BIODIVERSITAS
Volume 25, Number 10, October 2024
Pages: 3500-3509

ISSN: 1412-033X
E-ISSN: 2085-4722
DOI: 10.13057/biodiv/d251012

Mitochondrial DNA of CO1 and 16S rRNA genes for diversity and phylogenetic analysis of sardine (*Sardinella* spp.) from the Lombok Straits, Indonesia

H. MAHRUS♥, AGIL AL IDRUS, ABDUL SYUKUR
Program Study of Biology Education, Department of Sciences Education, Faculty of Teacher Training and Education, Universitas Mataram. Jl. Majapahit No. 62, Mataram 83125, West Nusa Tenggara, Indonesia. Tel.: +62-370-633007, ♥email: mahrus@unram.ac.id

Manuscript received: 29 July 2024. Revision accepted: 9 October 2024.

Abstract. *Mahrus H, Idrus AA, Syukur A. 2024. Mitochondrial DNA of CO1 and 16S rRNA genes for diversity and phylogenetic analysis of sardine (*Sardinella *spp.) from the Lombok Straits, Indonesia. Biodiversitas 25: 3500-3509.* Sardines in Indonesia consist of six species from around 21 species- world, having many similarities and difficulty distinguishing between one species and others caused the taxonomy of the Sardines species is still ambiguous affected the same names in two different species and the different names in one species because of morphological similarities. The most effective method for identifying animal species and subspecies is the molecular method using sequences of the cytochrome c oxidase subunit 1 (CO1) gene and the 16S rRNA gene. The study aims to determine the molecular characteristics, genetic diversity, and phylogenetic relationships of sardines in the Lombok Strait using a sequence of CO1 and 16S rRNA genes. The study has significant practical implications as it can potentially inform conservation efforts and fisheries management. The sardine samples were collected from the Lombok Strait by small-scale fishermen. The sampling method was random. It takes 55 individuals as a sample. Initially, the sardine samples were grouped based on morphological characteristics. Subsequently, DNA from the two genes of 20 sardine samples were separated and amplified using two pairs of primers. The DNA amplification yielded 671 bp for the CO1 gene and 625 bp for the 16S rRNA gene. The sequence data from the two genes were analyzed using Molecular Evolutionary Genetics Analysis Version 11 (MEGA 11). The CO1 gene sequence revealed that sardines from the Lombok Strait could be determined more accurately than the 16S rRNA. CO1 rRNA genes of 12 individuals indicated a close genetic relationship between sardines of Lombok Strait and *Sardinella aurita* Valenciennes 1847, and the 16S rRNA gene placed sardines from Lombok Strait as an out-group. It empirically demonstrates that the CO1 gene is more effective than the 16S rRNA gene in identifying and determining molecular characteristics, genetic diversity, and phylogenetic relationships of sardines in Lombok Strait.

Keywords: Amplification, characteristics, molecular, morphological, *Sardinella aurita*, sequence

INTRODUCTION

Sardines and herrings family Clupeidae are small pelagic tropical marine fish with significant economic value because of their benefactions to global food security based on their abundance, easy access, and very high nutrient content (Saher et al. 2018). They originate from the Mediterranean Sea and are widely caught in Indonesian waters (Kartika et al. 2017; Hunnam 2021). Sardines are the third largest commodity after tuna and shrimp, but their production has declined in most Indonesian marine waters (Jaya et al. 2022). This decline is not unique to Indonesian waters but is a global phenomenon. Overexploitation, pollution, habitat destruction, and climate change are causing a significant decrease in sardine production worldwide (Martínez-Huitle and Panizza 2018; Suherman et al. 2020).

Sardinella lemuru Bleeker, 1853, *Amblygaster sirm*, *Sardinella atricauda* Günther 1868, *Sardinella longiceps* Valenciennes 1847, *Sardinella fimbriata* Valenciennes 1847, and *Sardinella clupeoides* Bleeker 1849, all collectively known as *lemuru* (Suherman et al. 2020; Hunnam 2021). For instance, *S. longiceps* is mainly caught in the Bali Strait and is also known as *Clupea (Harengula*) *longiceps* (C.V.), *S. lemuru*, Bali Sardinela (international name), and *S. lemuru* is the correct and recognize name In Indonesia (Mahrus et al. 2012; Sartimbul et al. 2023). The genetic diversity of sardines is a fascinating aspect of their biology, with slightly higher diversity than other marine fish, despite low genetic distance and variation between populations, such as *S. lemuru* in the Bali Strait, northern, and southeastern Java (Thomas et al. 2014; Sartimbul et al. 2018).

