Bukti Korespondensi Jurnal Internasional Bereputasi terakreditasi Q3 (Ecology)

Judul : **Molecular Identification of Snapper (Perciformes: Lutjanidae) Landed at Pondokdadap Fishing Port of Sendang Biru, Malang, Indonesia**

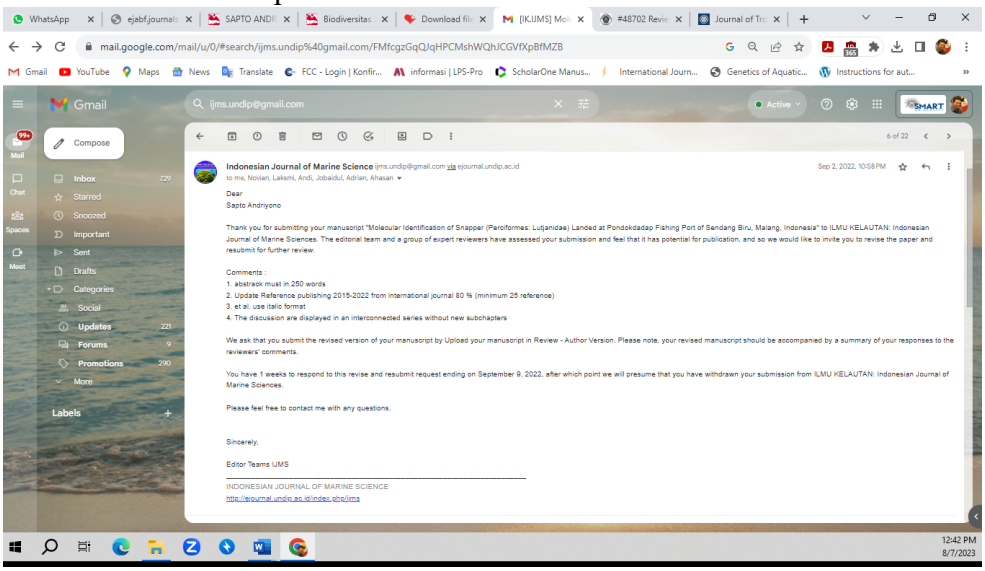
Jurnal : **ILMU KELAUTAN**

Penulis : **NOVIAN AJI PRADANA, LAKSMI SULMARTIWI, ANDI ALIAH HIDAYANI, MD. JOBAIDUL ALAM, ADRIAN DAMORA, AHASAN HABIB, SAPTO ANDRIYONO.**

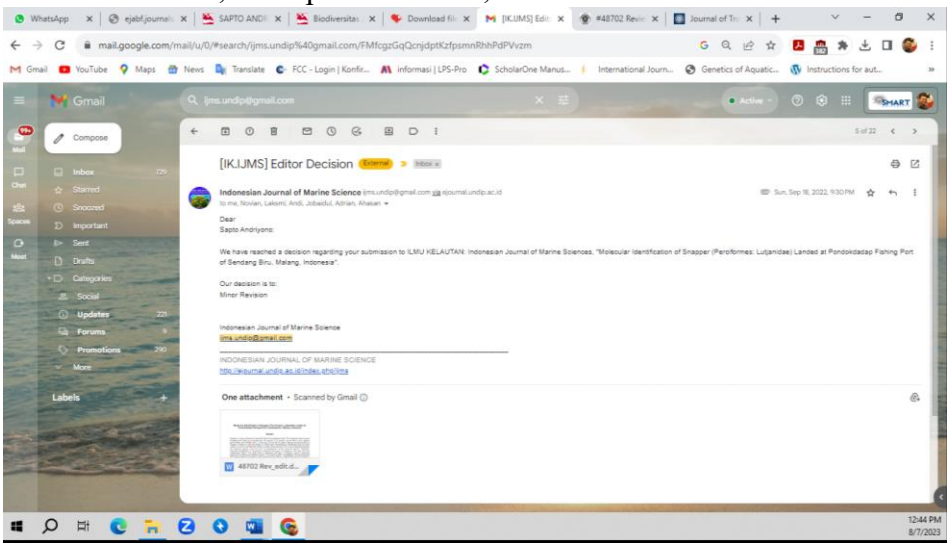
No.	Perihal	Tanggal	Keterangan
1	Bukti Submit dan Artikel yang di submit	2 September 2022	Page : 2, Lampiran 1
2	Bukti Review 1	2 September 2022	Page 2, Lampiran 2
3	Bukti Review 2	18 September 2022	Page 3, Lampiran 3
4	Bukti Proofread	25 November 2022	Page 3, Lampiran 4
5	Link Artikel terpublikasi	18 Juni 2022	Page 4, Lampiran 5

1. Bukti Submit dan Artikel yang disubmit 02 September 2022 disystem OJS

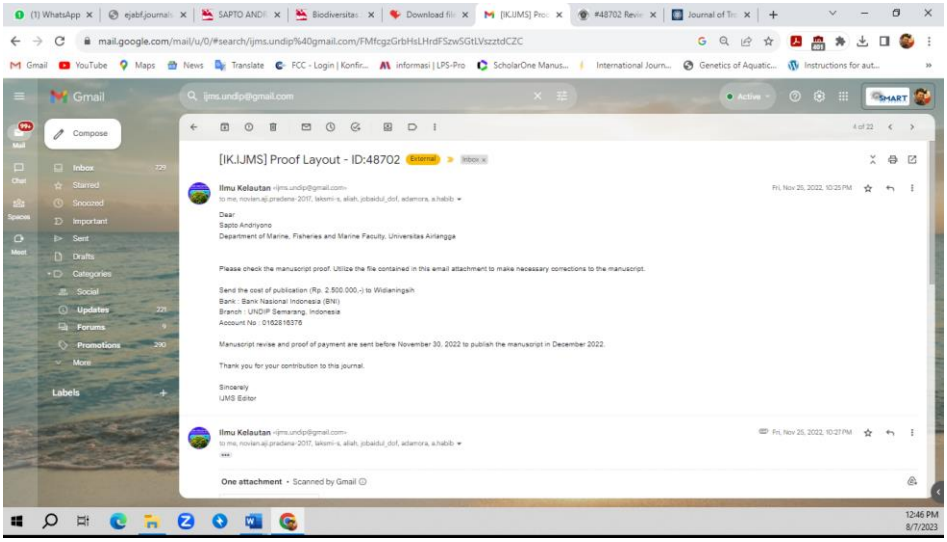
2. Review Roud 1. 2 September 2022



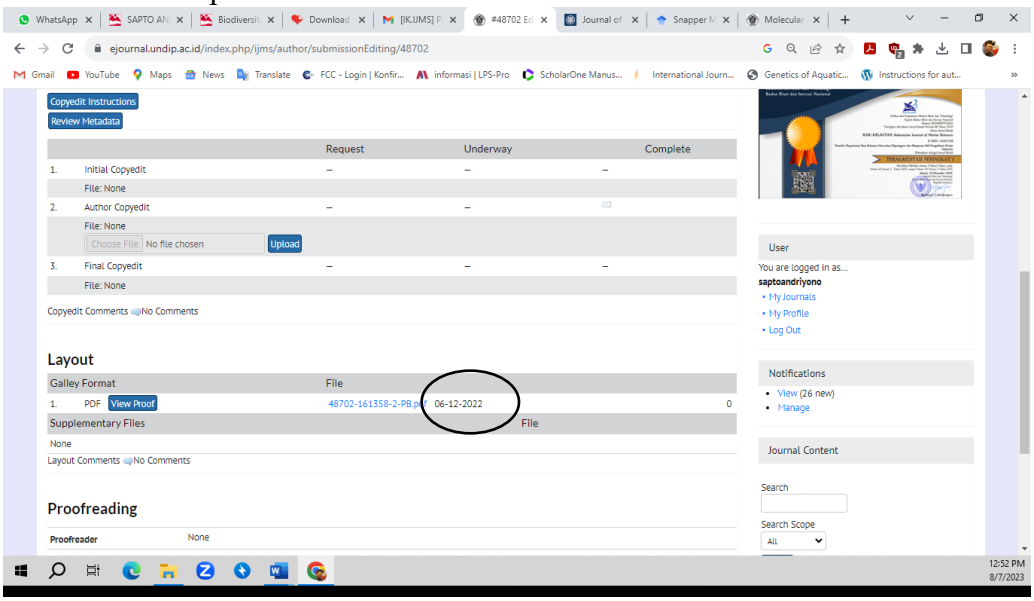
3. Review Round 2, 18 September 2022, Minor revision



4. Proofread. 25 November 2022



5. Link Artikel Terpublished 6 Desember 2022



Your article link: https://ejournal.undip.ac.id/index.php/ijms/article/view/48702

Molecular Identification of Snapper (Perciformes: Lutjanidae) Landed at Pondokdadap Fishing Port of Sendang Biru, Malang, Indonesia

Abstract

Snapper is a type of demersal marine fish from the Lutjanidae family. The Lutjanidae family spread throughout the world currently has 123 species in 21 genera, one of which is the Lutjanus genus (Miller and Thomas, 2007). To this day, the records of capture fisheries production data for snapper in Malang is still very limited to certain types. Morphological identification that has been carried out so far is still difficult to obtain accurate results because of the many similarities between the observed species and the loss of characteristics of the observed species. Therefore, molecular identification is necessary to determine the types of snappers in this area and their conservation status. This study aims to determine the types of snappers landed at Pondokdadap Fishing Port, Sendang Biru, South Malang to the species level using a molecular approach to the Cytochrome Oxidase subunit I (COI) gene marker and reconstruct the phylogenetic tree of snapper based on DNA sequence data and know their conservation status. This research method is an observation method. The nucleotide sequences in the COI gene were analyzed using Chromas, Clustal-W, Reverse-Complement and Mega X software. Phylogenetic tree reconstruction and genetic distance calculations were performed using Mega X software through the neighbour-joining (NJ) Algorithm with the addition of sequences from the NCBI database. The results of the identification of snapper based on a molecular approach with DNA barcoding revealed that the four species of snapper samples obtained were *L. gibbus, L. rufolineatus, L. bengalensis*, and *L. erythropterus*. Based on the results of the compilation of the phylogenetic tree, it can be seen that the L. bengalensis sample is closely related to *L. rufolineatus* while *L. gibbus*, and *L. erythropterus* each form a separate clade from the two previous Lutjanus species. Based on their conservation status at the IUCN, the four species of snapper found are in the Least Concern category, while based on their trading status on CITES, these four species are in the Not Evaluated category.

Keywords: diversity, gene, identification, phylogenetic, snapper

Introduction

Sendang Biru is one of the coastal areas that prioritise in efforts to manage marine fisheries resources in Malang Regency, East Java (Andriyono et al. 2019). The development makes Sendang Biru a centre for the capture industry (Aliviyanti et al. 2021). One of the fish catches obtained on this beach is snapper (Luthfi et al. 2016). Snapper is a type of demersal fish of the family Lutjanidae. The family Lutjanidae, spread throughout the world, currently has 123 species in 21 genera, one of which is the genus *Lutjanus* (Miller and Cribb 2007). Based on morphology and habitat characteristics, there are 30 species of snapper from the genus *Lutjanus* that live in Indonesian waters (Allen et al. 2013). Snapper in nature plays the role of one of the large-sized apex predatory fish that inhabit tropical coastal ecosystems around the world. Ecologically, the existence of this fish is important because it acts as a peak predator with extensive *food habits*. This fish can eat small fish, *cephalopods*, crabs, shrimps, and other benthic crustaceans to control the stability of the aquatic ecosystem in which it lives (España 2003). Snapper is also one of the capture fishery commodities which is usually used as consumption fish which is sold in the form of fresh fish, *fillets,* and processed products (Oktaviyani 2018). The production of this fish has increased every year. This follows data from the Central Statistics Agency (BPS) of Malang Regency (2020), where the total production of this fish reached 57.05 tons in 2018 and 2019 to 108.24 tons. Based on data from BPS Malang Regency (2020), recording data on the production of capture fisheries for snapper in Malang is still

very limited to certain types. This is due to the difficulty of identifying species in the field or at the time of simultaneous landing with other types of fish at the fish auction site. Identification of a species can be made morphologically as well as molecularly. Morphological identification that has been carried out so far is still difficult to obtain accurate results because of the many similarities between the observed specifications. In addition, the loss of distinctive features in observed species due to adaptation to the environment is also an obstacle in identifying a species morphologically (Prehadi et al. 2015).

One alternative to identification that can be done in addition to morphological identification is molecular identification by DNA *barcoding.* DNA *barcoding* is a globally agreed method for identifying plant and animal species based on DNA sequence variations (Coissac et al. 2016) from nitrogenous base pair regions in the *Cytochrome Oxidase* subunit I (COI) gene (Powers et al. 2018). Since its introduction in 2003, the DNA *barcoding* technique has become the *golden standard* or the main standard for molecular taxonomy (Fadli et al. 2020). DNA-based identification *barcoding* has been well received globally for its various advantages, such as being very simple and using a universal tool applicable to all organisms, both in fresh samples and processed products (Kress et al. 2015). Some examples of research that utilizes the DNA *barcoding* technique at this time include the use of DNA *barcoding* to identify fish larvae at different stages of development (Wibowo et al. 2018), identification of the discovery of new and cryptic fish species (Nurul Farhana et al. 2018) as well as identification of fish species that have similar morphological characters (Bingpeng et al. 2018).

DNA *barcoding* has been shown to be effective for identifying a species with fast and accurate results based on the *Cytochrome Oxidase* subunit I (COI) gene (Hebert et al. 2003). The COI gene is one of the protein-encoding genes found in mitochondria that has a distinctive character in each species so that it becomes a standard gene as a marker gene when identifying an animal species (Aprilia et al. 2014). The COI genes in DNA *barcoding* has two advantages, namely, this gene has a very sturdy primer so that it can recognise the 5' end of most animal species. Second, this gene has a high interspecific divergence because its molecular evolution rate is the highest and most complex than other protein-encoding genes in the mitochondria so that it can show differences between populations and individuals in one species (Vineesh et al. 2014). Therefore, research on the identification of types of snappers based on DNA markings of the COI gene needs to be carried out to provide genetic information in the form of a DNA sequence database as information material in the data design of the number of capture fisheries production of snapper based on their species and is expected to be supporting data in the management of conservation areas and fishing zones in the waters of South Malang, especially from Sendang Biru.

Materials and Methods

2.1 Sampling of Crabs

A total of 4 samples were collected from the Traditional fish market of Pondok dadap fishing port at Sendang Biru, Malang in the middle of march 2020. All samples collected from the local traditional fisherman were dead upon purchasing. The digital camera has taken the individual photograph before further treatments has been applied. Morphologically, identification and species confirmation have been carried out with molecular identification carried out in this study. No specific permit was required for this study,

2.2 DNA extraction and PCR condition

Each specimen has been collected based on the morphological characters and after collection directly preserved in 90% ethanol for further experimental purposes. Genomic DNA extracted using an Accuprep® Genomic DNA Extraction Kit (Bioneer) according to the product guidelines. The pereipod fin, around 1 cm tissues, was dissected and mix with 6X lysis buffer, which was further homogenized by the TissueLyser II (Qiagen). Quantification of purified genomic DNA performed by nanoDrop (Thermofisher Scientific D1000), aliquoted and stored at the -70^{o}C for further analysis.

One set universal fish primer targeting cytochrome c oxidase I (COI) region, BCL-BCH (Baldwin et al. 2009, Handy et al. 2011), used to obtain the partial sequences of each gene. The PCR mixture

(20µL) included 11.2 µL ultra-pure water, 1 µL primer forward and reverse (0.5 µM), 0.2 µL Ex Taq DNA polymerase (TaKaRa, Japan), 2 µL 10X ExTag Buffer, 2 µL dNTPs (1 µM, TaKaRa, Japan), and 2 µL genomic DNA as template. The PCR condition carried out under the following setting: 95^{o}C for 5 min in initial denaturation, followed by denaturation at 95^{o}C for 30 s in 40 cycles, 50^{o}C for 30 s in annealing, and 72^{o}C for 45 s in extension step, and a final extension at 72^{o}C for 5 min. The PCR products purified with the AccuPrep®Gel purification kit (Bioneer, Korea).

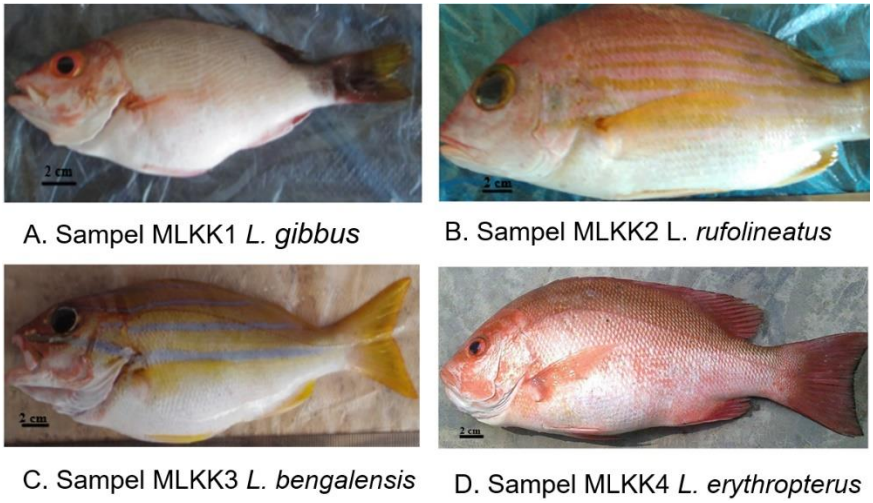
2.3 Data Analysis

All sequences were aligned to reference on GenBank database by BLASTN (https://blast.ncbi.nlm.nih.gov/Blast.cgi). The pairwise evolutionary distance among the family determined by the Kimura 2-Parameter method. The Neighbor-joining (NJ) tree constructed, and 1000 bootstrap analysis was carried by Mega X and genetic distance used a nucleotide substitution model by comparing a DNA sequence of one nucleotide with another nucleotide (Kumar et al. 2018).

Result and Discussion

3.1 Morphological Identification

The snapper samples obtained from the Sendang Biru traditional fish market were 4 snappers with different species consisting of *Lutjanus bengalengsis*, *Lutjanus rufolineatus*, *Lutjanus gibbus*, and *Lutjanus erythropterus*. The most striking difference between each species is the color and body pattern of each snapper sample (Figure 1). In addition to body color and pattern, morphological identification also observed morphometric and meristic characters in snapper samples (Table 1).



A. Sampel MLKK1 *L. gibbus*

B. Sampel MLKK2 L. *rufolineatus*

C. Sampel MLKK3 *L. bengalensis*

D. Sampel MLKK4 *L. erythropterus*

Figure 1. Snappers Landed at Pondok dadap Fishing Port on traditional fish market of Sendang South Malang.

