Demersal	Indo-West Pacific	NE
YK12	*Gazza minuta*	Leiognathidae	Chaetodontiformes	Toothpony	Demersal	Indo-Pacific	LC
YK13	*Caranx tille**	Carangidae	Carangiformes	Tille trevally	Small pelagic	Indo-West Pacific	LC
YK14	*Scomberomorus guttatus*	Scombridae	Scrombriformes	Indo-Pacific king mackerel	Large pelagic	Indo-West Pacific	DD
YK15	*Selar crumenopthalmus**	Carangidae	Carangiformes	Bigeye Scad	Small pelagic	Eastern Indian Ocean and Western Central Pacific Ocean	LC
YK16	*Scomber australasicus*	Scombridae	Scrombriformes	Blue mackerel	Large pelagic	Indo-West Pacific; East Pacific	LC

*Small pelagic fish

Table 3. Nucleotides differences between species of marine fish species from two landing sites in Yogyakarta

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1																
2	0,183															
3	0,234	0,233														
4	0,206	0,207	0,216													
5	0,196	0,223	0,259	0,238												
6	0,210	0,230	0,281	0,234	0,154											
7	0,246	0,255	0,247	0,239	0,250	0,253										
8	0,192	0,230	0,252	0,230	0,155	0,134	0,266									
9	0,205	0,233	0,256	0,251	0,223	0,201	0,250	0,221								
10	0,193	0,256	0,244	0,234	0,243	0,245	0,266	0,239	0,245							
11	0,169	0,212	0,236	0,208	0,226	0,247	0,267	0,251	0,235	0,218						
12	0,224	0,237	0,300	0,224	0,273	0,259	0,245	0,274	0,246	0,272	0,250					
13	0,172	0,103	0,213	0,211	0,207	0,239	0,215	0,243	0,242	0,257	0,198	0,238				
14	0,138	0,197	0,245	0,192	0,229	0,196	0,266	0,210	0,215	0,215	0,192	0,212	0,210			
15	0,169	0,185	0,241	0,231	0,246	0,225	0,247	0,251	0,236	0,235	0,182	0,256	0,158	0,193		
16	0,132	0,220	0,265	0,233	0,206	0,209	0,270	0,233	0,203	0,234	0,197	0,253	0,214	0,173	0,194	

Discussion

In this study, 16 species of commercial and edible fish species from Yogyakarta were identified. Nowadays, the molecular identification using DNA barcoding is indispensable in the field of marine and fisheries sciences. Previous study done with marine fish mainly focus on the morphological identification on specific species for example *Decapterus macrosoma* (Kusumanigrum *et al*., 2021). Fish species were determined using *COI* gene region. This region showed higher similarity of the same species than compared to the sequences sampled from different species (Andriyono *et al*., 2022; Ward *et al*., 2005). The genetic distance between different species (intrerspecies) was more than 0.1, similar with the average of previous results (Hebert *et al*., 2004; Li *et al*., 2025; Ward *et al*., 2008).

The species based on genetic similarity and identity using BLAST-N was identified. Most of our samples showed higher in both values than 97% which are predetermined as species border (Riani *et al*., 2021). In addition, high sequence similarity value ranging from 98-100% is one of the most accurate confirmations of the success of the barcoding approaches (Alcantara and Yambot, 2016; Bhattacharjee *et al*., 2012; Cerutti-Pereyra *et al*., 2012). Comparison to available DNA sequences in the database and the genetic distance analysis conducted in this research confirms the utility of the COI gene in the precise identification of marine fish species in Yogyakarta. Previous study for marine species from Kutaradja Port, Aceh, Indonesia successfully identified 37 species of fish (Andriyono *et al*., 2022).

However, this study is lacking in information about population structure which may supported the conservation of fish species. Although most species were identified as shown in the list of IUCN as LC category. In addition, there are also fish species that are categorized as Not Evaluated (NE), Data deficient (DD), Near Threatened (NT; for example *Protonibea diacanthus*) and even Endangered (EN; for example *Argyrosomus japonicus*). Thus, the research on marine fish species in Indonesia need to be expanded. The improvement including accurate species identification could lead to better fisheries management and conservation. In the future study, COI and additional barcoding marker will be employed to evaluate the intraspecies genetic diversity. The study would be very useful for conservation and fisheries management in Indonesia.

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Conflict of interest

The authors declare that they have no conflict of interest regarding the publication of this research article.