The other small pelagic fish, like anchovies (*Spratelloides delicatulus* Bennett 1832) caught in the waters south of Lombok, exhibit high diversity and excessive exploitation, leading to high adaptability to environmental conditions (Hendiari et al. 2020; Mahrus et al. 2022). The most well-known sardine area in Indonesia is the Bali Strait. *S. lemuru* Bleeker is a dominant small pelagic species experiencing productivity fluctuations (Tinungki and Sirajang 2019). The Bali Strait, between Java and Bali, connects the Bali Sea to the Indian Ocean. The Indonesian islands interconnect with the Pacific and Indian Oceans, making Indonesia's sea a place of interchange for both. The Bali Strait is close to the Lombok Strait (between Lombok and Bali) and links the Java Sea to the Indian Ocean as a water mass flow passage in the Indonesian waters. Water also occurs in the central Indian and Pacific Oceans along

the south coast of Sumatra-Java, and the southern waters of Lombok experience upwelling in the east season. This upwelling increases chlorophyll levels, enhancing water fertility and biomass productivity, including *Sardinella* spp. (Eisele et al. 2021).

The genetic diversity of *Sardine* is slightly higher than that of other marine fish, despite low genetic distance and variation between their populations, such as *S. lemuru* Bleeker 1853 in the Bali Strait, northern and southeast Java (Thomas et al. 2014; Sartimbul et al. 2018). However, *S. lemuru* and *S. aurita* in the Lombok Strait show many similarities based on the 12S rRNA gene sequence (Mahrus et al. 2012). Molecular techniques for species identification, such as DNA, are an alternative method (Devanand et al. 2020a). Studies have reported that the use of DNA barcoding with the cytochrome c oxidase subunit 1 (CO1) and 16S rRNA genes is effective for identifying species quickly and accurately (Wang et al. 2018; Dong et al. 2021). The CO1 and 16S rRNA genes exhibit low intraspecific variation but high interspecific variation, especially in closely related taxa (Choi et al. 2020; Chan et al. 2022). Fauna in Indonesia with DNA barcodes is minimal, including mammals, birds, Komodo dragons (*Varanus komodoensis* Ouwens, 1912), and insect pests (Rahayu and Jannah 2019; Chen et al. 2021). The CO1 gene is more functional than the 16S rRNA gene for DNA barcoding of Indian carp (Mohanty et al. 2015).

The diversity of *Sardinella* spp., both molecularly and morphologically, has sparked significant debates regarding taxonomy in recent years (Queiroz et al. 2020). The taxonomy of sardines worldwide consists of five genera and at least 21 unique species, which remains ambiguous (Thomas et al. 2014). The number and names of *Sardine* species in the Lombok Strait, based on molecular characters using CO1 and 16S rRNA genes, have not yet been reported, leading to taxonomic ambiguity. This research aims to determine the molecular characteristics, genetic diversity, and phylogenetic relationships of sardines in the Lombok Strait using CO1 and 16S rRNA gene sequences. This research will be crucial in elucidating these aspects (i) ambiguous taxonomy: (ii) number and name of sardines, molecular characteristics; and (iii) genetic diversity.

MATERIALS AND METHODS

Location of sampling and sample collection

Sardine sampling was conducted at five locations in the Lombok Strait waters, Indonesia (Figure 1). Samples of sardine caught by local fishing groups using surface gillnet and purse seine fishing gear from May 5 to August 30, 2023, amounted to 55 individuals and were sorted for the research using random methods. Next, it used 25 sardine samples (four sardines for each location) for molecular identification based on the consideration that the morphological characteristics of the sardine samples were generally the same. Sardine samples used for molecular genetic analysis were mantle tissue (±1 cm) cut using surgical scissors, placed into 5 mL sample tubes, preserved with 96% alcohol, and stored at the Immunology Laboratory, Faculty of Mathematics and Natural Sciences, Universitas Mataram. This method is almost the same as the method used by Eviasta et al. (2018).