Table 1. Morphometric and Meristic Measurements of Snapper Samples

Parameters	Total Length	Standard Length	Head Length	Tail Length

3.2 Molecular Identification

Molecular identification of snapper samples was carried out using the DNA barcoding method. The sequence data obtained were then analyzed and matched with the sequences found in GenBank at NCBI (National Center for Biotechnology Information) using BLASTN (Basic Local Alignment Search Tool Nucleotide) based on the degree of similarity (Table 2). Based on the results of the BLASTN analysis, sample MLKK1 was identified as having 96.72% similarity to the species *Lutjanus gibbus* (Humpback red snapper) access number MF409615, sample MLKK2 was identified to have 99.19% similarity to the species *Lutjanus rufolineatus* (Yellow lined snapper) access number MN870411, sample MLKK3 was identified as having 99.54% similarity to *Lutjanus bengalensis* (Bengal snapper) access number EU600137, while MLKK4 sample was identified to have 100% similarity to *Lutjanus erythropterus* (Crimson snapper) species access number GU673841.

Table 2. BLASTN Results of Snapper Samples with NCBI GenBank Database

No.	Sample Code	Species Name/ Common Name	No. Access GenBank	Identity (%)
1.	MLKK1	*Lutjanus gibbus* / Humpback red snapper	MF409615	96,72%
2.	MLKK2	*Lutjanus rufolineatus* / Yellow lined snapper	MN870411	99,19%
3.	MLKK3	*Lutjanus bengalensis* / Bengal snapper	EU600137	99,54%
4.	MLKK4	*Lutjanus erythropterus* / Crimson snapper	GU673841	100%

3.3 Phylogentic tree reconstruction

Based on the results of the phylogenetic tree reconstruction (Figure 2), samples of snapper landed at the Pondokdadap Sendang Biru Fishing Port, obtained 4 clades formed in the family Lutjanidae with the genus Lutjanus. The clade *L. bengalensis* is phylogenetically close to the clade *L. rufolineatus*, while the clade *L. gibbus* is closely related to the clade *L. erythropterus*.

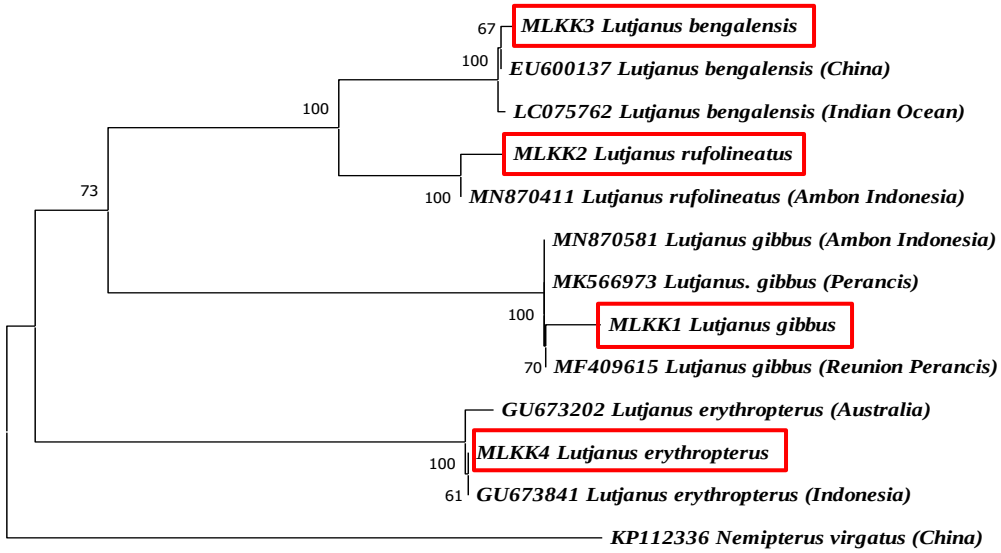


Figure 2. Reconstruction of Phylogenetic Tree based on several COI sequences of Snapper including COI sequences from the NCBI GenBank

Discussion

The diversity of potential marine fish in Indonesia needs serious attention (Suman et al. 2017). Not only in sustainable management (Atmaja and Nugroho 2017), accurate species determination is also a must in providing a valid data base at the species level. Many morphological identifications have been carried out, however, in marine fish species there are morphological similarities in both shape and color which causes confusion and inconsistency in naming fish species. In this study, apart from observing specimens based on morphological characteristics, a molecular approach was used to improve data accuracy in identification at the species level. Of the 4 specimens collected, the morphological characteristics showed that the specimens were able to be identified based on their morphometric characteristics, so that all samples were identified as *Lutjanus bengalensis, Lutjanus rufolineatus, Lutjanus gibbus,* and *Lutjanus erythropterus*. To increase accuracy in identification, we also carried out molecular identification of the COI gene section (Andriyono and Suciyono 2020) which has been agreed as a universal area for identification at the species level (Allen et al. 2013).

Morphological observations on the four samples showed that the MLKK1 sample had similarities with *Lutjanus gibbus*/ humpback red snapper or also known as jinaha snapper. The distinctive features or key morphological identification of this fish are having a compressed body shape with a grayish red body color, the caudal fin is clearly branched with dark red rounded lobes, and on the dorsal fin there are 10 hard spines and 13-14 soft spines (Thi et al. 2015). The MLKK2 sample has similarities with *Lutjanus rufolineatus*/yellow lined snapper or also known as badur snapper. The key to morphological identification of the *L. rufolineatus* species is that there are 6 yellow stripes on each side of its body, it has a pale red compressed body shape, the tail is brownish yellow, and on the dorsal fin there are 10 hard spines and 12-13 soft spines (Allen et al. 2013).

The MLKK3 sample has similarities with the *Lutjanus bengalensis*/bengal snapper species or also known as yellow snapper with a key Identification. According to Iwatsuki et al. (2016) the body of this fish is compressed, the body color is bright yellow with 4 grayish white stripes on each side of the body, there is a deep groove on the front operculum, the caudal fin is broad with a straight tip, has 11 spines and 12- 14 soft rays on the dorsal fin (Iwatsuki et al. 2016). According to Sarkar et al. (2021), the MLKK4 sample has similarities to the *Lutjanus erythropterus*/ crimson snapper or often referred to as the red snapper with the identification key in common in the form of having a pink to dark red compressed body shape from the tip of the head to the tail, the tip of the snout is slightly pointed and relatively small, the preoperculum notch is not very pronounced and on the dorsal fin there are 11 hard spines and 16-17 soft spines (Sarkar et al. 2021).

Reconstruction of the phylogenetic tree of snapper samples landed at the Pondokdadap Fishing Port, Sendang Biru, obtained 4 clades formed in the family Lutjanidae with the genus Lutjanus. The clade *L. bengalensis* is phylogenetically close to the clade *L. rufolineatus*, while the clade *L. gibbus* is closely related to the clade *L. erythropterus*. In the phylogenetic tree reconstruction, there is also a clade of the Nemipterus virgatus species as a comparison or outgroup. Phylogenetic tree analysis is an analysis that aims to compile phylogenetic relationships which are generally described in a branching line like a tree which is commonly referred to as a phylogenetic tree (Irawan 2013). Reconstruction of phylogenetic trees is supported by the results of genetic distance analysis in a species (Akbar and Labenua 2018). The results of the genetic distance analysis showed that the MLKK3 sample was close to the *L. bengalensis* EU600137 (China) and LC075762 (Indian Ocean) samples, with a genetic distance of 0.00 (zero). The MLKK2 specimen was closely related to the *L. rufolineatus* specimen MN870411 (Indonesia) and had a genetic distance of 0.01. The MLKK1 specimen was closely related to *Lutjanus gibbus* MN870581 (Ambon, Indonesia), MK566973 (France) and MF409615 (Reunion) with a genetic distance of 0.01 each. The MLKK4 specimen was closely related to *L*. *erythropterus* specimens GU673841 (Indonesia) and GU673202 (Australia) with a genetic distance of 0.00 and 0.01, respectively. Research on the Lutjanidae species in peninsular Malaysia (Malacca Strait and South China Sea) also shows that there is a variation in genetic distance (Halim et al. 2022).

Based on the conservation status that refers to the IUCN (International Union for the Conservation of Nature and Natural Resources), *L. gibbus*, *L. rufolineatus*, *L. bengalensis*, and *L. erythropterus* species are included in the Least Concern or low risk category (IUCN Red List, 2021). Least Concern is a species that has been evaluated but its status is still under the status of almost endangered or it can be said that it does not fall into any category. The IUCN conservation status categories include the category of extinction (EX), category of extinction in the wild (EW), category of critically (CR), category of threatened or critical (EN), category of vulnerable (VU), category of near threatened (NT), the category of low risk (LC) and the category of lack of information (DD) (https://www.iucnredlist.org/). Then based on their trading status according to CITES, these four snapper species are included in the Not Evaluated category, so that they are still classified as safe for international trade.

Conclusion

Based on morphological and molecular identification, the types of snappers that landed from the waters of Sendang Biru, South Malang were *Lutjanus bengalensis*, *Lutjanus rufolineatus*, *Lutjanus gibbus*, and *Lutjanus erythropterus*. Based on the results of the compilation of the phylogenetic tree, it can be seen that the *L. bengalensis* sample is closely related to *L. rufolineatus* while *L. gibbus*, and *L. erythropterus* each form a separate clade from the two previous Lutjanus species. Based on their conservation status at the IUCN, the four species of snapper found are in the Least Concern category, while based on their trading status on CITES, these four species are in the Not Evaluated category.

Acknowledgement

We would like to deliver our gratitude to the PUF Research Grant 2020 internal fund research program from Faculty of Fisheries and Marine Affairs, Universitas Airlangga has been providing support in this research. We also thank the research team colleagues who have helped in the sample collection in South Malang, East Java

Table 3. Genetic Distance of Snapper COI gene Sequences from Sendang Biru with Snapper COI gene Sequences on NCBI GenBank

No.	Name of Spesies	1	2	3	4	5	6	7	8	9	10	11	12	13
1.	MLKK1 *Lutjanus gibbus*													
2.	MN870581 *L. gibbus* (Ambon)	0,01												
3.	MK566973 *L. gibbus* (Perancis)	0,01	0,00											
4.	MF409615 *L. gibbus* (Reunion)	0,01	0,00	0,00										
5.	MLKK2 *Lutjanus rufolineatus*	0,17	0,16	0,16	0,16									
6.	MN870411 *L.rufolineatus* (Ambon)	0,16	0,15	0,15	0,15	0,01								
7.	MLKK3 *Lutjanus bengalensis*	0,17	0,16	0,16	0,16	0,07	0,06							
8.	EU600137 *L.bengalensis* (China)	0,17	0,16	0,16	0,16	0,06	0,05	0,00						
9.	LC075762 *L. bengalensis* (Indian Ocean)	0,17	0,16	0,16	0,16	0,07	0,06	0,00	0,00					
10.	MLKK4 *Lutjanus erythropterus*	0,18	0,17	0,17	0,17	0,17	0,16	0,18	0,17	0,18				
11.	GU673841 *L. erythropterus* (Australia)	0,18	0,17	0,17	0,17	0,17	0,16	0,18	0,17	0,18	0,18	0,00		
12.	GU67202 *L. erythropterus* (Malaysia)	0,19	0,18	0,18	0,18	0,18	0,17	0,19	0,18	0,19	0,19	0,01	0,01	
13.	KP112336 *Nemipterus virgatus* (China)	0,24	0,23	0,23	0,23	0,21	0,20	0,21	0,21	0,21	0,21	0,21	0,21	0,20

References

Akbar NN, Labenua R. 2018. Keragaman genetik ikan cakalang (Katsuwonus pelamis) di perairan laut Maluku Utara. Depik 7: 164-176.

Aliviyanti D, Semedi B, Yona D, Asadi MA, Kasitowati RD, Dewi CSU, Lutfi OM, Isdianto A. 2021. Upaya Penguatan Manajemen Pemasaran Hasil Perikanan Berbasis Media Online di TPI Sendangbiru, Kabupaten Malang, Indonesia. Abdi Geomedisains: 59-67.

Allen GR, Erdmann MV, Randall JE, Ching P, Rauzon MJ, Hayashi LA, Thomas M, Robertson D, Taylor L, Coste M. 2013. Reef fishes of the East Indies. Philosophy East and West 63.

Andriyono S, Suciyono S. 2020. Molecular Identification and Phylogenetic Tree Reconstruction of Marine Fish from the Essential Wetland Area of Banyuwangi, Indonesia. Egyptian Journal of Aquatic Biology and Fisheries 24: 427-439.

Andriyono S, Alam MJ, KIM H-W. 2019. Environmental DNA (eDNA) metabarcoding: Diversity study around the Pondok Dadap fish landing station, Malang, Indonesia. Biodiversitas Journal of Biological Diversity 20.

Aprilia FE, Soewondo A, Widodo N. 2014. Amplifikasi Gen COI dan 16s rRNA dari Invertebrata Laut Plakobranchus ocellatus. Biotropika: Journal of Tropical Biology 2: 276-278.

Atmaja SB, Nugroho D. 2017. Upaya-upaya pengelolaan sumber daya ikan yang berkelanjutan di Indonesia. Jurnal Kebijakan Perikanan Indonesia 3: 101-113.

Baldwin CC, Mounts JH, Smith DG, Weigt 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.

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

Coissac E, Hollingsworth PM, Lavergne S, Taberlet P. 2016. From barcodes to genomes: extending the concept of DNA barcoding: Wiley Online Library.

España HP. 2003. Ecological importance of snappers in the stability of modeled coastal ecosystems. Ecological Modelling 168: 13-24.

Fadli N, Nor SAM, Othman AS, Sofyan H, Muchlisin ZA. 2020. DNA barcoding of commercially important reef fishes in Weh Island, Aceh, Indonesia. PeerJ 8: e9641.

Halim LJ, Rahim I, Mahboob S, Al-Ghanim K, Asmiaty A, Naim DM. 2022. Phylogenetic relationships of the commercial red snapper (Lutjanidae sp.) from three marine regions. Journal of King Saud University-Science 34: 101756.

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

Hebert PD, Cywinska A, Ball SL, DeWaard JR. 2003. Biological identifications through DNA barcodes. Proceedings of the Royal Society of London. Series B: Biological Sciences 270: 313-321.

Irawan B. 2013. Karsinologi: Airlangga University Press.

Iwatsuki Y, Al-Mamry JM, Heemstra PC. 2016. Validity of a blue stripe snapper, Lutjanus octolineatus (Cuvier 1828) and a related species, L. bengalensis (Bloch 1790) with a new species (Pisces; Lutjanidae) from the Arabian Sea. Zootaxa 4098: 511-528.

Kress WJ, García-Robledo C, Uriarte M, Erickson DL. 2015. DNA barcodes for ecology, evolution, and conservation. Trends in ecology & evolution 30: 25-35.

Kumar S, Stecher G, Li M, Knyaz C, Tamura K. 2018. MEGA X: molecular evolutionary genetics analysis across computing platforms. J Molecular biology evolution 35: 1547.

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. Jurnal Kelautan: Indonesian Journal of Marine Science and Technology 9: 43-49.

Miller TL, Cribb TH. 2007. Phylogenetic relationships of some common Indo-Pacific snappers (Perciformes: Lutjanidae) based on mitochondrial DNA sequences, with comments on the taxonomic position of the Caesioninae. Molecular phylogenetics and evolution 44: 450-460.

Nurul Farhana S, Muchlisin ZA, Duong TY, Tanyaros S, Page LM, Zhao Y, Adamson EA, Khaironizam M, de Bruyn M, Siti Azizah MN. 2018. Exploring hidden diversity in Southeast Asia's Dermogenys spp.(Beloniformes: Zenarchopteridae) through DNA barcoding. Scientific Reports 8: 1-11.

Oktaviyani S. 2018. Mengenal marga Lutjanus, salah satu komoditas unggulan dalam perikanan tangkap. Oseana 43: 29-39.

Powers T, Harris T, Higgins R, Mullin P, Powers K. 2018. Discovery and Identification of Species Using COI DNA Barcoding. Journal of Nematology 50: 399-412.

Prehadi P, Sembiring A, Kurniasih EM, Rahmad R, Arafat D, Subhan B, Madduppa HH. 2015. DNA barcoding and phylogenetic reconstruction of shark species landed in Muncar fisheries landing site in comparison with Southern Java fishing port. Biodiversitas Journal of Biological Diversity 16.

Sarkar P, Islam MJ, Habib A, Neogi AK, Habib KA. 2021. Two New Records of Snapper (Perciformes, Lutjanidae) from Saint Martin's Island, Bangladesh. Journal of Ocean University of China 20: 439-444.

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

Thi OT, Ha QVD, Thuy BD. 2015. Phylogenetic relationships of emperors (Lethrinidae) and Snappers (Lutjanidae) in Vietnam based on mitochondrial DNA sequences. Pages 15-16. International Conference on Biological, Environment and Food Engineering,(BEFE-2015) Singapore May.

Vineesh N, Mohitha C, Bineesh K, Kumar RG, Gopalakrishnan A, Basheer V. 2014. DNA barcoding and taxonomic notes on the genus Macolor (Perciformes: Lutjanidae) from Indian waters.

Wibowo A, Panggabean AS, Zamroni A, Priatna A, Yusuf HN. 2018. Using DNA barcode to improve the identification of marine fish larvae, case study coastal water near Jakarta and Banda Sea, Indonesia. Indonesian Fisheries Research Journal 24: 23-30.

Molecular Identification of Snapper (Perciformes: Lutjanidae) Landed at Pondokdadap Fishing Port of Sendang Biru, Malang, Indonesia

Abstract

Snapper is a type of demersal marine fish from the Lutjanidae family. The Lutjanidae family spread throughout the world currently has 123 species in 21 genera, one of which is the Lutjanus genus (Miller and Thomas, 2007). To this day, the records of capture fisheries production data for snapper in Malang is still very limited to certain types. Morphological identification that has been carried out so far is still difficult to obtain accurate results because of the many similarities between the observed species and the loss of characteristics of the observed species. Therefore, molecular identification is necessary to determine the types of snappers in this area and their conservation status. This study aims to determine the types of snappers landed at Pondokdadap Fishing Port, Sendang Biru, South Malang to the species level using a molecular approach to the Cytochrome Oxidase subunit I (COI) gene marker and reconstruct the phylogenetic tree of snapper based on DNA sequence data and know their conservation status. This research method is an observation method. The nucleotide sequences in the COI gene were analyzed using Chromas, Clustal-W, Reverse-Complement and Mega X software. Phylogenetic tree reconstruction and genetic distance calculations were performed using Mega X software through the neighbour-joining (NJ) Algorithm with the addition of sequences from the NCBI database. The results of the identification of snapper based on a molecular approach with DNA barcoding revealed that the four species of snapper samples obtained were *L. gibbus, L. rufolineatus, L. bengalensis*, and *L. erythropterus*. Based on the results of the compilation of the phylogenetic tree, it can be seen that the L. bengalensis sample is closely related to *L. rufolineatus* while *L. gibbus*, and *L. erythropterus* each form a separate clade from the two previous Lutjanus species. Based on their conservation status at the IUCN, the four species of snapper found are in the Least Concern category, while based on their trading status on CITES, these four species are in the Not Evaluated category.