References

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

Andriyono, S., Damora, A. and Kim, H. W. (2022). Molecular identification and phylogenetic tree reconstruction of marine fish species from the fishing port of Kutaradja, Banda Aceh. Journal of Tropical Biodiversity and Biotechnology, 7:71955. https://doi.org/10.22146/jtbb.71955

Antil, S., Abraham, J. S., Sripoorna, S., Maurya, S., Dagar, J., Makhija, S., Bhagat, P., Gupta, R., Sood, U., Lal, R. and Toteja, R. (2023). DNA barcoding, an effective tool for species identification: A review. Molecular Biology Reports, 50:761-775. https://doi.org/10.1007/s11033-022-08015-7

Arisuryanti, T., Waskito A. K., Shabrina, F. N., Febriyanti, D., Daryono, B. S. and Priyono, D. S. (2024). Phylogenetic and genetic variation of common mudskippers (*Periophthalmus kalolo* Lesson, 1831) from the southern coast of Java, Indonesia inferred from the COI mitochondrial gene. Journal of Genetic Engineering and Biotechnology, 22:100335. https://doi.org/10.1016/j.jgeb.2023.100335

Azrita and Syandri, H. (2015). Morphological character among five strains of giant gourami, *Oshpronemus gouramy* Lacepede, 1801 (Actinopterygii: Perciformes: Osphronemidae) using a truss morphometric system. International Journal of Fisheries and Aquatic Studies, 2:344-350.

Bhattacharjee, M. J., Laskar, B. A., Dhar, B. and Ghosh, S. K. (2012). Identification and re-evaluation of freshwater catfishes through DNA barcoding. PLoS ONE, 7:e49950. https://doi.org/10.1371/journal.pone.0049950

Cerutti-Pereyra, F., Meekan, M. G., Wei, N. W. V., O'Shea, O., Bradshaw, C. J. A. and Austin, C. M. (2012). Identification of rays through dna barcoding: an application for ecologists. PLoS ONE, 7:e36479. https://doi.org/10.1371/journal.pone.0036479

Fitrian, T. and Madduppa, H. (2020). Determination of shortfin scad (*Decapterus macrosoma*) using morphological analysis methods and dna barcoding from Muara Baru fish market North Jakarta. Bawal Widya Riset Perikanan Tangkap, 12:127-135. http://dx.doi.org/10.15578/bawal.12.3.2020.127-135

Hebert, P. D. N., Ratnasingham, S. and De Waard, J. R. (2003). Barcoding animal life: cytochrome *c* oxidase subunit 1 divergences among closely related species. Proceedings

of the Royal Society of London. Series B: Biological Sciences, 270:S96-S99. https://doi.org/10.1098/rsbl.2003.0025

Hebert, P. D. N., Stoeckle, M. Y., Zemlak, T. S. and Francis, C. M. (2004). Identification of birds through DNA barcodes. PLoS Biology, 2:e312. https://doi.org/10.1371/journal.pbio.0020312

Khatami, A. M., Yonvitner and Setyobudiandi, I. (2019). Biological characteristics and exploitation rates of small pelagic fish in the northern waters of Java. Jurnal Ilmu dan Teknologi Kelautan Tropis, 11:637-651. https://doi.org/10.29244/jitkt.v11i3.19159 (In Indonesian).

Kusumanigrum, R. C., Alfiatunnisa, N., Murwantoko, M. and Setyobudi, E. (2021). Morphometric and meristic characteristics of the flying fish (*Decapterus macrosoma* Bleeker, 1851) on the southern coast of the Special Region of Yogyakarta, Indonesia. Jurnal Perikanan Universitas Gadjah Mada, 23:1. https://doi.org/10.22146/jfs.52348 (In Indonesian).

Li, H., Cheng, H., Huang, R., Qiu, Z. and Zhang, R. (2025). DNA barcoding of the Genus Discogobio (Teleostei, Cyprinidae) in China. Fishes, 10:157. https://doi.org/10.3390/fishes10040157

Modeel, S., Negi, R. K., Sharma, M., Dolkar, P., Yadav, S., Siwach, S., Yadav, P. and Negi, T. (2024). A comprehensive DNA barcoding of Indian freshwater fishes of the Indus River system, Beas. Scientific Reports: 14:2763. https://doi.org/10.1038/s41598-024-52519-0

Riani, S., Prabowo, R. E. and Nuryanto, A. (2021). Molecular characteristics and taxonomic status of morphologically similar barnacles (Amphibalanus) assessed using the cytochrome c oxidase 1 gene. Biodiversitas Journal of Biological Diversity, 22:1-7. https://doi.org/10.13057/biodiv/d220349

Tamura, K., Stecher, G. and Kumar, S. (2021). MEGA11: Molecular Evolutionary Genetics Analysis Version 11. Molecular Biology and Evolution, 38:3022-3027. https://doi.org/10.1093/molbev/msab120

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

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

Ward, R. D., Holmes, B. H., White, W. T. and Last, P. R. (2008). DNA barcoding Australasian chondrichthyans: Results and potential uses in conservation. Marine and Freshwater Research, 59:57-71. https://doi.org/10.1071/MF07148

Zhang, L., Zhang, J., Song, P., Liu, S., Liu, P., Liu, C., Lin, L. and Li, Y. (2020). Reidentification of *Decapterus macarellus* and *D. macrosoma* (Carangidae) reveals inconsistencies with current morphological taxonomy in China. ZooKeys, 995:81-96. https://doi.org/10.3897/zookeys.995.58092

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