Keywords: diversity, gene, identification, phylogenetic, snapper

Introduction

Sendang Biru is one of the coastal areas that prioritise in efforts to manage marine fisheries resources in Malang Regency, East Java (Andriyono et al. 2019). The development makes Sendang Biru a centre for the capture industry (Aliviyanti et al. 2021). One of the fish catches obtained on this beach is snapper (Luthfi et al. 2016). Snapper is a type of demersal fish of the family Lutjanidae. The family Lutjanidae, spread throughout the world, currently has 123 species in 21 genera, one of which is the genus *Lutjanus* (Miller and Cribb 2007). Based on morphology and habitat characteristics, there are 30 species of snapper from the genus *Lutjanus* that live in Indonesian waters (Allen et al. 2013). Snapper in nature plays the role of one of the large-sized apex predatory fish that inhabit tropical coastal ecosystems around the world. Ecologically, the existence of this fish is important because it acts as a peak predator with extensive *food habits*. This fish can eat small fish, *cephalopods*, crabs, shrimps, and other benthic crustaceans to control the stability of the aquatic ecosystem in which it lives (España 2003). Snapper is also one of the capture fishery commodities which is usually used as consumption fish which is sold in the form of fresh fish, *fillets,* and processed products (Oktaviyani 2018). The production of this fish has increased every year. This follows data from the Central Statistics Agency (BPS) of Malang Regency (2020), where the total production of this fish reached 57.05 tons in 2018 and 2019 to 108.24 tons. Based on data from BPS Malang Regency (2020), recording data on the production of capture fisheries for snapper in Malang is still

very limited to certain types. This is due to the difficulty of identifying species in the field or at the time of simultaneous landing with other types of fish at the fish auction site. Identification of a species can be made morphologically as well as molecularly. Morphological identification that has been carried out so far is still difficult to obtain accurate results because of the many similarities between the observed specifications. In addition, the loss of distinctive features in observed species due to adaptation to the environment is also an obstacle in identifying a species morphologically (Prehadi et al. 2015).

One alternative to identification that can be done in addition to morphological identification is molecular identification by DNA *barcoding.* DNA *barcoding* is a globally agreed method for identifying plant and animal species based on DNA sequence variations (Coissac et al. 2016) from nitrogenous base pair regions in the *Cytochrome Oxidase* subunit I (COI) gene (Powers et al. 2018). Since its introduction in 2003, the DNA *barcoding* technique has become the *golden standard* or the main standard for molecular taxonomy (Fadli et al. 2020). DNA-based identification *barcoding* has been well received globally for its various advantages, such as being very simple and using a universal tool applicable to all organisms, both in fresh samples and processed products (Kress et al. 2015). Some examples of research that utilizes the DNA *barcoding* technique at this time include the use of DNA *barcoding* to identify fish larvae at different stages of development (Wibowo et al. 2018), identification of the discovery of new and cryptic fish species (Nurul Farhana et al. 2018) as well as identification of fish species that have similar morphological characters (Bingpeng et al. 2018).

DNA *barcoding* has been shown to be effective for identifying a species with fast and accurate results based on the *Cytochrome Oxidase* subunit I (COI) gene (Hebert et al. 2003). The COI gene is one of the protein-encoding genes found in mitochondria that has a distinctive character in each species so that it becomes a standard gene as a marker gene when identifying an animal species (Aprilia et al. 2014). The COI genes in DNA *barcoding* has two advantages, namely, this gene has a very sturdy primer so that it can recognise the 5' end of most animal species. Second, this gene has a high interspecific divergence because its molecular evolution rate is the highest and most complex than other protein-encoding genes in the mitochondria so that it can show differences between populations and individuals in one species (Vineesh et al. 2014). Therefore, research on the identification of types of snappers based on DNA markings of the COI gene needs to be carried out to provide genetic information in the form of a DNA sequence database as information material in the data design of the number of capture fisheries production of snapper based on their species and is expected to be supporting data in the management of conservation areas and fishing zones in the waters of South Malang, especially from Sendang Biru.

Materials and Methods

2.1 Sampling of Crabs

A total of 4 samples were collected from the Traditional fish market of Pondok dadap fishing port at Sendang Biru, Malang in the middle of march 2020. All samples collected from the local traditional fisherman were dead upon purchasing. The digital camera has taken the individual photograph before further treatments has been applied. Morphologically, identification and species confirmation have been carried out with molecular identification carried out in this study. No specific permit was required for this study,

2.2 DNA extraction and PCR condition

Each specimen has been collected based on the morphological characters and after collection directly preserved in 90% ethanol for further experimental purposes. Genomic DNA extracted using an Accuprep® Genomic DNA Extraction Kit (Bioneer) according to the product guidelines. The pereipod fin, around 1 cm tissues, was dissected and mix with 6X lysis buffer, which was further homogenized by the TissueLyser II (Qiagen). Quantification of purified genomic DNA performed by nanoDrop (Thermofisher Scientific D1000), aliquoted and stored at the -70^{o}C for further analysis.

One set universal fish primer targeting cytochrome c oxidase I (COI) region, BCL-BCH (Baldwin et al. 2009, Handy et al. 2011), used to obtain the partial sequences of each gene. The PCR mixture

(20µL) included 11.2 µL ultra-pure water, 1 µL primer forward and reverse (0.5 µM), 0.2 µL Ex Taq DNA polymerase (TaKaRa, Japan), 2 µL 10X ExTag Buffer, 2 µL dNTPs (1 µM, TaKaRa, Japan), and 2 µL genomic DNA as template. The PCR condition carried out under the following setting: 95^{o}C for 5 min in initial denaturation, followed by denaturation at 95^{o}C for 30 s in 40 cycles, 50^{o}C for 30 s in annealing, and 72^{o}C for 45 s in extension step, and a final extension at 72^{o}C for 5 min. The PCR products purified with the AccuPrep®Gel purification kit (Bioneer, Korea).

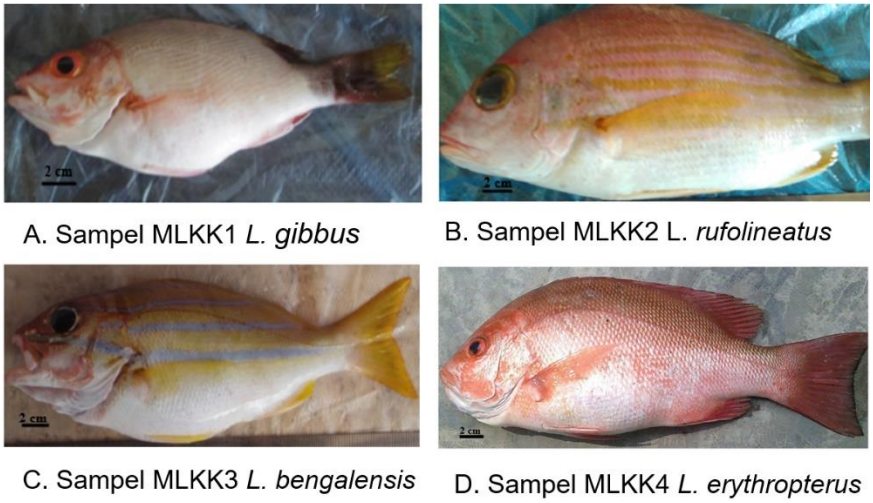
2.3 Data Analysis

All sequences were aligned to reference on GenBank database by BLASTN (https://blast.ncbi.nlm.nih.gov/Blast.cgi). The pairwise evolutionary distance among the family determined by the Kimura 2-Parameter method. The Neighbor-joining (NJ) tree constructed, and 1000 bootstrap analysis was carried by Mega X and genetic distance used a nucleotide substitution model by comparing a DNA sequence of one nucleotide with another nucleotide (Kumar et al. 2018).

Result and Discussion

3.1 Morphological Identification

The snapper samples obtained from the Sendang Biru traditional fish market were 4 snappers with different species consisting of *Lutjanus bengalengsis*, *Lutjanus rufolineatus*, *Lutjanus gibbus*, and *Lutjanus erythropterus*. The most striking difference between each species is the color and body pattern of each snapper sample (Figure 1). In addition to body color and pattern, morphological identification also observed morphometric and meristic characters in snapper samples (Table 1).



A. Sampel MLKK1 *L. gibbus*

B. Sampel MLKK2 L. *rufolineatus*

C. Sampel MLKK3 *L. bengalensis*

D. Sampel MLKK4 *L. erythropterus*

Figure 1. Snappers Landed at Pondok dadap Fishing Port on traditional fish market of Sendang South Malang.

Table 1. Morphometric and Meristic Measurements of Snapper Samples

Parameters	Total Length	Standard Length	Head Length	Tail Length

3.2 Molecular Identification

Molecular identification of snapper samples was carried out using the DNA barcoding method. The sequence data obtained were then analyzed and matched with the sequences found in GenBank at NCBI (National Center for Biotechnology Information) using BLASTN (Basic Local Alignment Search Tool Nucleotide) based on the degree of similarity (Table 2). Based on the results of the BLASTN analysis, sample MLKK1 was identified as having 96.72% similarity to the species *Lutjanus gibbus* (Humpback red snapper) access number MF409615, sample MLKK2 was identified to have 99.19% similarity to the species *Lutjanus rufolineatus* (Yellow lined snapper) access number MN870411, sample MLKK3 was identified as having 99.54% similarity to *Lutjanus bengalensis* (Bengal snapper) access number EU600137, while MLKK4 sample was identified to have 100% similarity to *Lutjanus erythropterus* (Crimson snapper) species access number GU673841.

Table 2. BLASTN Results of Snapper Samples with NCBI GenBank Database

No.	Sample Code	Species Name/ Common Name	No. Access GenBank	Identity (%)
1.	MLKK1	*Lutjanus gibbus* / Humpback red snapper	MF409615	96,72%
2.	MLKK2	*Lutjanus rufolineatus* / Yellow lined snapper	MN870411	99,19%
3.	MLKK3	*Lutjanus bengalensis* / Bengal snapper	EU600137	99,54%
4.	MLKK4	*Lutjanus erythropterus* / Crimson snapper	GU673841	100%

3.3 Phylogentic tree reconstruction

Based on the results of the phylogenetic tree reconstruction (Figure 2), samples of snapper landed at the Pondokdadap Sendang Biru Fishing Port, obtained 4 clades formed in the family Lutjanidae with the genus Lutjanus. The clade *L. bengalensis* is phylogenetically close to the clade *L. rufolineatus*, while the clade *L. gibbus* is closely related to the clade *L. erythropterus*.

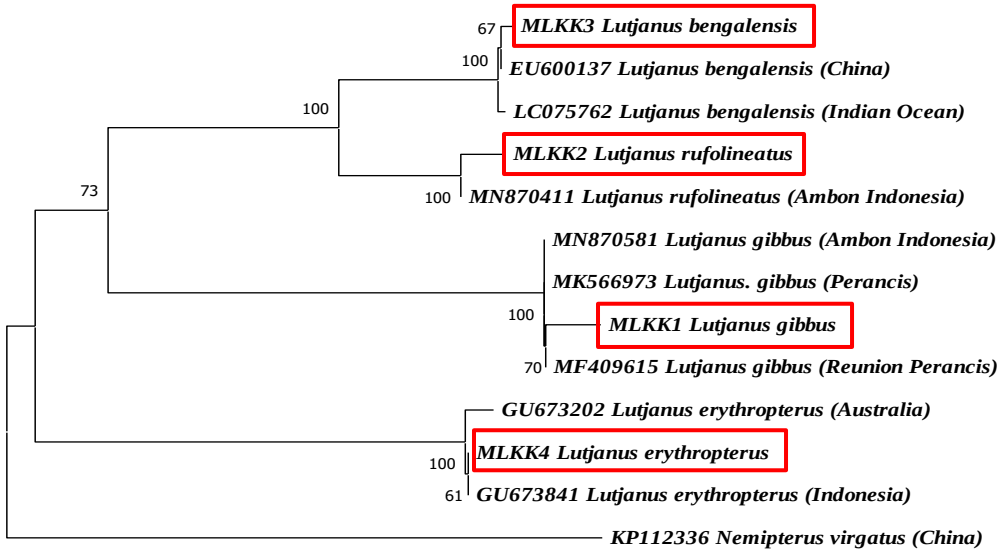


Figure 2. Reconstruction of Phylogenetic Tree based on several COI sequences of Snapper including COI sequences from the NCBI GenBank

Discussion

The diversity of potential marine fish in Indonesia needs serious attention (Suman et al. 2017). Not only in sustainable management (Atmaja and Nugroho 2017), accurate species determination is also a must in providing a valid data base at the species level. Many morphological identifications have been carried out, however, in marine fish species there are morphological similarities in both shape and color which causes confusion and inconsistency in naming fish species. In this study, apart from observing specimens based on morphological characteristics, a molecular approach was used to improve data accuracy in identification at the species level. Of the 4 specimens collected, the morphological characteristics showed that the specimens were able to be identified based on their morphometric characteristics, so that all samples were identified as *Lutjanus bengalensis, Lutjanus rufolineatus, Lutjanus gibbus,* and *Lutjanus erythropterus*. To increase accuracy in identification, we also carried out molecular identification of the COI gene section (Andriyono and Suciyono 2020) which has been agreed as a universal area for identification at the species level (Allen et al. 2013).

Morphological observations on the four samples showed that the MLKK1 sample had similarities with *Lutjanus gibbus*/ humpback red snapper or also known as jinaha snapper. The distinctive features or key morphological identification of this fish are having a compressed body shape with a grayish red body color, the caudal fin is clearly branched with dark red rounded lobes, and on the dorsal fin there are 10 hard spines and 13-14 soft spines (Thi et al. 2015). The MLKK2 sample has similarities with *Lutjanus rufolineatus*/yellow lined snapper or also known as badur snapper. The key to morphological identification of the *L. rufolineatus* species is that there are 6 yellow stripes on each side of its body, it has a pale red compressed body shape, the tail is brownish yellow, and on the dorsal fin there are 10 hard spines and 12-13 soft spines (Allen et al. 2013).

The MLKK3 sample has similarities with the *Lutjanus bengalensis*/bengal snapper species or also known as yellow snapper with a key Identification. According to Iwatsuki et al. (2016) the body of this fish is compressed, the body color is bright yellow with 4 grayish white stripes on each side of the body, there is a deep groove on the front operculum, the caudal fin is broad with a straight tip, has 11 spines and 12- 14 soft rays on the dorsal fin (Iwatsuki et al. 2016). According to Sarkar et al. (2021), the MLKK4 sample has similarities to the *Lutjanus erythropterus*/ crimson snapper or often referred to as the red snapper with the identification key in common in the form of having a pink to dark red compressed body shape from the tip of the head to the tail, the tip of the snout is slightly pointed and relatively small, the preoperculum notch is not very pronounced and on the dorsal fin there are 11 hard spines and 16-17 soft spines (Sarkar et al. 2021).

Reconstruction of the phylogenetic tree of snapper samples landed at the Pondokdadap Fishing Port, Sendang Biru, obtained 4 clades formed in the family Lutjanidae with the genus Lutjanus. The clade *L. bengalensis* is phylogenetically close to the clade *L. rufolineatus*, while the clade *L. gibbus* is closely related to the clade *L. erythropterus*. In the phylogenetic tree reconstruction, there is also a clade of the Nemipterus virgatus species as a comparison or outgroup. Phylogenetic tree analysis is an analysis that aims to compile phylogenetic relationships which are generally described in a branching line like a tree which is commonly referred to as a phylogenetic tree (Irawan 2013). Reconstruction of phylogenetic trees is supported by the results of genetic distance analysis in a species (Akbar and Labenua 2018). The results of the genetic distance analysis showed that the MLKK3 sample was close to the *L. bengalensis* EU600137 (China) and LC075762 (Indian Ocean) samples, with a genetic distance of 0.00 (zero). The MLKK2 specimen was closely related to the *L. rufolineatus* specimen MN870411 (Indonesia) and had a genetic distance of 0.01. The MLKK1 specimen was closely related to *Lutjanus gibbus* MN870581 (Ambon, Indonesia), MK566973 (France) and MF409615 (Reunion) with a genetic distance of 0.01 each. The MLKK4 specimen was closely related to *L*. *erythropterus* specimens GU673841 (Indonesia) and GU673202 (Australia) with a genetic distance of 0.00 and 0.01, respectively. Research on the Lutjanidae species in peninsular Malaysia (Malacca Strait and South China Sea) also shows that there is a variation in genetic distance (Halim et al. 2022).

Based on the conservation status that refers to the IUCN (International Union for the Conservation of Nature and Natural Resources), *L. gibbus*, *L. rufolineatus*, *L. bengalensis*, and *L. erythropterus* species are included in the Least Concern or low risk category (IUCN Red List, 2021). Least Concern is a species that has been evaluated but its status is still under the status of almost endangered or it can be said that it does not fall into any category. The IUCN conservation status categories include the category of extinction (EX), category of extinction in the wild (EW), category of critically (CR), category of threatened or critical (EN), category of vulnerable (VU), category of near threatened (NT), the category of low risk (LC) and the category of lack of information (DD) (https://www.iucnredlist.org/). Then based on their trading status according to CITES, these four snapper species are included in the Not Evaluated category, so that they are still classified as safe for international trade.

Conclusion

Based on morphological and molecular identification, the types of snappers that landed from the waters of Sendang Biru, South Malang were *Lutjanus bengalensis*, *Lutjanus rufolineatus*, *Lutjanus gibbus*, and *Lutjanus erythropterus*. Based on the results of the compilation of the phylogenetic tree, it can be seen that the *L. bengalensis* sample is closely related to *L. rufolineatus* while *L. gibbus*, and *L. erythropterus* each form a separate clade from the two previous Lutjanus species. Based on their conservation status at the IUCN, the four species of snapper found are in the Least Concern category, while based on their trading status on CITES, these four species are in the Not Evaluated category.

Acknowledgement

We would like to deliver our gratitude to the PUF Research Grant 2020 internal fund research program from Faculty of Fisheries and Marine Affairs, Universitas Airlangga has been providing support in this research. We also thank the research team colleagues who have helped in the sample collection in South Malang, East Java

Table 3. Genetic Distance of Snapper COI gene Sequences from Sendang Biru with Snapper COI gene Sequences on NCBI GenBank

No.	Name of Spesies	1	2	3	4	5	6	7	8	9	10	11	12	13
1.	MLKK1 *Lutjanus gibbus*													
2.	MN870581 *L. gibbus* (Ambon)	0,01												
3.	MK566973 *L. gibbus* (Perancis)	0,01	0,00											
4.	MF409615 *L. gibbus* (Reunion)	0,01	0,00	0,00										
5.	MLKK2 *Lutjanus rufolineatus*	0,17	0,16	0,16	0,16									
6.	MN870411 *L.rufolineatus* (Ambon)	0,16	0,15	0,15	0,15	0,01								
7.	MLKK3 *Lutjanus bengalensis*	0,17	0,16	0,16	0,16	0,07	0,06							
8.	EU600137 *L.bengalensis* (China)	0,17	0,16	0,16	0,16	0,06	0,05	0,00						
9.	LC075762 *L. bengalensis* (Indian Ocean)	0,17	0,16	0,16	0,16	0,07	0,06	0,00	0,00					
10.	MLKK4 *Lutjanus erythropterus*	0,18	0,17	0,17	0,17	0,17	0,16	0,18	0,17	0,18				
11.	GU673841 *L. erythropterus* (Australia)	0,18	0,17	0,17	0,17	0,17	0,16	0,18	0,17	0,18	0,18	0,00		
12.	GU67202 *L. erythropterus* (Malaysia)	0,19	0,18	0,18	0,18	0,18	0,17	0,19	0,18	0,19	0,19	0,01	0,01	
13.	KP112336 *Nemipterus virgatus* (China)	0,24	0,23	0,23	0,23	0,21	0,20	0,21	0,21	0,21	0,21	0,21	0,21	0,20

References

Akbar NN, Labenua R. 2018. Keragaman genetik ikan cakalang (Katsuwonus pelamis) di perairan laut Maluku Utara. Depik 7: 164-176.

Aliviyanti D, Semedi B, Yona D, Asadi MA, Kasitowati RD, Dewi CSU, Lutfi OM, Isdianto A. 2021. Upaya Penguatan Manajemen Pemasaran Hasil Perikanan Berbasis Media Online di TPI Sendangbiru, Kabupaten Malang, Indonesia. Abdi Geomedisains: 59-67.

Allen GR, Erdmann MV, Randall JE, Ching P, Rauzon MJ, Hayashi LA, Thomas M, Robertson D, Taylor L, Coste M. 2013. Reef fishes of the East Indies. Philosophy East and West 63.

Andriyono S, Suciyono S. 2020. Molecular Identification and Phylogenetic Tree Reconstruction of Marine Fish from the Essential Wetland Area of Banyuwangi, Indonesia. Egyptian Journal of Aquatic Biology and Fisheries 24: 427-439.

Andriyono S, Alam MJ, KIM H-W. 2019. Environmental DNA (eDNA) metabarcoding: Diversity study around the Pondok Dadap fish landing station, Malang, Indonesia. Biodiversitas Journal of Biological Diversity 20.

Aprilia FE, Soewondo A, Widodo N. 2014. Amplifikasi Gen COI dan 16s rRNA dari Invertebrata Laut Plakobranchus ocellatus. Biotropika: Journal of Tropical Biology 2: 276-278.

Atmaja SB, Nugroho D. 2017. Upaya-upaya pengelolaan sumber daya ikan yang berkelanjutan di Indonesia. Jurnal Kebijakan Perikanan Indonesia 3: 101-113.

Baldwin CC, Mounts JH, Smith DG, Weigt 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.

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

Coissac E, Hollingsworth PM, Lavergne S, Taberlet P. 2016. From barcodes to genomes: extending the concept of DNA barcoding: Wiley Online Library.

España HP. 2003. Ecological importance of snappers in the stability of modeled coastal ecosystems. Ecological Modelling 168: 13-24.

Fadli N, Nor SAM, Othman AS, Sofyan H, Muchlisin ZA. 2020. DNA barcoding of commercially important reef fishes in Weh Island, Aceh, Indonesia. PeerJ 8: e9641.

Halim LJ, Rahim I, Mahboob S, Al-Ghanim K, Asmiaty A, Naim DM. 2022. Phylogenetic relationships of the commercial red snapper (Lutjanidae sp.) from three marine regions. Journal of King Saud University-Science 34: 101756.

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

Hebert PD, Cywinska A, Ball SL, DeWaard JR. 2003. Biological identifications through DNA barcodes. Proceedings of the Royal Society of London. Series B: Biological Sciences 270: 313-321.

Irawan B. 2013. Karsinologi: Airlangga University Press.

Iwatsuki Y, Al-Mamry JM, Heemstra PC. 2016. Validity of a blue stripe snapper, Lutjanus octolineatus (Cuvier 1828) and a related species, L. bengalensis (Bloch 1790) with a new species (Pisces; Lutjanidae) from the Arabian Sea. Zootaxa 4098: 511-528.

Kress WJ, García-Robledo C, Uriarte M, Erickson DL. 2015. DNA barcodes for ecology, evolution, and conservation. Trends in ecology & evolution 30: 25-35.

Kumar S, Stecher G, Li M, Knyaz C, Tamura K. 2018. MEGA X: molecular evolutionary genetics analysis across computing platforms. J Molecular biology evolution 35: 1547.

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. Jurnal Kelautan: Indonesian Journal of Marine Science and Technology 9: 43-49.

Miller TL, Cribb TH. 2007. Phylogenetic relationships of some common Indo-Pacific snappers (Perciformes: Lutjanidae) based on mitochondrial DNA sequences, with comments on the taxonomic position of the Caesioninae. Molecular phylogenetics and evolution 44: 450-460.

Nurul Farhana S, Muchlisin ZA, Duong TY, Tanyaros S, Page LM, Zhao Y, Adamson EA, Khaironizam M, de Bruyn M, Siti Azizah MN. 2018. Exploring hidden diversity in Southeast Asia's Dermogenys spp.(Beloniformes: Zenarchopteridae) through DNA barcoding. Scientific Reports 8: 1-11.

Oktaviyani S. 2018. Mengenal marga Lutjanus, salah satu komoditas unggulan dalam perikanan tangkap. Oseana 43: 29-39.

Powers T, Harris T, Higgins R, Mullin P, Powers K. 2018. Discovery and Identification of Species Using COI DNA Barcoding. Journal of Nematology 50: 399-412.

Prehadi P, Sembiring A, Kurniasih EM, Rahmad R, Arafat D, Subhan B, Madduppa HH. 2015. DNA barcoding and phylogenetic reconstruction of shark species landed in Muncar fisheries landing site in comparison with Southern Java fishing port. Biodiversitas Journal of Biological Diversity 16.

Sarkar P, Islam MJ, Habib A, Neogi AK, Habib KA. 2021. Two New Records of Snapper (Perciformes, Lutjanidae) from Saint Martin's Island, Bangladesh. Journal of Ocean University of China 20: 439-444.

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

Thi OT, Ha QVD, Thuy BD. 2015. Phylogenetic relationships of emperors (Lethrinidae) and Snappers (Lutjanidae) in Vietnam based on mitochondrial DNA sequences. Pages 15-16. International Conference on Biological, Environment and Food Engineering,(BEFE-2015) Singapore May.

Vineesh N, Mohitha C, Bineesh K, Kumar RG, Gopalakrishnan A, Basheer V. 2014. DNA barcoding and taxonomic notes on the genus Macolor (Perciformes: Lutjanidae) from Indian waters.

Wibowo A, Panggabean AS, Zamroni A, Priatna A, Yusuf HN. 2018. Using DNA barcode to improve the identification of marine fish larvae, case study coastal water near Jakarta and Banda Sea, Indonesia. Indonesian Fisheries Research Journal 24: 23-30.

Artikel 4. Lampiran 3

Molecular Identification of Snapper (Perciformes: Lutjanidae) Landed at Pondokdadap Fishing Port of Sendang Biru, Malang, Indonesia

Abstract

Snapper is a type of demersal marine fish from the Lutjanidae family. The Lutjanidae family spread throughout the world and currently has 123 species in 21 genera, one of which is the Lutjanus genus (Miller and Thomas, 2007). To this day, the records of capture fisheries production data for snapper in Malang is still very limited to certain types. Morphological identification that has been carried out so far is still difficult to obtain accurate results because of the many similarities between the observed species orand the loss of characteristics of the observed species. Therefore, molecular identification is necessary to determine the types of snappers in this area and their conservation status. This study aims to determine the types of snappers using a molecular approach by Cytochrome Oxidase subunit I (COI) gene marker. Phylogenetic tree reconstruction and genetic distance calculations were performed using Mega X software through the neighbour-joining (NJ) aAlgorithm. The results of the identification of snapper based on a molecular approach with DNA barcoding revealed that the four species of snapper samples obtained were *L. gibbus, L. rufolineatus, L. bengalensis*, and *L. erythropterus*. Based on the results of the compilation of the phylogenetic tree, it can be seen that the *L. bengalensis* sample is closely related to *L. rufolineatus* while *L. gibbus*, and *L. erythropterus* each form a separate clade from the two previous *Lutjanus* species. Based on their conservation status at the IUCN, the four species of snapper found are in the Least Concern category.

Keywords: diversity, gene, identification, phylogenetic, snapper

Introduction

Sendang Biru is one of the coastal areas that prioritise in efforts to manage marine fisheries resources in Malang Regency, East Java (Andriyono *et al*. 2019). The development makes Sendang Biru a centre for the capture industry (Aliviyanti *et al*. 2021). One of the fish catches obtained on this beach is snapper (Luthfi *et al*. 2016). Snapper is a type of demersal fish of the family Lutjanidae. The family Lutjanidae, spread throughout the world, currently has 123 species in 21 genera, one of which is the genus *Lutjanus* (Miller and Cribb 2007). Based on morphology and habitat characteristics, there are 30 species of snapper from the genus *Lutjanus* that live in Indonesian waters (Allen et al. 2013, Halim Abdul et al. 2020). Snapper in nature plays the role of one of the large-sized apex predatory fish that inhabit tropical coastal ecosystems around the world. Ecologically, the existence of this fish is important because it acts as a peak predator with extensive *food habits*. This fish can eat small fish, *cephalopods*, crabs, shrimps, and other benthic crustaceans to control the stability of the aquatic ecosystem in which it lives (Simonsen *et al*. 2015). Snapper is also one of the capture fishery commodities which is usually used as consumption fish which is sold in the form of fresh fish, *fillets,* and processed products (Oktaviyani 2018). The production of this fish has increased every year. This follows data from the Central Statistics Agency (BPS) of Malang Regency (2020), where the total production of this fish reached 57.05 tons in 2018 and 2019 to 108.24 tons. Based on data from BPS Malang Regency (2020), recording data on the production of capture fisheries for snapper in Malang is still very limited to certain types. This is due to the difficulty of identifying species in the field or at the time of simultaneous landing with other types of fish at the fish auction site. Identification of a species can be made morphologically as well as molecularly. Morphological identification that has been carried out so far is still difficult to obtain accurate results because of the many similarities between the observed specifications. In addition, the loss of

distinctive features in observed species due to adaptation to the environment is also an obstacle in identifying a species morphologically (Prehadi *et al*. 2015).
One alternative to identification that can be done in addition to morphological identification is molecular identification by DNA *barcoding.* DNA *barcoding* is a globally agreed method for identifying plant and animal species based on DNA sequence variations (Coissac *et al*. 2016) from nitrogenous base pair regions in the *Cytochrome Oxidase* subunit I (COI) gene (Powers et al. 2018). Since its introduction in 2003, the DNA *barcoding* technique has become the *golden standard* or the main standard for molecular taxonomy (Fadli *et al*. 2020). DNA-based identification *barcoding* has been well received globally for its various advantages, such as being very simple and using a universal tool applicable to all organisms, both in fresh samples and processed products (Kress *et al*. 2015). Some examples of research that utilizes the DNA *barcoding* technique at this time include the use of DNA *barcoding* to identify fish larvae at different stages of development (Wibowo et al. 2018), identification of the discovery of new and cryptic fish species (Farhana *et al*. 2018) as well as identification of fish species that have similar morphological characters (Bingpeng *et al*. 2018).
DNA *barcoding* has been shown to be effective for identifying a species with fast and accurate results based on the *Cytochrome Oxidase* subunit I (COI), even specimen are larvae (Li *et al*. 2016). The COI gene is one of the protein-encoding genes found in mitochondria that has a distinctive character in each species so that it becomes a standard gene as a marker gene when identifying an animal species (Pentinsaari *et al*. 2016). The COI genes in DNA *barcoding* has two advantages, not only for species identification and metabarcoding as well (Andújar *et al*. 2018).Therefore, research on the identification of types of snappers based on DNA markings of the COI gene needs to be carried out to provide genetic information in the form of a DNA sequence database as information material in the data design of the number of capture fisheries production of snapper based on their species and is expected to be supporting data in the management of conservation areas and fishing zones in the waters of South Malang, especially from Sendang Biru.

Materials and Methods

2.1 Sampling of fish~~Crabs~~

A total of 4 samples were collected from the Traditional fish market of Pondok dadap fishing port at Sendang Biru, Malang in the middle of march 2020. All samples collected from the local traditional fisherman were dead upon purchasing. The digital camera has taken the individual photograph before further treatments has been applied. Morphologically, identification and species confirmation have been carried out with molecular identification carried out in this study. No specific permit was required for this study,

2.2 DNA extraction and PCR condition

Each specimen has been collected based on the morphological characters and ~~after collection~~ directly preserved in 90% ethanol for further experimental purposes. Genomic DNA extracted using an Accuprep® Genomic DNA Extraction Kit (Bioneer) according to the product guidelines. The pereipod fin, around 1 cm tissues, was dissected and mix with 6X lysis buffer, which was further homogenized by the TissueLyser II (Qiagen). Quantification of purified genomic DNA performed by nanoDrop (Thermofisher Scientific D1000), aliquoted and stored at the -70ºC for further analysis.
One set universal fish primer targeting cytochrome c oxidase I (COI) region, BCL-BCH were used to obtain the partial sequences of each gene (Madduppa *et al*. 2016). The PCR mixture (20µL) included 11.2 µL ultra-pure water, 1 µL primer forward and reverse (0.5 µM), 0.2 µL Ex Taq DNA polymerase (TaKaRa, Japan), 2 µL 10X ExTag Buffer, 2 µL dNTPs (1 µM, TaKaRa, Japan), and 2 µL genomic DNA as template. The PCR condition carried out under the following setting: 95ºC for 5 min in initial denaturation, followed by denaturation at 95ºC for 30 s in 40 cycles, 50ºC for 30 s in annealing, and 72ºC for 45 s in extension step, and a final extension at 72ºC for 5 min. The PCR products purified with the AccuPrep®Gel purification kit (Bioneer, Korea).

2.3 Data Analysis

Forward and reverse sequence were edited and aligned using MEGA X (REFF). All sequences were then aligned to the reference on GenBank database by BLASTN (https://blast.ncbi.nlm.nih.gov/Blast.cgi). The pairwise evolutionary distance among the family determined by the Kimura 2-Parameter method. The Neighbor-joining (NJ) tree constructed, and 1000 bootstrap analysis was carried by Mega X and genetic distance used a nucleotide substitution model by comparing a DNA sequence of one nucleotide with another nucleotide (Kumar *et al*. 2018).

Result and Discussion

3.1 Morphological Identification

The snapper samples obtained from the Sendang Biru traditional fish market were 4 snappers with different species consisting of *Lutjanus bengalengsis*, *Lutjanus rufolineatus*, *Lutjanus gibbus*, and *Lutjanus erythropterus*. The most striking difference between each species is the color and body pattern of each snapper sample (Figure 1). In addition to body color and pattern, morphological identification also observed morphometric and meristic characters in snapper samples (Table 1).

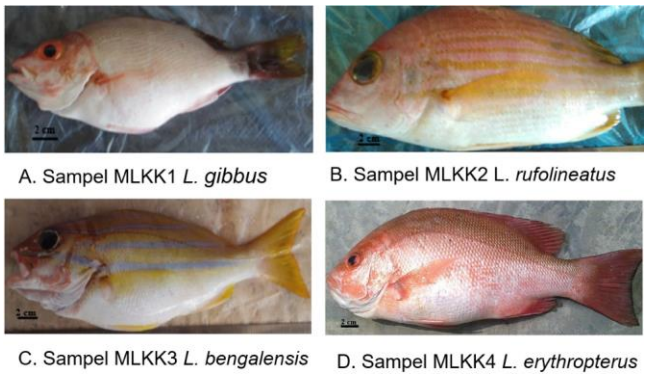


Figure 1. Four species of Snappers Landed at Pondok dadap Fishing Port on traditional fish market of Sendang South Malang.

Table 1. Morphometric and Meristic Measurements of Snapper Samples

Parameters	
Total	

3.2 Molecular Identification

Molecular identification of snapper samples was carried out using the DNA barcoding method. The sequence data obtained were then analyzed and matched with the sequences found in GenBank at NCBI (National Center for Biotechnology Information) using BLASTN (Basic Local Alignment Search Tool Nucleotide) based on the degree of similarity (Table 2). Based on the results of the BLASTN analysis, sample MLKK1 was identified as having 96.72% similarity to the species *Lutjanus gibbus* (Humpback red snapper) access number MF409615, sample MLKK2 was identified to have 99.19% similarity to the species *Lutjanus rufolineatus* (Yellow lined snapper) access number MN870411, sample MLKK3 was identified as having 99.54% similarity to *Lutjanus bengalensis* (Bengal snapper) access number EU600137, while MLKK4 sample was identified to have 100% similarity to *Lutjanus erythropterus* (Crimson snapper) species access number GU673841.

Table 2. BLASTN Results of Snapper Samples with NCBI GenBank Database

No.	Sample Code	Species Name/ Common Name	No. Access GenBank	Identity (%)
1.	MLKK1	*Lutjanus gibbus* / Humpback red snapper	MF409615	96,72%
2.	MLKK2	*Lutjanus rufolineatus* / Yellow lined snapper	MN870411	99,19%
3.	MLKK3	*Lutjanus bengalensis* / Bengal snapper	EU600137	99,54%
4.	MLKK4	*Lutjanus erythropterus* / Crimson snapper	GU673841	100%

3.3 Phylogentic tree reconstruction

Based on the results of the phylogenetic tree reconstruction (Figure 2), samples of snapper landed at the Pondokdadap Sendang Biru Fishing Port, obtained 4 clades formed in the family Lutjanidae with the genus Lutjanus. The clade *L. bengalensis* is phylogenetically close to the clade *L. rufolineatus*, while the clade *L. gibbus* is closely related to the clade *L. erythropterus*.

Commented [CD1]: Maybe you can talk a little bit about the distance between species.

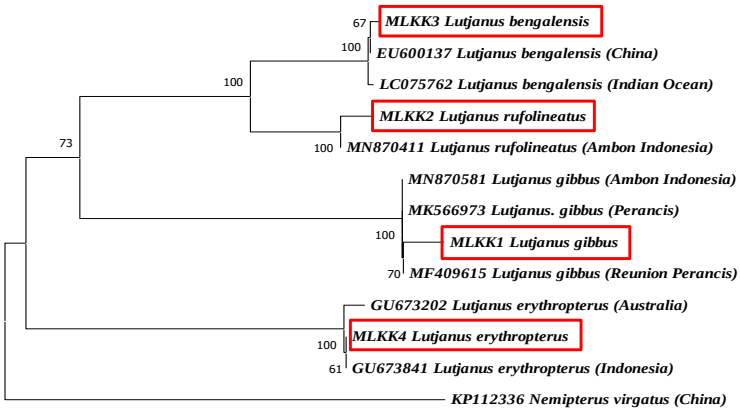


Figure 2. Reconstruction of Phylogenetic Tree based on several COI sequences of Snapper including COI sequences from the NCBI GenBank

The diversity of potential marine fish in Indonesia needs serious attention (Suman *et al*. 2017). Not only in sustainable management (Atmaja and Nugroho 2017), accurate species determination is also a must in providing a valid data base at the species level. Many morphological identifications have been carried out, however, in marine fish species there are morphological similarities in both shape and color which causes confusion and inconsistency in naming fish species. In this study, apart from observing specimens based on morphological characteristics, a molecular approach was used to improve data accuracy in identification at the species level. Of the 4 specimens collected, the morphological characteristics showed that the specimens were able to be identified based on their morphometric characteristics, so that all samples were identified as *Lutjanus bengalensis, Lutjanus rufolineatus, Lutjanus gibbus,* and *Lutjanus erythropterus*. To increase accuracy in identification, we also carried out molecular identification of the COI gene section (Andriyono and Suciyono 2020) which has been agreed as a universal area for identification at the species level.

Morphological observations on the four samples showed that the MLKK1 sample had similarities with *Lutjanus gibbus*/ humpback red snapper or also known as jinaha snapper. The distinctive features or key morphological identification of this fish are having a compressed body shape with a grayish red body color, the caudal fin is clearly branched with dark red rounded lobes, and on the dorsal fin there are 10 hard spines and 13-14 soft spines (Thi *et al*. 2015). The MLKK2 sample has similarities with *Lutjanus rufolineatus*/yellow lined snapper or also known as badur snapper. The key to morphological identification of the *L. rufolineatus* species is that there are 6 yellow stripes on each side of its body, it has a pale red compressed body shape, the tail is brownish yellow, and on the dorsal fin there are 10 hard spines and 12-13 soft spines (Allen *et al*. 2013).

The MLKK3 sample has similarities with the *Lutjanus bengalensis*/bengal snapper species or also known as yellow snapper with a key Identification. The body of this fish is compressed, the body color is bright yellow with 4 grayish white stripes on each side of the body, there is a deep groove on the front operculum, the caudal fin is broad with a straight tip, has 11 spines and 12- 14 soft rays on the dorsal fin (Iwatsuki *et al*. 2016). The MLKK4 sample has similarities to the *Lutjanus erythropterus*/ crimson snapper or often referred to as the red snapper with the identification key in common in the form of having a pink to dark red compressed body shape from the tip of the head to the tail, the tip of the snout is slightly pointed and relatively small, the preoperculum notch is not very pronounced and on the dorsal fin there are 11 hard spines and 16-17 soft spines (Sarkar *et al*. 2021).

Reconstruction of the phylogenetic tree of snapper samples landed at the Pondokdadap Fishing Port, Sendang Biru, obtained 4 clades formed in the family Lutjanidae with the genus Lutjanus. The clade *L. bengalensis* is phylogenetically close to the clade *L. rufolineatus*, while the clade *L. gibbus*

is closely related to the clade *L. erythropterus*. In the phylogenetic tree reconstruction, there is also a clade of the *Nemipterus virgatus* species as a comparison or outgroup. Reconstruction of phylogenetic trees is supported by the results of genetic distance analysis in a species (Akbar and Labenua 2018). The results of the genetic distance analysis showed that the MLKK3 sample was close to the *L. bengalensis* EU600137 (China) and LC075762 (Indian Ocean) samples, with a genetic distance of 0.00 (zero). The MLKK2 specimen was closely related to the *L. rufolineatus* specimen MN870411 (Indonesia) and had a genetic distance of 0.01. The MLKK1 specimen was closely related to *Lutjanus gibbus* MN870581 (Ambon, Indonesia), MK566973 (France) and MF409615 (Reunion) with a genetic distance of 0.01 each. The MLKK4 specimen was closely related to *L. erythropterus* specimens GU673841 (Indonesia) and GU673202 (Australia) with a genetic distance of 0.00 and 0.01, respectively. Research on the Lutjanidae species in peninsular Malaysia (Malacca Strait and South China Sea) also shows that there is a variation in genetic distance (Halim *et al*. 2022).

Based on the conservation status that refers to the IUCN (International Union for the Conservation of Nature and Natural Resources), *L. gibbus*, *L. rufolineatus*, *L. bengalensis*, and *L. erythropterus* species are included in the Least Concern or low risk category (IUCN Red List, 2021). Least Concern is a species that has been evaluated but its status is still under the status of almost endangered or it can be said that it does not fall into any category. The IUCN conservation status categories include the category of extinction (EX), category of extinction in the wild (EW), category of critically (CR), category of threatened or critical (EN), category of vulnerable (VU), category of near threatened (NT), the category of low risk (LC) and the category of lack of information (DD) (https://www.iucnredlist.org/). Then based on their trading status according to CITES, these four snapper species are included in the Not Evaluated category, so that they are still classified as safe for international trade.

Conclusion

Based on morphological and molecular identification, the types of snappers that landed from the waters of Sendang Biru, South Malang were *Lutjanus bengalensis*, *Lutjanus rufolineatus*, *Lutjanus gibbus*, and *Lutjanus erythropterus*. Based on the results of the compilation of the phylogenetic tree, it can be seen that the *L. bengalensis* sample is closely related to *L. rufolineatus* while *L. gibbus*, and *L. erythropterus* each form a separate clade from the two previous Lutjanus species. Based on their conservation status at the IUCN, the four species of snapper found are in the Least Concern category, while based on their trading status on CITES, these four species are in the Not Evaluated category.

Acknowledgement

We would like to deliver our gratitude to the PUF Research Grant 2020 internal fund research program from Faculty of Fisheries and Marine Affairs, Universitas Airlangga has been providing support in this research. We also thank the research team colleagues who have helped in the sample collection in South Malang, East Java

Table 3. Genetic Distance of Snapper COI gene Sequences from Sendang Biru with Snapper COI gene Sequences on NCBI GenBank

No.	Name of Spesies	1	2	3	4	5	6	7	8	9	10	11	12	13
1.	MLKK1 *Lutjanus gibbus*													
2.	MN870581 *L. gibbus* (Ambon)	0,01												
3.	MK566973 *L. gibbus* (Perancis)	0,01	0,00											
4.	MF409615 *L. gibbus* (Reunion)	0,01	0,00	0,00										
5.	MLKK2 *Lutjanus rufolineatus*	0,17	0,16	0,16	0,16									
6.	MN870411 *L.rufolineatus* (Ambon)	0,16	0,15	0,15	0,15	0,01								
7.	MLKK3 *Lutjanus bengalensis*	0,17	0,16	0,16	0,16	0,07	0,06							
8.	EU600137 *L.bengalensis* (China)	0,17	0,16	0,16	0,16	0,06	0,05	0,00						
9.	LC075762 *L. bengalensis* (Indian Ocean)	0,17	0,16	0,16	0,16	0,07	0,06	0,00	0,00					
10.	MLKK4 *Lutjanus erythropterus*	0,18	0,17	0,17	0,17	0,17	0,16	0,18	0,17	0,18				
11.	GU673841 *L. erythropterus* (Australia)	0,18	0,17	0,17	0,17	0,17	0,16	0,18	0,17	0,18	0,18	0,00		
12.	GU67202 *L. erythropterus* (Malaysia)	0,19	0,18	0,18	0,18	0,18	0,17	0,19	0,18	0,19	0,19	0,01	0,01	
13.	KP112336 *Nemipterus virgatus* (China)	0,24	0,23	0,23	0,23	0,21	0,20	0,21	0,21	0,21	0,21	0,21	0,21	0,20

References

Commented [RV2]: Put DOI number

Akbar NN, Labenua R. 2018. Keragaman genetik ikan cakalang (Katsuwonus pelamis) di perairan laut Maluku Utara. Depik 7: 164-176.

Aliviyanti D, Semedi B, Yona D, Asadi MA, Kasitowati RD, Dewi CSU, Lutfi OM, Isdianto A. 2021. Upaya Penguatan Manajemen Pemasaran Hasil Perikanan Berbasis Media Online di TPI Sendangbiru, Kabupaten Malang, Indonesia. Abdi Geomedisains: 59-67.

Allen GR, Erdmann MV, Randall JE, Ching P, Rauzon MJ, Hayashi LA, Thomas M, Robertson D, Taylor L, Coste M. 2013. Reef fishes of the East Indies. Philosophy East and West 63.no?? pages??

Andriyono S, Suciyono S. 2020. Molecular Identification and Phylogenetic Tree Reconstruction of Marine Fish from the Essential Wetland Area of Banyuwangi, Indonesia. Egyptian Journal of Aquatic Biology and Fisheries 24: 427-439.

Andriyono S, Alam MJ, KIM H-W. 2019. Environmental DNA (eDNA) metabarcoding: Diversity study around the Pondok Dadap fish landing station, Malang, Indonesia. Biodiversitas Journal of Biological Diversity 20.no?? pages??

Andújar C, Arribas P, Yu DW, Vogler AP, Emerson BC. 2018. Why the COI barcode should be the community DNA metabarcode for the metazoa: Wiley Online Library. pages??

Atmaja SB, Nugroho D. 2017. Upaya-upaya pengelolaan sumber daya ikan yang berkelanjutan di Indonesia. Jurnal Kebijakan Perikanan Indonesia 3: 101-113.

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

Coissac E, Hollingsworth PM, Lavergne S, Taberlet P. 2016. From barcodes to genomes: extending the concept of DNA barcoding: Wiley Online Library. pages??

Fadli N, Nor SAM, Othman AS, Sofyan H, Muchlisin ZA. 2020. DNA barcoding of commercially important reef fishes in Weh Island, Aceh, Indonesia. PeerJ 8: e9641.

Farhana NS, Muchlisin ZA, Duong TY, Tanyaros S, Page LM, Zhao Y, Adamson EA, Khaironizam M, de Bruyn M, Siti Azizah MN. 2018. Exploring hidden diversity in Southeast Asia's Dermogenys spp.(Beloniformes: Zenarchopteridae) through DNA barcoding. Scientific Reports 8: 1-11.

Halim, Rahim I, Mahboob S, Al-Ghanim K, Asmiaty A, Naim DM. 2022. Phylogenetic relationships of the commercial red snapper (Lutjanidae sp.) from three marine regions. Journal of King Saud University-Science 34: 101756.

Halim A, Loneragan NR, Wiryawan B, Hordyk AR, Sondita MFA, Yulianto I. 2020. Evaluating data-limited fisheries for grouper (Serranidae) and snapper (Lutjanidae) in the Coral Triangle, eastern Indonesia. Regional Studies in Marine Science 38: 101388.

Iwatsuki Y, Al-Mamry JM, Heemstra PC. 2016. Validity of a blue stripe snapper, Lutjanus octolineatus (Cuvier 1828) and a related species, L. bengalensis (Bloch 1790) with a new species (Pisces; Lutjanidae) from the Arabian Sea. Zootaxa 4098: 511-528.

Kress WJ, García-Robledo C, Uriarte M, Erickson DL. 2015. DNA barcodes for ecology, evolution, and conservation. Trends in ecology & evolution 30: 25-35.

Kumar S, Stecher G, Li M, Knyaz C, Tamura K. 2018. MEGA X: molecular evolutionary genetics analysis across computing platforms. J Molecular biology evolution 35: 1547.

Li Q, Li M, Li D, Shi W, Li J, Ning T, Duan Y. 2016. Identification of the Larvae of Helicoverpa assulta and H. Armigera (Lepidoptera: Noctuidae) Through Morphological Difference and DNA Barcoding. ~~Pages 306-309.~~ 3rd International Conference on Smart Materials and Nanotechnology in Engineering. Pages 306-309.

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. Jurnal Kelautan: Indonesian Journal of Marine Science and Technology 9: 43-49.

Madduppa H, Ayuningtyas RU, Subhan B, Arafat D. 2016. Exploited but unevaluated: DNA barcoding reveals skates and stingrays (Chordata, Chondrichthyes) species landed in the Indonesian fish market. IJMS (complete Journal name?) 21: 77-84.

Miller TL, Cribb TH. 2007. Phylogenetic relationships of some common Indo-Pacific snappers (Perciformes: Lutjanidae) based on mitochondrial DNA sequences, with comments on the taxonomic position of the Caesioninae. Molecular phylogenetics and evolution 44: 450-460.
Oktaviyani S. 2018. Mengenal marga Lutjanus, salah satu komoditas unggulan dalam perikanan tangkap. Oseana 43: 29-39.
Pentinsaari M, Salmela H, Mutanen M, Roslin T. 2016. Molecular evolution of a widely-adopted taxonomic marker (COI) across the animal tree of life. Scientific reports 6: 1-12.
Powers T, Harris T, Higgins R, Mullin P, Powers K. 2018. Discovery and Identification of Species Using COI DNA Barcoding. Journal of Nematology 50: 399-412.
Prehadi P, Sembiring A, Kurniasih EM, Rahmad R, Arafat D, Subhan B, Madduppa HH. 2015. DNA barcoding and phylogenetic reconstruction of shark species landed in Muncar fisheries landing site in comparison with Southern Java fishing port. Biodiversitas Journal of Biological Diversity 16. no?? pages???
Sarkar P, Islam MJ, Habib A, Neogi AK, Habib KA. 2021. Two New Records of Snapper (Perciformes, Lutjanidae) from Saint Martin's Island, Bangladesh. Journal of Ocean University of China 20: 439-444.
Simonsen KA, Cowan JH, Boswell KM. 2015. Habitat differences in the feeding ecology of red snapper (Lutjanus campechanus, Poey 1860): a comparison between artificial and natural reefs in the northern Gulf of Mexico. Environmental Biology of Fishes 98: 811-824.
Suman A, Irianto HE, Satria F, Amri K. 2017. Potensi dan tingkat pemanfaatan sumber daya ikan di wilayah pengelolaan perikanan Negara Republik Indonesia (WPP NRI) Tahun 2015 serta Opsi Pengelolaannya. Jurnal Kebijakan Perikanan Indonesia 8: 97-100.
Thi OT, Ha QVD, Thuy BD. 2015. Phylogenetic relationships of emperors (Lethrinidae) and Snappers (Lutjanidae) in Vietnam based on mitochondrial DNA sequences. Pages 15-16. International Conference on Biological, Environment and Food Engineering,(BEFE-2015) Singapore May. pages??
Wibowo A, Panggabean AS, Zamroni A, Priatna A, Yusuf HN. 2018. Using DNA barcode to improve the identification of marine fish larvae, case study coastal water near Jakarta and Banda Sea, Indonesia. Indonesian Fisheries Research Journal 24: 23-30.

Molecular Identification of Snapper (Perciformes: Lutjanidae) Landed at Pondokdadap Fishing Port of Sendang Biru, Malang, Indonesia

Abstract

Snapper is a type of demersal marine fish from the Lutjanidae family. The Lutjanidae family spread throughout the world and currently has 123 species in 21 genera, one of which is the Lutjanus genus (Miller and Thomas, 2007). To this day, the records of capture fisheries production data for snapper in Malang are still very limited to certain types. Morphological identification that has been carried out so far is still challenging to obtain accurate results because of the many similarities between the observed species or the loss of characteristics. Therefore, molecular identification is necessary to determine the types of snappers in this area and their conservation status. This study aims to determine the types of snappers using a molecular approach by Cytochrome Oxidase subunit I (COI) gene marker. Phylogenetic tree reconstruction and genetic distance calculations were performed using Mega X software through the neighbour-joining (NJ) algorithm. The results of the identification snapper based on a molecular approach with DNA barcoding revealed that the four snapper samples were *L. gibbus, L. rufolineatus, L. bengalensis*, and *L. erythropterus*. Based on the results of the compilation of the phylogenetic tree, it can be seen that the *L. bengalensis* sample is closely related to *L. rufolineatus* while *L. gibbus*, and *L. erythropterus* each form a separate clade from the two previous *Lutjanus* species. Based on their conservation status at the IUCN, the four species of snapper found are in the Least Concern category.

Keywords: diversity, gene, identification, phylogenetic, snapper

Introduction

Sendang Biru is one of the coastal areas that prioritise managing marine fisheries resources in Malang Regency, East Java (Andriyono *et al*. 2019). The development makes Sendang Biru a centre for the capture industry (Aliviyanti *et al*. 2021). Snapper is one of the fish catches obtained on this region (Luthfi *et al*. 2016). Snapper is a type of demersal fish of the family Lutjanidae. The family Lutjanidae, spread throughout the world, currently has 123 species in 21 genera, one of which is the genus *Lutjanus* (Miller and Cribb 2007). Based on morphology and habitat characteristics, 30 species of snapper from the genus Lutjanus found in Indonesian waters (Allen et al. 2013, Halim Abdul et al. 2020). Snapper in nature plays the role of one of the large-sized apex predatory fish that inhabit tropical coastal ecosystems around the world. Ecologically, the existence of this fish is important because it acts as a peak predator with extensive food habits. This fish can eat other small fish, *cephalopods*, crabs, shrimps, and other benthic crustaceans to control the stability of the aquatic ecosystem in which it lives (Simonsen *et al*. 2015). Snapper is also one of the captured fishery commodities that is usually used as consumption fish sold in the form of fresh fish, fillets, and processed products (Oktaviyani 2018). The production of this fish has increased every year. This follows data from the Central Statistics Agency (BPS) of Malang Regency (2020), where the total production of this fish reached 57.05 tons in 2018 and 2019 to 108.24 tons. Based on data from BPS Malang Regency (2020), recording data on the production of capture fisheries for snapper in Malang is still very limited to certain types. This is due to the difficulty of identifying species in the field and at the time of simultaneous landing with other types of fish at the fish auction site. Identification of a species can be made morphologically as well as molecularly. Morphological identification that has been carried out so far is still challenging to obtain accurate results because of the many similarities between the observed specifications. In addition, the loss of distinctive features in observed species

due to adaptation to the environment is also an obstacle in identifying a species morphologically (Prehadi *et al*. 2015).

One alternative to identification that can be done in addition to morphological identification is molecular identification by DNA barcoding. DNA *barcoding* is a globally agreed method for identifying plant and animal species based on DNA sequence variations (Coissac *et al*. 2016) from nitrogenous base pair regions in the *Cytochrome Oxidase* subunit I (COI) gene (Powers et al. 2018). Since its introduction in 2003, the DNA barcoding technique has become the golden standard or the main standard for molecular taxonomy (Fadli *et al*. 2020). DNA-based identification barcoding has been well received globally for its various advantages, such as being very simple and using a universal tool applicable to all organisms, both in fresh samples and processed products (Kress *et al*. 2015). Some examples of research that utilizes the DNA barcoding technique include the use of DNA barcoding to identify fish larvae at different stages of development (Wibowo et al. 2018), identification of the discovery of new and cryptic fish species (Farhana *et al*. 2018), identification of fish species that have similar morphological characters (Bingpeng *et al*. 2018).

DNA barcoding is effective for identifying a species with fast and accurate results based on the *Cytochrome Oxidase* subunit I (COI), even if specimen are larvae (Li et al. 2016). The COI gene is one of the protein-encoding genes found in mitochondria that has a distinctive character in each species so it becomes a standard gene as a marker gene when identifying an animal species (Pentinsaari *et al*. 2016). Therefore, the COI genes in DNA barcoding has two advantages, not only for species identification and for metabarcoding (Andújar *et al*. 2018), (Tan et al., 2019) as well.Therefore, research on the identification of snappers based on DNA markings of the COI gene needs to be carried out to provide genetic information.. It is expected to be supporting data in the management of conservation areas and fishing zones in the waters of South Malang, especially from Sendang Biru.

Materials and Methods

2.1 Sampling of fish

A total of 4 samples were collected from the Traditional fish market of Pondok dadap fishing port at Sendang Biru, Malang in the middle of march 2020. All samples collected from the local traditional fisherman were dead upon purchase. The digital camera has taken the individual photograph before further treatments has been applied. Morphologically, identification and species confirmation have been carried out with molecular identification in this study. No specific permit was required for this study,

2.2 DNA extraction and PCR amplification

Each specimen has been collected based on the morphological characters and directly preserved in 90% ethanol for further experimental purposes. Genomic DNA extracted using an Accuprep® Genomic DNA Extraction Kit (Bioneer) according to the product guidelines. The pereiopod fin, around 1 cm tissues, was dissected and mixed with 6X lysis buffer, which was further homogenized by the TissueLyser II (Qiagen). Quantification of purified genomic DNA performed by nanoDrop (Thermofisher Scientific D1000), aliquoted and stored at the -70^{o}C for further analysis.

One set of universal fish primer targeting cytochrome c oxidase I (COI) region, BCL-BCH were used to obtain the partial sequences of each gene (Madduppa *et al*. 2016). The PCR mixture (20µL) included 11.2 µL ultra-pure water, 1 µL primer forward and reverse (0.5 µM), 0.2 µL Ex Taq DNA polymerase (TaKaRa, Japan), 2 µL 10X ExTag Buffer, 2 µL dNTPs (1 µM, TaKaRa, Japan), and 2 µL genomic DNA as template. The PCR condition carried out under the following setting: 95^{o}C for 5 min in initial denaturation, followed by denaturation at 95^{o}C for 30 s in 40 cycles, 50^{o}C for 30 s in annealing, and 72^{o}C for 45 s in extension step, and a final extension at 72^{o}C for 5 min. The PCR products purified with the AccuPrep®Gel purification kit (Bioneer, Korea).

2.3 Data Analysis

Forward and reverse sequence were edited and aligned using MEGAX (Kumar et al. 2018). All sequences were then aligned to the reference on GenBank database by BLASTN (https://blast.ncbi.nlm.nih.gov/Blast.cgi). The pairwise evolutionary distance among the family determined by the Kimura 2-Parameter method. The Neighbor-joining (NJ) tree constructed, and 1000 bootstrap analysis was carried by Mega X and genetic distance used a nucleotide substitution model by comparing a DNA sequence of one nucleotide with another nucleotide (Kumar *et al*. 2018).

Result and Discussion

3.1 Morphological Identification

The snapper samples obtained from the Sendang Biru traditional fish market were 4 snappers with different species consisting of *Lutjanus bengalengsis*, *Lutjanus rufolineatus*, *Lutjanus gibbus*, and *Lutjanus erythropterus*. The most striking difference between each species is each snapper sample's color and body pattern (Figure 1). In addition to body color and pattern, morphological identification also observed morphometric and meristic characters in snapper samples (Table 1).

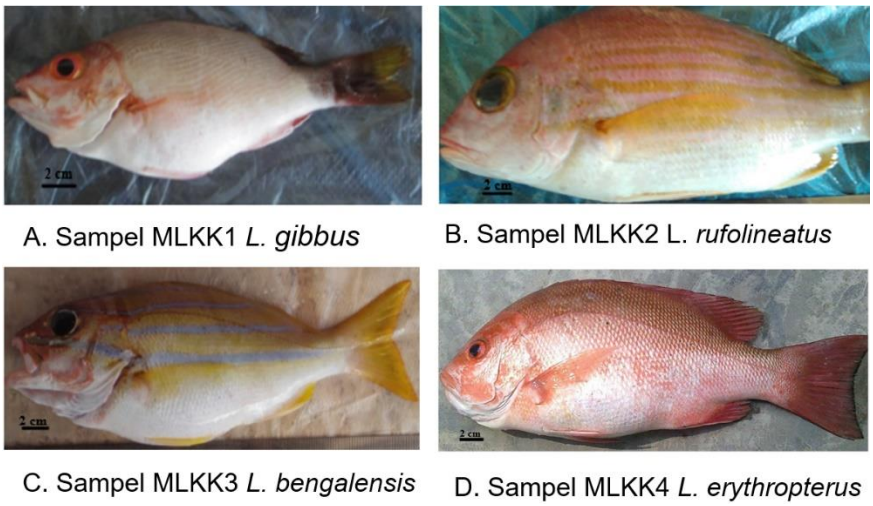


Figure 1. Four species of Snappers Landed at Pondok dadap Fishing Port in traditional fish market of Sendang Biru, South Malang.

Table 1. Morphometric and Meristic Measurements of Snapper Samples

Parameters	Total Length	Standard Length	Head Length	Tail Length

3.2 Molecular Identification

Molecular identification of snapper samples was carried out using the DNA barcoding method. The sequence data obtained were then analyzed and matched with the sequences found in GenBank at NCBI (National Center for Biotechnology Information) using BLASTN (Basic Local Alignment Search Tool Nucleotide) based on the degree of similarity (Table 2). Based on the results of the BLASTN analysis, sample MLKK1 was identified as having 96.72% similarity to the species *Lutjanus gibbus* (Humpback red snapper) access number MF409615, sample MLKK2 was identified to have 99.19% similarity to the species *Lutjanus rufolineatus* (Yellow lined snapper) access number MN870411, sample MLKK3 was identified as having 99.54% similarity to *Lutjanus bengalensis* (Bengal snapper) access number EU600137, while MLKK4 sample was identified to have 100% similarity to *Lutjanus erythropterus* (Crimson snapper) species access number GU673841.

Table 2. BLASTN Results of Snapper Samples with NCBI GenBank Database

No.	Sample Code	Species Name/ Common Name	No. Access GenBank	Identity (%)
1.	MLKK1	*Lutjanus gibbus* / Humpback red snapper	MF409615	96,72%
2.	MLKK2	*Lutjanus rufolineatus* / Yellow lined snapper	MN870411	99,19%
3.	MLKK3	*Lutjanus bengalensis* / Bengal snapper	EU600137	99,54%
4.	MLKK4	*Lutjanus erythropterus* / Crimson snapper	GU673841	100%

3.3 Phylogentic tree reconstruction

Based on the results of the phylogenetic tree reconstruction (Figure 2), samples of snapper landed at the Pondokdadap Sendang Biru Fishing Port, obtained 4 clades formed in the family Lutjanidae with the genus Lutjanus. The clade *L. bengalensis* is phylogenetically close to the clade *L. rufolineatus*, while the clade *L. gibbus* is closely related to the clade *L. erythropterus*. Both *L. bengalensis* (MLKK3) and *L. rufolineatus* (MLKK2) has low genetic distance with close species from other region, but *L erythropterus* from Malang has sifnificant genetic distance with sam species from Australia (0.18) and Malaysia (0.19). As a fish associated with coral reef ecosystems, *Lutjanus* fish species make coral reefs a habitat for rearing ground and feeding ground (Halim Abdul et al. 2020,

Tony et al. 2020). The coral reef habitat will experience different speciation in each region. The Indian Ocean area has different characteristics from the Malaysian waters (South China Sea) and the Australian area which is influenced by the Pacific Ocean which is the main barrier in the distribution of shallow marine fish species. This pattern is known as allopatric speciation (Rocha and Bowen 2008).

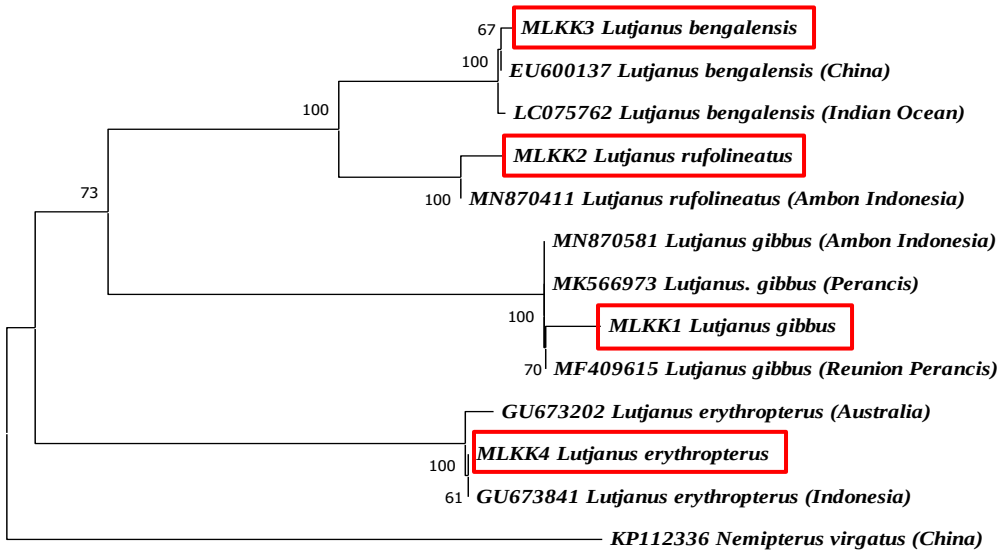


Figure 2. Reconstruction of Phylogenetic Tree based on several COI sequences of Snapper including COI sequences from the NCBI GenBank

The diversity of potential marine fish in Indonesia needs serious attention (Suman *et al*. 2017). Not only in sustainable management (Atmaja and Nugroho 2017), accurate species determination is also a must in providing a valid database at the species level. Many morphological identifications have been carried out. However, in marine fish species there are morphological similarities in both shape and color which causes confusion and inconsistency in naming fish species. In this study, apart from observing specimens based on morphological characteristics, a molecular approach was used to improve data accuracy in identification at the species level. Of the 4 specimens collected, the morphological characteristics showed that the specimens were able to be identified based on their morphometric characteristics, so that all samples were identified as *Lutjanus bengalensis, Lutjanus rufolineatus, Lutjanus gibbus,* and *Lutjanus erythropterus*. To increase accuracy in identification, we also carried out molecular identification of the COI gene section (Andriyono and Suciyono 2020) which has been agreed as a universal area for identification at the species level.

Morphological observations on the four samples showed that the MLKK1 sample had similarities with *Lutjanus gibbus*/ humpback red snapper, also known as jinaha snapper. The distinctive features or key morphological identification of this fish are having a compressed body shape with a grayish red body color, the caudal fin is clearly branched with dark red rounded lobes, and on the dorsal fin there are 10 hard spines and 13-14 soft spines (Thi *et al*. 2015). The MLKK2 sample has similarities with *Lutjanus rufolineatus*/yellow lined snapper, also known as badur snapper. The key to morphological identification of the *L. rufolineatus* species is that there are 6 yellow stripes on each side of its body, it has a pale red compressed body shape, the tail is brownish yellow, and on the dorsal fin there are 10 hard spines and 12-13 soft spines (Allen *et al*. 2013).

The MLKK3 sample is similar to the Lutjanus bengalensis/bengal snapper species, also known as yellow snapper with a key Identification. The body of this fish is compressed, the body color is bright yellow with 4 grayish white stripes on each side of the body, there is a deep groove on the front operculum, the caudal fin is broad with a straight tip, has 11 spines and 12- 14 soft rays on the dorsal fin (Iwatsuki *et al*. 2016). The MLKK4 sample has similarities to the *Lutjanus erythropterus*/ crimson snapper or often referred to as the red snapper. The identification key in common in the form of

having a pink to dark red compressed body shape from the tip of the head to the tail. Another characteristic are the tip of the snout is slightly pointed and relatively small. Besides, the preoperculum notch is not very pronounced and on the dorsal fin there are 11 hard spines and 16-17 soft spines (Sarkar *et al*. 2021).

Reconstruction of the phylogenetic tree of snapper samples landed at the Pondokdadap Fishing Port, Sendang Biru, obtained 4 clades formed in the family Lutjanidae with the genus Lutjanus. The clade *L. bengalensis* is phylogenetically close to the clade *L. rufolineatus*, while the clade *L. gibbus* is closely related to the clade *L. erythropterus*. In the phylogenetic tree reconstruction, there is also a clade of the *Nemipterus virgatus* species as a comparison or outgroup. Reconstruction of phylogenetic trees is supported by the results of genetic distance analysis in a species (Akbar and Labenua 2018). The results of the genetic distance analysis showed that the MLKK3 sample was close to the *L. bengalensis* EU600137 (China) and LC075762 (Indian Ocean) samples, with a genetic distance of 0.00 (zero). The MLKK2 specimen was closely related to the *L. rufolineatus* specimen MN870411 (Indonesia) and had a genetic distance of 0.01. The MLKK1 specimen was closely related to *Lutjanus gibbus* MN870581 (Ambon, Indonesia), MK566973 (France) and MF409615 (Reunion) with a genetic distance of 0.01 each. The MLKK4 specimen was closely related to *L*. *erythropterus* specimens GU673841 (Indonesia) and GU673202 (Australia) with a genetic distance of 0.00 and 0.01, respectively. Research on the Lutjanidae species in peninsular Malaysia (Malacca Strait and South China Sea) also shows that there is a variation in genetic distance (Halim *et al*. 2022).

Based on the conservation status that refers to the IUCN (International Union for the Conservation of Nature and Natural Resources), *L. gibbus*, *L. rufolineatus*, *L. bengalensis*, and *L. erythropterus* species are included in the Least Concern or low risk category (IUCN Red List, 2021). Least Concern is a species that has been evaluated but its status is still under the status of almost endangered or it can be said that it does not fall into any category. The IUCN conservation status categories include the category of extinction (EX), category of extinction in the wild (EW), category of critically (CR), category of threatened or critical (EN), category of vulnerable (VU), category of near threatened (NT), the category of low risk (LC) and the category of lack of information (DD) (https://www.iucnredlist.org/). Then based on their trading status according to CITES, these four snapper species are included in the Not Evaluated category, so that they are still classified as safe for international trade.

Conclusion

Based on morphological and molecular identification, the types of snappers that landed from Sendang Biru, South Malang waters were *Lutjanus bengalensis*, *Lutjanus rufolineatus*, *Lutjanus gibbus*, and *Lutjanus erythropterus*. Based on the results of the compilation of the phylogenetic tree, it can be seen that the *L. bengalensis* sample is closely related to *L. rufolineatus* while *L. gibbus*, and *L. erythropterus* each form a separate clade from the two previous Lutjanus species. Based on their conservation status at the IUCN, the four species of snapper found are in the Least Concern category, while based on their trading status on CITES, these four species are in the Not Evaluated category.

Acknowledgement

We would like to deliver our gratitude to the PUF Research Grant 2020 internal fund research program from Faculty of Fisheries and Marine Affairs, Universitas Airlangga has been providing support in this research. We also thank the research team colleagues who have helped in the sample collection in South Malang, East Java

Table 3. Genetic Distance of Snapper COI gene Sequences from Sendang Biru with Snapper COI gene Sequences on NCBI GenBank

No.	Name of Spesies	1	2	3	4	5	6	7	8	9	10	11	12	13
1.	MLKK1 *Lutjanus gibbus*													
2.	MN870581 *L. gibbus* (Ambon)	0,01												
3.	MK566973 *L. gibbus* (Perancis)	0,01	0,00											
4.	MF409615 *L. gibbus* (Reunion)	0,01	0,00	0,00										
5.	MLKK2 *Lutjanus rufolineatus*	0,17	0,16	0,16	0,16									
6.	MN870411 *L.rufolineatus* (Ambon)	0,16	0,15	0,15	0,15	0,01								
7.	MLKK3 *Lutjanus bengalensis*	0,17	0,16	0,16	0,16	0,07	0,06							
8.	EU600137 *L.bengalensis* (China)	0,17	0,16	0,16	0,16	0,06	0,05	0,00						
9.	LC075762 *L. bengalensis* (Indian Ocean)	0,17	0,16	0,16	0,16	0,07	0,06	0,00	0,00					
10.	MLKK4 *Lutjanus erythropterus*	0,18	0,17	0,17	0,17	0,17	0,16	0,18	0,17	0,18				
11.	GU673841 *L. erythropterus* (Australia)	0,18	0,17	0,17	0,17	0,17	0,16	0,18	0,17	0,18	0,18	0,00		
12.	GU67202 *L. erythropterus* (Malaysia)	0,19	0,18	0,18	0,18	0,18	0,17	0,19	0,18	0,19	0,19	0,01	0,01	
13.	KP112336 *Nemipterus virgatus* (China)	0,24	0,23	0,23	0,23	0,21	0,20	0,21	0,21	0,21	0,21	0,21	0,21	0,20

References

Akbar NN, Labenua R. 2018. Keragaman genetik ikan cakalang (Katsuwonus pelamis) di perairan laut Maluku Utara. Depik 7: 164-176. DOI: 10.13170/depik.7.2.11156

Aliviyanti D, Semedi B, Yona D, Asadi MA, Kasitowati RD, Dewi CSU, Lutfi OM, Isdianto A. 2021. Upaya Penguatan Manajemen Pemasaran Hasil Perikanan Berbasis Media Online di TPI Sendangbiru, Kabupaten Malang, Indonesia. Abdi Geomedisains: 59-67. DOI: 10.23917/abdigeomedisains.v1i2.199

Allen GR, Erdmann MV, Randall JE, Ching P, Rauzon MJ, Hayashi LA, Thomas M, Robertson D, Taylor L, Coste M. 2013. Reef fishes of the East Indies. Philosophy East and West 63 (2), 1292 pages.

Andriyono S, Suciyono S. 2020. Molecular Identification and Phylogenetic Tree Reconstruction of Marine Fish from the Essential Wetland Area of Banyuwangi, Indonesia. Egyptian Journal of Aquatic Biology and Fisheries 24: 427-439. DOI: 10.21608/ejabf.2020.87032

Andriyono S, Alam MJ, KIM H-W. 2019. Environmental DNA (eDNA) metabarcoding: Diversity study around the Pondok Dadap fish landing station, Malang, Indonesia. Biodiversitas Journal of Biological Diversity 20 (12) page 3772-3781. DOI: 10.13057/biodiv/d201241

Andújar C, Arribas P, Yu DW, Vogler AP, Emerson BC. 2018. Why the COI barcode should be the community DNA metabarcode for the metazoa: Wiley Online Library. 27:3968–3975 DOI: 10.1111/mec.14844

Atmaja SB, Nugroho D. 2017. Upaya-upaya pengelolaan sumber daya ikan yang berkelanjutan di Indonesia. Jurnal Kebijakan Perikanan Indonesia 3: 101-113. DOI: 10.15578/jkpi.3.2.2011.101-113

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

Coissac E, Hollingsworth PM, Lavergne S, Taberlet P. 2016. From barcodes to genomes: extending the concept of DNA barcoding: Wiley Online Library. 25: 1423–1428 DOI: 10.1111/mec.13549

Fadli N, Nor SAM, Othman AS, Sofyan H, Muchlisin ZA. 2020. DNA barcoding of commercially important reef fishes in Weh Island, Aceh, Indonesia. PeerJ 8: e9641. DOI: 10.7717/peerj.9641

Farhana NS, Muchlisin ZA, Duong TY, Tanyaros S, Page LM, Zhao Y, Adamson EA, Khaironizam M, de Bruyn M, Siti Azizah MN. 2018. Exploring hidden diversity in Southeast Asia's Dermogenys spp.(Beloniformes: Zenarchopteridae) through DNA barcoding. Scientific Reports 8: 1-11. DOI: 10.1038/s41598-018-29049-7

Halim, Rahim I, Mahboob S, Al-Ghanim K, Asmiaty A, Naim DM. 2022. Phylogenetic relationships of the commercial red snapper (Lutjanidae sp.) from three marine regions. Journal of King Saud University-Science 34: 101756. DOI: 10.1016/j.jksus.2021.101756

Halim A, Loneragan NR, Wiryawan B, Hordyk AR, Sondita MFA, Yulianto I. 2020. Evaluating data-limited fisheries for grouper (Serranidae) and snapper (Lutjanidae) in the Coral Triangle, eastern Indonesia. Regional Studies in Marine Science 38: 101388. DOI: 10.1016/j.rsma.2020.101388

Iwatsuki Y, Al-Mamry JM, Heemstra PC. 2016. Validity of a blue stripe snapper, Lutjanus octolineatus (Cuvier 1828) and a related species, L. bengalensis (Bloch 1790) with a new species (Pisces; Lutjanidae) from the Arabian Sea. Zootaxa 4098: 511-528. DOI: 10.11646/zootaxa.4098.3.5

Kress WJ, García-Robledo C, Uriarte M, Erickson DL. 2015. DNA barcodes for ecology, evolution, and conservation. Trends in ecology & evolution 30: 25-35. DOI: 10.1016/j.tree.2014.10.008

Kumar S, Stecher G, Li M, Knyaz C, Tamura K. 2018. MEGA X: molecular evolutionary genetics analysis across computing platforms. J Molecular biology evolution 35: 1547. DOI: 10.1093/molbev/msy096

Li Q, Li M, Li D, Shi W, Li J, Ning T, Duan Y. 2016. Identification of the Larvae of Helicoverpa assulta and H. Armigera (Lepidoptera: Noctuidae) Through Morphological Difference and DNA Barcoding. 3rd International Conference on Smart Materials and Nanotechnology in Engineering. Pages 306-309.

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. Jurnal Kelautan: Indonesian Journal of Marine Science and Technology 9: 43-49. DOI: 10.21107/jk.v9i1.1019

Madduppa H, Ayuningtyas RU, Subhan B, Arafat D. 2016. Exploited but unevaluated: DNA barcoding reveals skates and stingrays (Chordata, Chondrichthyes) species landed in the Indonesian fish market. IJMS (complete Journal name?) 21: 77-84. DOI:10.14710/ik.ijms.21.2.77-84

Miller TL, Cribb TH. 2007. Phylogenetic relationships of some common Indo-Pacific snappers (Perciformes: Lutjanidae) based on mitochondrial DNA sequences, with comments on the taxonomic position of the Caesioninae. Molecular phylogenetics and evolution 44: 450-460. DOI: 10.1016/j.ympev.2006.10.029

Oktaviyani S. 2018. Mengenal marga Lutjanus, salah satu komoditas unggulan dalam perikanan tangkap. Oseana 43: 29-39. DOI: 10.14203/oseana.2018.Vol.43No.3.61

Pentinsaari M, Salmela H, Mutanen M, Roslin T. 2016. Molecular evolution of a widely-adopted taxonomic marker (COI) across the animal tree of life. Scientific reports 6: 1-12. DOI:10.1038/srep35275

Powers T, Harris T, Higgins R, Mullin P, Powers K. 2018. Discovery and Identification of Species Using COI DNA Barcoding. Journal of Nematology 50: 399-412. DOI: 10.21307/jofnem-2018-029

Prehadi P, Sembiring A, Kurniasih EM, Rahmad R, Arafat D, Subhan B, Madduppa HH. 2015. DNA barcoding and phylogenetic reconstruction of shark species landed in Muncar fisheries landing site in comparison with Southern Java fishing port. Biodiversitas Journal of Biological Diversity 16 (1) page: 55-61. DOI: 10.13057/biodiv/d160107

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

Sarkar P, Islam MJ, Habib A, Neogi AK, Habib KA. 2021. Two New Records of Snapper (Perciformes, Lutjanidae) from Saint Martin's Island, Bangladesh. Journal of Ocean University of China 20: 439-444. DOI: 10.1007/s11802-021-4566-x

Simonsen KA, Cowan JH, Boswell KM. 2015. Habitat differences in the feeding ecology of red snapper (Lutjanus campechanus, Poey 1860): a comparison between artificial and natural reefs in the northern Gulf of Mexico. Environmental Biology of Fishes 98: 811-824. DOI:10.1007/s10641-014-0317-9

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

Thi OT, Ha QVD, Thuy BD. 2015. Phylogenetic relationships of emperors (Lethrinidae) and Snappers (Lutjanidae) in Vietnam based on mitochondrial DNA sequences. Pages 15-16. International Conference on Biological, Environment and Food Engineering,(BEFE-2015) Singapore May. Pages15-16.

Tony F, Soemarno S, Wiadnya DGR, Hakim L. 2020. Diversity of reef fish in Halang Melingkau Island, South Kalimantan, Indonesia. Biodiversitas Journal of Biological Diversity 21 (10). DOI: 10.13057/biodiv/d211046.

Wibowo A, Panggabean AS, Zamroni A, Priatna A, Yusuf HN. 2018. Using DNA barcode to improve the identification of marine fish larvae, case study coastal water near Jakarta and Banda Sea, Indonesia. Indonesian Fisheries Research Journal 24: 23-30. DOI:10.15578/ifrj.24.1.2018.37-44

ILMU KELAUTAN: Indonesian Journal of Marine Sciences *December 2022 Vol 27(4):307-314* *e-ISSN 2406-7598*

Molecular Identification of Snapper (Perciformes: Lutjanidae) Landed at Pondokdadap Fishing Port of Sendang Biru, Malang, Indonesia

Sapto Andriyono[1*], Novian Aji Pradana[2], Laksmi Sulmartiwi[1], Andi Aliah Hidayani[3], Md. Jobaidul Alam[4], Adrian Damora[5], Ahasan Habib[6]

[1]Department of Marine, Fisheries and Marine Faculty, Universitas Airlangga
[2]Aquaculture Study Program, Faculty of Fisheries and Marine, Universitas Airlangga
Jl. Wijaya Kusuma No. 13 Banyuwangi, Jawa Timur 68411 Indonesia
[3]Fisheries Department, Faculty of Marine Science and Fisheries, Hasanuddin University
Jl. Perintis Kemerdekaan No.Km. 10 Makassar, Sulawesi Selatan 90245 Indonesia
[4]Department of Fisheries, Ministry of Fisheries and Livestock, Dhaka, Bangladesh
Dhaka 1205, Bangladesh
[5]Department of Aquaculture, Faculty of Marine and Fisheries, Universitas Syiah Kuala
Jl. Teuku Nyak Arief No.441, Kopelma Darussalam, Syiah Kuala, Kota Banda Aceh, Aceh 23111 Indonesia
[6]Faculty of Fisheries and Food Science, Universiti Malaysia Terengganu
21030 Kuala Nerus, Terengganu, Malaysia
Email: sapto.andriyono@fpk.unair.ac.id

Abstract

Snapper is a type of demersal marine fish from the Lutjanidae family. The Lutjanidae family spread throughout the world and currently has 123 species in 21 genera, one of which is the Lutjanus genus (Miller and Thomas, 2007). To this day, the records of capture fisheries production data for snapper in Malang are still very limited to certain types. Morphological identification that has been carried out so far is still challenging to obtain accurate results because of the many similarities between the observed species or the loss of characteristics. Therefore, molecular identification is necessary to determine the types of snappers in this area and their conservation status. This study aims to determine the types of snappers using a molecular approach by Cytochrome Oxidase subunit I (COI) gene marker. Phylogenetic tree reconstruction and genetic distance calculations were performed using Mega X software through the neighbour-joining (NJ) algorithm. The results of the identification snapper based on a molecular approach with DNA barcoding revealed that the four snapper samples were L. gibbus, L. rufolineatus, L. bengalensis, and L. erythropterus. Based on the results of the compilation of the phylogenetic tree, it can be seen that the L. bengalensis sample is closely related to L. rufolineatus while L. gibbus, and L. erythropterus each form a separate clade from the two previous Lutjanus species. Based on their conservation status at the IUCN, the four species of snapper found are in the Least Concern category.

Keywords: diversity, gene, identification, phylogenetic, snapper

Introduction

Sendang Biru is one of the coastal areas that prioritise managing marine fisheries resources in Malang Regency, East Java (Andriyono *et al.,* 2019). The development makes Sendang Biru a centre for the capture industry (Aliviyanti *et al.,* 2021). Snapper is one of the fish catches obtained on this region (Luthfi *et al.,* 2016). Snapper is a type of demersal fish of the family Lutjanidae. The family Lutjanidae, spread throughout the world, currently has 123 species in 21 genera, one of which is the genus *Lutjanus* (Miller and Cribb, 2007). Based on morphology and habitat characteristics, 30 species of snapper from the genus Lutjanus found in Indonesian waters (Allen *et al*., 2013, Halim *et al*., 2020).

Snapper in nature plays the role as one of the large-sized apex predatory fishes that inhabit tropical coastal ecosystems around the world. Ecologically, the existence of this fish is important because it acts as a peak predator with extensive food habits. This fish can eat other small fish, *cephalopods*, crabs, shrimps, and other benthic crustaceans to control the stability of the aquatic ecosystem in which it lives (Simonsen *et al.,* 2015). Snapper is also one of the captured fishery commodities that is usually used as consumption fish sold in the form of fresh fish, fillets, and processed products (Oktaviyani, 2018). The production of this fish has increased every year. This follows data from the Central Statistics Agency (BPS) of Malang Regency (2020), where the total production of this fish reached 57.05 tons in 2018 and 2019 to 108.24 tons. Based on data from BPS

*) Corresponding author

https://ejournal.undip.ac.id/index.php/ijms
DOI: 10.14710/ik.ijms.27.4.307-314
Received : 02-09-2022
Accepted : 19-11-2022

Malang Regency (2020), recording data on the production of capture fisheries for snapper in Malang is still very limited to certain types. This is due to the difficulty of identifying species in the field and at the time of simultaneous landing with other types of fish at the fish auction site. Identification of a species can be made morphologically as well as molecularly. Morphological identification that has been carried out so far is still challenging to obtain accurate results because of the many similarities between the observed specifications. In addition, the loss of distinctive features in observed species due to adaptation to the environment is also an obstacle in identifying a species morphologically (Prehadi *et al.,* 2015).

One alternative to identify that can be done in addition to morphological is molecular identification by DNA barcoding. DNA *barcoding* is a globally agreed method for identifying plant and animal species based on DNA sequence variations (Coissac *et al.,* 2016) from nitrogenous base pair regions in the *Cytochrome Oxidase* subunit I (COI) gene (Powers *et al*., 2018). Since its introduction in 2003, the DNA barcoding technique has become the golden standard or the main standard for molecular taxonomy (Fadli *et al.,* 2020). DNA-based identification barcoding has been well received globally for its various advantages, such as being very simple and using a universal tool applicable to all organisms, both in fresh samples and processed products (Kress *et al.,* 2015). Some examples of research that utilizes the DNA barcoding technique include the use of DNA barcoding to identify fish larvae at different stages of development (Wibowo et al., 2018), identification of the discovery of new and cryptic fish species (Farhana *et al.,* 2018), identification of fish species that have similar morphological characters (Bingpeng *et al.,* 2018).

DNA barcoding is effective for identifying a species with fast and accurate results based on the *Cytochrome Oxidase* subunit I (COI), even if specimen are larvae (Li *et al.*, 2016). The COI gene is one of the protein-encoding genes found in mitochondria that has a distinctive character in each species so it becomes a standard gene as a marker gene when identifying an animal species (Pentinsaari *et al.,* 2016). Therefore, the COI genes in DNA barcoding has two advantages, not only for species identification and for metabarcoding (Andújar *et al.,* 2018), (Tan *et al*., 2019) as well. Therefore, research on the identification of snappers based on DNA markings of the COI gene needs to be carried out to provide genetic information. It is expected to be supporting data in the management of conservation areas and fishing zones in the waters of South Malang, especially from Sendang Biru.

Materials and Methods

Sampling of fish

A total of 4 samples were collected from traditional fish market of Pondokdadap fishing port at Sendang Biru, Malang in the middle of March 2020. All samples collected from the local traditional fisherman were dead upon purchase. The digital camera was used to take the individual photograph before further treatments. Morphologically, identification and species confirmation were carried out with molecular identification in this study. No specific permit was required for this study,

DNA extraction and PCR amplification

Each specimen has been collected based on the morphological characters and directly preserved in 90% ethanol for further experimental purposes. Genomic DNA extracted using an Accuprep® Genomic DNA Extraction Kit (Bioneer) according to the product guidelines. The pereiopod fin, around 1 cm tissues, was dissected and mixed with 6X lysis buffer, which was further homogenized by the TissueLyser II (Qiagen). Quantification of purified genomic DNA performed by nanoDrop (Thermofisher Scientific D1000), aliquoted and stored at the -70^{o}C for further analysis.

One set of universal fish primer targeting cytochrome c oxidase I (COI) region, BCL-BCH were used to obtain the partial sequences of each gene (Madduppa *et al.,* 2016). The PCR mixture (20µL) included 11.2 µL ultra-pure water, 1 µL primer forward and reverse (0.5 µM), 0.2 µL Ex Taq DNA polymerase (TaKaRa, Japan), 2 µL 10X ExTag Buffer, 2 µL dNTPs (1 µM, TaKaRa, Japan), and 2 µL genomic DNA as template. The PCR condition carried out under the following setting: 95^{o}C for 5 min in initial denaturation, followed by denaturation at 95^{o}C for 30 s in 40 cycles, 50^{o}C for 30 s in annealing, and 72^{o}C for 45 s in extension step, and a final extension at 72^{o}C for 5 min. The PCR products purified with the AccuPrep®Gel purification kit (Bioneer, Korea).

Data analysis

Forward and reverse sequence were edited and aligned using MEGAX (Kumar *et al*., 2018). All sequences were then aligned to the reference on GenBank database by BLASTN (https://blast.ncbi.nlm.nih.gov/Blast.cgi). The pairwise evolutionary distance among the family determined by the Kimura 2-Parameter method. The Neighbor-joining (NJ) tree constructed, and 1000 bootstrap analysis was carried by Mega X and genetic distance used a nucleotide substitution model by comparing a DNA sequence of one nucleotide with another nucleotide (Kumar *et al.,* 2018).

Result and Discussion

Morphological identification

The samples obtained from the Sendang Biru traditional fish market were 4 snappers with different species consisting of *Lutjanus bengalengsis*, *L. rufolineatus*, *L. gibbus*, and *L. erythropterus*. The most striking difference between each species is each snapper sample's color and body pattern (Figure 1.). In addition to body color and pattern, morphological identification also observed morphometric and meristic characters in snapper samples (Table 1.).

Molecular identification

Molecular identification of snapper samples was carried out using the DNA barcoding method. The sequence data obtained were then analyzed and matched with the sequences found in GenBank at NCBI (National Center for Biotechnology Information) using BLASTN (Basic Local Alignment Search Tool Nucleotide) based on the degree of similarity (Table 2). Based on the results of the BLASTN analysis, sample MLKK1 was identified as having 96.72% similarity to the species *L. gibbus* (Humpback red snapper) access number MF409615, sample MLKK2 was identified to have 99.19% similarity to the species *L. rufolineatus* (Yellow lined snapper) access number MN870411, sample MLKK3 was identified as having 99.54% similarity to *L. bengalensis* (Bengal snapper) access number EU600137, while MLKK4 sample was identified to have 100% similarity to *L. erythropterus* (Crimson snapper) species access number GU673841.

Phylogentic tree reconstruction

Based on the results of the phylogenetic tree reconstruction (Figure 2.), samples of snapper landed at the Pondokdadap Sendang Biru Fishing Port, obtained 4 clades formed in the family Lutjanidae with the genus Lutjanus. The clade *L. bengalensis* is phylogenetically close to the clade *L. rufolineatus*, while the clade *L. gibbus* is closely related to the clade *L. erythropterus*. Both *L. bengalensis* (MLKK3) and *L. rufolineatus* (MLKK2) has low genetic distance with close species from other region (Table 3.), but *L erythropterus* from Malang has sifnificant genetic distance with sam species from Australia (0.18) and Malaysia (0.19). As a fish associated with coral reef ecosystems, *Lutjanus* fish species make coral reefs a habitat for rearing ground and feeding ground (Halim *et al*., 2020, Tony *et al*., 2020). The coral reef habitat will experience different speciation in each region. The Indian Ocean area has different characteristics from the Malaysian waters (South China Sea) and the Australian area which is influenced by the Pacific Ocean which is the main barrier in the distribution of shallow marine fish species. This pattern is known as allopatric speciation (Rocha and Bowen, 2008).

The diversity of potential marine fish in Indonesia needs serious attention (Suman *et al.,* 2017). Not only in sustainable management (Atmaja and Nugroho, 2017), accurate species determination is also a must in providing a valid database at the species level. Many morphological identifications have been carried out. However, in marine fish species there are morphological similarities in both shape and color which causes confusion and inconsistency in naming fish species. In this study,

Table 1. Morphometric and Meristic Measurements of Snapper Samples

Parameters	Sample ID			
	MLKK1	MLKK2	MLKK3	MLKK4
Total length	26,3 cm	26,8 cm	27,5 cm	26,4 cm
Standard length	21,5 cm	21,5 cm	22,5 cm	20,5 cm
Head length	8 cm	8,3 cm	8,6 cm	8,2 cm
Height	8,5 cm	8,6 cm	10,8 cm	11,2 cm
Head height	6,5 cm	6,8 cm	9,2 cm	8,6 cm
Tail base height	2,5 cm	2,8 cm	3,0 cm	2,8 cm
Dorsal fin	D.X, 14	D.X, 14	D.XI, 14	D.XI, 14
Pectoral fin	P.17	P.16	P.16	P.17
ventral fin	V.I,6	V.I,6	V.I,6	V.I,5
anal fin	A.III.8	A.III, 8	A.III, 8	A.III, 9
caudal fin	C.18	C.18	C.20	C.18

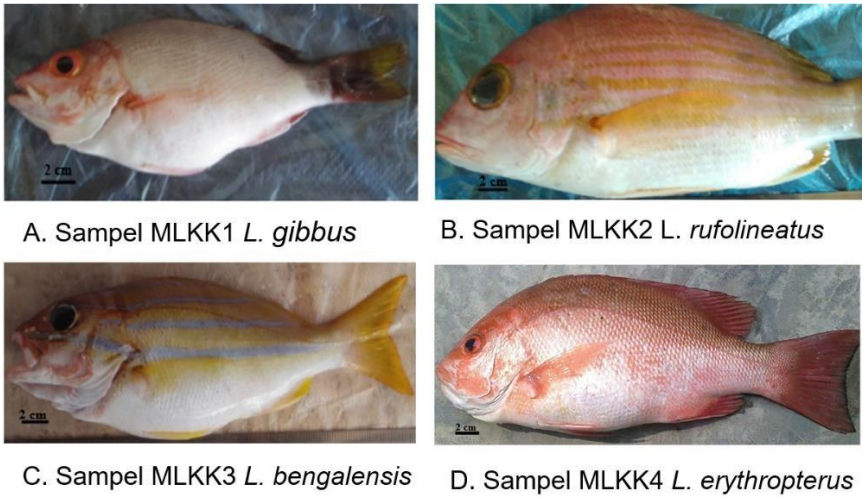


Figure 1. Four species of Snappers Landed at Pondokdadap Fishing Port in traditional fish market of Sendang Biru, South Malang.

Table 2. BLASTN Results of Snapper Samples with NCBI GenBank Database

No.	Sample Code	Species Name/ Common Name	No. Access GenBank	Identity (%)
1.	MLKK1	*Lutjanus gibbus* / Humpback red snapper	MF409615	96,72%
2.	MLKK2	*Lutjanus rufolineatus* / Yellow lined snapper	MN870411	99,19%
3.	MLKK3	*Lutjanus bengalensis* / Bengal snapper	EU600137	99,54%
4.	MLKK4	*Lutjanus erythropterus* / Crimson snapper	GU673841	100%

apart from observing specimens based on morphological characteristics, a molecular approach was used to improve data accuracy in identification at the species level. Of the 4 specimens collected, the morphological characteristics showed that the specimens were able to be identified based on their morphometric characteristics, so that all samples were identified as *L. bengalensis, L. rufolineatus, L. gibbus,* and *L. erythropterus*. To increase accuracy in identification, we also carried out molecular identification of the COI gene section (Andriyono and Suciyono, 2020) which has been agreed as a universal area for identification at the species level.

Morphological observations on the four samples showed that the MLKK1 sample had similarities with *Lutjanus gibbus*/ humpback red snapper, also known as jinaha snapper. The distinctive features or key morphological identification of this fish are having a compressed body shape with a grayish red body color, the caudal fin is clearly branched with dark red rounded lobes, and on the dorsal fin there are 10 hard spines and 13-14 soft spines (Thi *et al.,* 2015). The MLKK2 sample has similarities with *Lutjanus rufolineatus*/ yellow lined snapper, also known as badur snapper. The key to morphological identification of the *L. rufolineatus* species is that there are 6 yellow stripes on each side of its body, it has a pale red compressed body shape, the tail is brownish yellow, and on the dorsal fin there are 10 hard spines and 12-13 soft spines (Allen *et al.,* 2013).

The MLKK3 sample is similar to the Lutjanus bengalensis/bengal snapper species, also known as yellow snapper with a key Identification. The body of this fish is compressed, the body color is bright yellow with 4 grayish white stripes on each side of the body, there is a deep groove on the front operculum, the caudal fin is broad with a straight tip, has 11 spines and 12- 14 soft rays on the dorsal fin (Iwatsuki *et al.,* 2016). The MLKK4 sample has similarities to the *L. erythropterus*/ crimson snapper or often referred to as the red snapper. The identification key in common in the form of having a pink to dark red compressed body shape from the tip of the head to the tail. Another characteristic are the tip of the snout is slightly pointed and relatively small. Besides, the preoperculum notch is not very pronounced and on the dorsal fin there are 11 hard spines and 16-17 soft spines (Sarkar *et al.,* 2021).

Reconstruction of the phylogenetic tree of snapper samples landed at the Pondokdadap Fishing Port, Sendang Biru, obtained 4 clades formed in the family Lutjanidae with the genus Lutjanus. The clade *L. bengalensis* is phylogenetically close to the clade *L. rufolineatus*, while the clade *L. gibbus* is closely related to the clade *L. erythropterus*. In the phylogenetic tree reconstruction, there is also a clade

of the *Nemipterus virgatus* species as a comparison or outgroup. Reconstruction of phylogenetic trees is supported by the results of genetic distance analysis in a species (Akbar and Labenua 2018). The results of the genetic distance analysis showed that the MLKK3 sample was close to the *L. bengalensis* EU600137 (China) and LC075762 (Indian Ocean) samples, with a genetic distance of 0.00 (zero). The MLKK2 specimen was closely related to the *L. rufolineatus* specimen MN870411 (Indonesia) and had a genetic distance of 0.01. The MLKK1 specimen was closely related to *L. gibbus* MN870581 (Ambon, Indonesia), MK566973 (France) and MF409615 (Reunion) with a genetic distance of 0.01 each. The MLKK4 specimen was closely related to *L. erythropterus* specimens GU673841 (Indonesia) and GU673202 (Australia) with a genetic distance of 0.00 and 0.01, respectively. Research on the Lutjanidae species in peninsular Malaysia (Malacca Strait and South China Sea) also shows that there is a variation in genetic distance (Halim *et al.,* 2022).

Based on the conservation status that refers to the IUCN (International Union for the Conservation of Nature and Natural Resources), *L. gibbus*, *L. rufolineatus*, *L. bengalensis*, and *L. erythropterus* species are included in the Least Concern or low risk category (IUCN Red List, 2021). Least Concern is a species that has been evaluated but its status is still under the status of almost endangered or it can be said that it does not fall into any category. The IUCN conservation status categories include the category of extinction (EX), category of extinction in the wild (EW), category of critically (CR), category of threatened or critical (EN), category of vulnerable (VU), category of near threatened (NT), the category of low risk (LC) and the category of lack of information (DD) (https://www.iucnredlist.org/). Then based on their trading status according to CITES, these four snapper species are included in the Not Evaluated category, so that they are still classified as safe for international trade.

Table 3. Genetic Distance of Snapper COI gene Sequences from Sendang Biru with Snapper COI gene Sequences on NCBI GenBank

No.	Name of Spesies	1	2	3	4	5	6	7	8	9	10	11	12	13
1.	MLKK1 *Lutjanus gibbus*													
2.	MN870581 *L. gibbus* (Ambon)	0,01												
3.	MK566973 *L. gibbus* (Perancis)	0,01	0,00											
4.	MF409615 *L. gibbus* (Reunion)	0,01	0,00	0,00										
5.	MLKK2 *Lutjanus rufolineatus*	0,17	0,16	0,16	0,16									
6.	MN870411 *L.rufolineatus* (Ambon)	0,16	0,15	0,15	0,15	0,01								
7.	MLKK3 *Lutjanus bengalensis*	0,17	0,16	0,16	0,16	0,07	0,06							
8.	EU600137 *L.bengalensis* (China)	0,17	0,16	0,16	0,16	0,06	0,05	0,00						
9.	LC075762 *L. bengalensis* (Indian Ocean)	0,17	0,16	0,16	0,16	0,07	0,06	0,00	0,00					
10.	MLKK4 *Lutjanus erythropterus*	0,18	0,17	0,17	0,17	0,17	0,16	0,18	0,17	0,18				
11.	GU673841 *L. erythropterus* (Australia)	0,18	0,17	0,17	0,17	0,17	0,16	0,18	0,17	0,18	0,18	0,00		
12.	GU67202 *L. erythropterus* (Malaysia)	0,19	0,18	0,18	0,18	0,18	0,17	0,19	0,18	0,19	0,19	0,01	0,01	
13.	KP112336 *Nemipterus virgatus* (China)	0,24	0,23	0,23	0,23	0,21	0,20	0,21	0,21	0,21	0,21	0,21	0,21	0,20

Conclusion

Based on morphological and molecular identification, the types of snappers that landed from Sendang Biru, South Malang waters were *Lutjanus bengalensis*, *L. rufolineatus*, *L. gibbus*, and *L. erythropterus*. Based on the results of the compilation of the phylogenetic tree, it can be seen that the *L. bengalensis* sample is closely related to *L. rufolineatus* while *L. gibbus*, and *L. erythropterus* each form a separate clade from the two previous Lutjanus species. Based on their conservation status at the IUCN, the four species of snapper found are in the Least Concern category, while based on their trading status on CITES, these four species are in the Not Evaluated category.

Acknowledgement

We would like to deliver our gratitude to the PUF Research Grant 2020 internal fund research program from Faculty of Fisheries and Marine Affairs, Universitas Airlangga has been providing support in this research. We also thank the research team colleagues who have helped in the sample collection in South Malang, East Java

References

Akbar, N.N. & Labenua, R. 2018. Keragaman genetik ikan cakalang (*Katsuwonus pelamis*) di perairan laut Maluku Utara. *Depik* 7: 164-176. https://doi.org/10.13170/depik.7.2.11156

Aliviyanti, D., Semedi, B., Yona, D., Asadi, M.A., Kasitowati, R.D., Dewi, C.S.U., Lutfi, O.M. & Isdianto, A., 2021. Upaya Penguatan Manajemen Pemasaran Hasil Perikanan Berbasis Media Online di TPI Sendangbiru, Kabupaten Malang, Indonesia. *Abdi Geomedisains,* 1(2): 59-67. https://doi.org/10.23917/abdigeomedisains.v1i2.199

Allen, G.R., Erdmann, M.V., Randall, J.E., Ching, P., Rauzon, M.J., Hayashi, L.A., Thomas, M.D., Robertson, D.R., Taylor, L. & Coste, M., 2013. Reef fishes of the East Indies. *Philosophy East and West,* 63(2): p. 1292

Andriyono, S. & Suciyono, S. 2020. Molecular Identification and Phylogenetic Tree Reconstruction of Marine Fish from the Essential Wetland Area of Banyuwangi, Indonesia. *Egypt. J. Aquat. Biol. Fish,* 24: 427-439. https://doi.org/10.21608/ejabf.2020.87032

Andriyono, S., Alam, M.J. & Kim, H.W. 2019. Environmental DNA (eDNA) metabarcoding: Diversity study around the Pondok Dadap fish landing station, Malang, Indonesia. *Biodivers. J.* 20(12): 3772-3781. https://doi.org/10.13057/biodiv/d201241

Andújar, C., Arribas, P., Yu, D.W., Vogler, A.P. & Emerson, B.C. 2018. Why the COI barcode should be the community DNA metabarcode for the metazoa. *Mol. Ecol.,* 27: 3968–3975 https://doi.org/10.1111/mec.14844

Atmaja, S.B. & Nugroho, D. 2017. Upaya-upaya pengelolaan sumber daya ikan yang berkelanjutan di Indonesia. *J. Kebijakan Perikanan Indonesia*, 3: 101-113. https://doi.org/10.15578/jkpi.3.2.2011.101-113

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

Coissac, E., Hollingsworth, P.M., Lavergne, S. & Taberlet, P. 2016. From barcodes to genomes: extending the concept of DNA barcoding. *Mol. Ecol.,* 25: 1423–1428 https://doi.org/10.1111/mec.13549

Fadli, N., Nor, S.A.M., Othman, A.S., Sofyan, H. & Muchlisin, 2020. DNA barcoding of commercially important reef fishes in Weh Island, Aceh, Indonesia. *Peer J.* 8: e9641. https://doi.org/10.7717/peerj.9641

Farhana, N.S., Muchlisin, Z.A., Duong, T.Y., Tanyaros, S., Page, L.M., Zhao, Y., Adamson, E.A., Khaironizam, M., de Bruyn, M. & Siti A.M.N., 2018. Exploring hidden diversity in Southeast Asia's Dermogenys spp. (Beloniformes: Zenarchopteridae) through DNA barcoding. *Sci. Rep.*, 8: 1-11. https://doi.org/10.1038/s415 9 8-018-29049-7

Halim, L.J., Rahim, I., Mahboob, S., Al-Ghanim, K.A., Asmiaty, A.M.A.T. & Naim, D.M. 2022. Phylogenetic relationships of the commercial red snapper (*Lutjanidae* sp.) from three marine regions. *J. King Saud. Univ. Sci.,* 34: p.101756. https://doi.org/10.1016/j.jksus.2021.101756

Halim, A., Loneragan, N.R., Wiryawan, B., Hordyk, A.R., Sondita, M.F.A. & Yulianto, I., 2020. Evaluating data-limited fisheries for grouper (Serranidae) and snapper (Lutjanidae) in the Coral Triangle, eastern Indonesia. *Reg. Stud. Mar. Sci.,* 38: p.101388. https://doi.org/10.1016/j.rsma.2020.101388

Iwatsuki, Y.U.K.I.O., Al-Mamry, J.M. & Heemstra, P.C. 2016. Validity of a blue stripe snapper, *Lutjanus octolineatus* (Cuvier 1828) and a related species, *L. bengalensis* (Bloch 1790) with a new species (Pisces; Lutjanidae) from the Arabian Sea. *Zootaxa*, 4098: 511-528. https://doi.org/10.11646/zootaxa.4098.3.5

Kress, W.J., García-Robledo, C., Uriarte, M. & Erickson, D.L., 2015. DNA barcodes for ecology, evolution, and conservation. *Trends Ecol. Evol.,* 30: 25-35. https://doi.org/10.1016/j.tree.2014.10.008

Kumar, S., Stecher, G., Li, M., Knyaz, C. & Tamura, K.. 2018. MEGA X: molecular evolutionary genetics analysis across computing platforms. *Mol. Biol. Evol.,* 35(6): p.1547. https://doi.org/10.1093/molbev/msy096

Li, Q., Li, M., Li, D., Shi, W., Li, J., Ning, T. & Duan, Y. 2016. Identification of the Larvae of Helicoverpa assulta and H. Armigera (Lepidoptera: Noctuidae) Through Morphological Difference and DNA Barcoding. *3rd Int. Conf. Smart Mat. Nanotechnol. Eng.* pp.306-309.

Luthfi, O.M., Pujarahayu, P., Wahyudiarto, A., Fakri, S.R., Sofyan, M., Ramadhan, F., Murian, S., Tovani, I., Mahmud, M., Adi, D. & Abdi, F. 2016. Biodiversitas dan populasi ikan karang di perairan selat sempu sendang biru kabupaten malang jawa timur. *J. Kelautan: Indonesian J. Mar. Sci. Technol.* 9: 43-49. https://doi.org/10.21107/jk.v9i1.1019

Madduppa, H., Ayuningtyas, R.U., Subhan, B. & Arafat, D. 2016. Exploited but unevaluated: DNA barcoding reveals skates and stingrays (Chordata, Chondrichthyes) species landed in the Indonesian fish market. *Ilmu Kelautan : Indonesian Journal of Marine Science,* 21: 77-84. https://doi.org/10.14710/ik.ijms.21.2.77-84

Miller, T.L. & Cribb, T.H. 2007. Phylogenetic relationships of some common Indo-Pacific snappers (Perciformes: Lutjanidae) based on mitochondrial DNA sequences, with comments on the taxonomic position of the Caesioninae. *Mol. Phylogenet. Evol.,* 44: 450-460. https://doi.org/10.1016/j.ympev.2006.10.029

Oktaviyani, S. 2018. Mengenal marga Lutjanus, salah satu komoditas unggulan dalam perikanan tangkap. *Oseana* 43: 29-39. https://doi.org/10.14203/oseana.2018.Vol.43No.3.61

Pentinsaari, M., Salmela, H., Mutanen, M. & Roslin, T. 2016. Molecular evolution of a widely-adopted taxonomic marker (COI) across the animal tree of life. *Sci. Rep.* 6: 1-12. https://doi.org/10.1038/srep35275

Powers, T., Harris, T., Higgins, R., Mullin, P. & Powers, K. 2018. Discovery and Identification of Species Using COI DNA Barcoding. *J. Nematol.* 50: 399-412. https://doi.org/10.21307/jofnem-2018-029

Prehadi, P., Sembiring, A., Kurniasih, E.M., Rahmad, R., Arafat, D., Subhan, B. & Madduppa, H.H. 2015. DNA barcoding and phylogenetic reconstruction of shark species landed in Muncar fisheries landing site in comparison with Southern Java fishing port. *Biodivers. J.,* 16(1): 55-61. https://doi.org/10.13057/biodiv/d160107

Rocha, L. & Bowen, B. 2008. Speciation in coral-reef fishes. *J. Fish Biol.* 72: 1101-1121. https://doi.org/10.1111/j.1095-8649.2007.01770.x

Sarkar, P., Islam, M.J., Habib, A.H.M., Neogi, A.K. & Habib, K.A. 2021. Two New Records of Snapper (Perciformes, Lutjanidae) from Saint Martin's Island, Bangladesh. *J. Ocean Univ. China,* 20: 439-444. https://doi.org/10.1007/s11802-021-4566-x

Simonsen, K.A., Cowan, J.H. & Boswell, K.M. 2015. Habitat differences in the feeding ecology of red snapper (*Lutjanus campechanus*, Poey 1860): a comparison between artificial and natural reefs in the northern Gulf of Mexico. *Environ. Biol. Fishes,* 98: 811-824. https://doi.org/10.1007/s10641-014-0317-9

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

Thi, O.T., Ha, Q.V.D. & Thuy, B.D. 2015. Phylogenetic relationships of emperors (Lethrinidae) and Snappers (Lutjanidae) in Vietnam based on mitochondrial DNA sequences. Pages 15-16. *Int. Conf. Biol. Environ. Food Eng. Singapore May.* 15-16 pages.

Tony, F., Soemarno, S., Wiadnya, D.G.R. & Hakim, L. 2020. Diversity of reef fish in Halang Melingkau Island, South Kalimantan, Indonesia. *Biodiv. J.* 21 (10): 4804-4812. https://doi.org/10.13057/biodiv/d211046.

Wibowo, A., Panggabean, A.S., Zamroni, A., Priatna, A. & Yusuf, H.N. 2018. Using DNA barcode to improve the identification of marine fish larvae, case study coastal water near Jakarta and Banda Sea, Indonesia. *Indones. Fish. Res. J.,* 24: 23-30. https://doi.org/10.15578/ifrj.24.1.2018.37-44