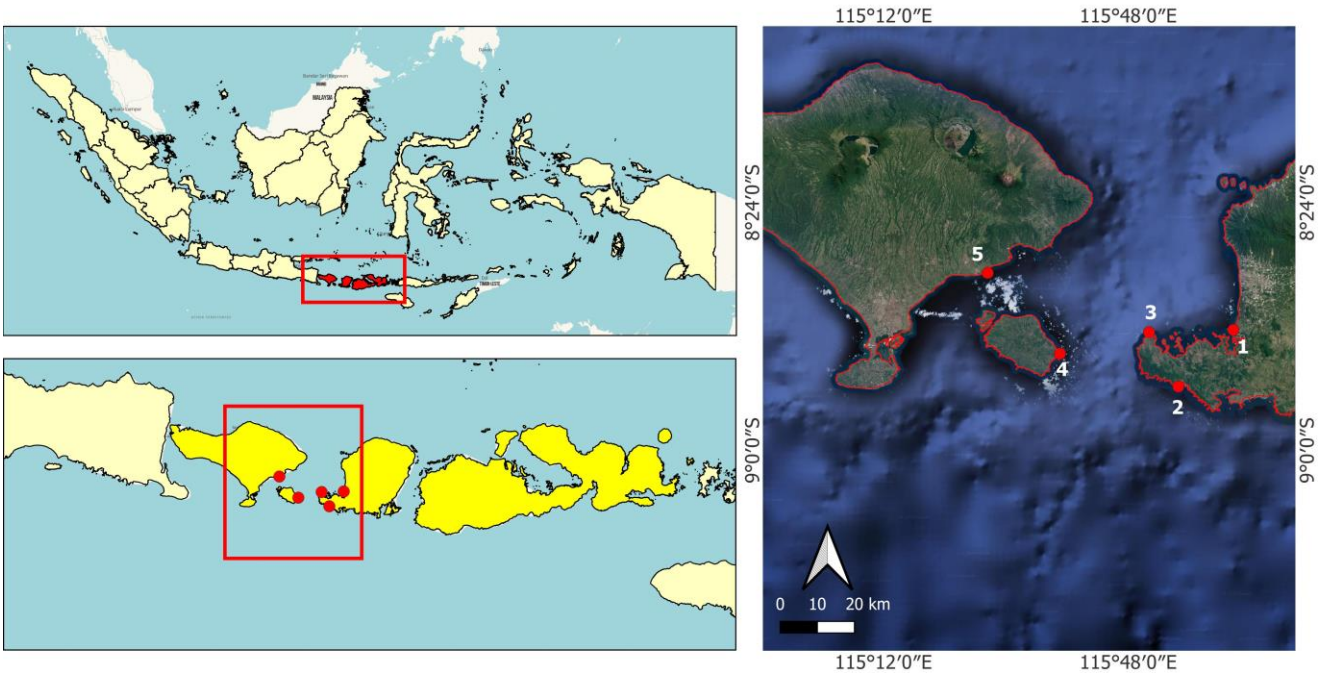

Figure 1. Sampling locations of sardines in Lombok Strait. The geographical positions of sampling locations included: 1: Cemare Beach (-8.7075,116.0574); 2: Mekaki Beach (-8.8450,115.9226); 3: Bangko-Bangko -8.7133,115.8508); 4: Sebele Beach (-8.7654,115.6325); 5: Kusamba Beach (-8.5699,115.4552)

Morphological character identification

Taxonomic identification was performed at the species level whenever possible. Generic diagnosis, including meristic counts and proportional measurements of collected specimens, was performed by following Sidiq et al. (2021). Morphological characters were observed and recorded for each sample on a premade datasheet. Determination of fish species nomenclature followed the Food and Agriculture Organization (FAO) Fish Identification Sheets (Keat-Chuan et al. 2017).

Molecular characteristics identification

This study used 25 samples for molecular characteristics identification. Firstly, fixation in alcohol 96% and stored in the Immunology Laboratory, Faculty of Sciences, Universitas Mataram for one month. Molecular identification in this study used CO1 and 16S gene sequences for 12 *Sardinella* spp.. These genes are currently the best solution for identifying various fish and other fauna species quickly and accurately (Bingpeng et al. 2018; Buckwalter et al. 2019). Additionally, these genes can identify species with conservative nucleotide base sequences that undergo only minor variations, deletions, and insertions (Miya et al. 2015; Phillips et al. 2019). This study used 12 *Sardinella* spp. to test genetic diversity and phylogenetic relationships. This group of fish is often called *lemuru* in Indonesia.

Extraction and amplification of CO1 and 16S gene DNA

Extraction and amplification of sardine DNA have the potential to serve as candidate DNA markers for sardine species. It can be obtained using a commercial kit (DNeasy Blood and Tissue Kit, Qiagen Cat. No. 69504) with several modifications according to tissue type or using a 10% Chelex solution (Barrientos-Villalobos and Schmitter-Soto 2019; Yang et al. 2022) at 95°C. The technique combines both methods to optimize DNA quality, especially when tissue samples are in a poor environment or dirty environmental conditions such as the existing pollution, contaminants-microbes, extreme temperatures, or uncontrolled biological hazards that have a crucial impact on DNA quality. Otherwise, the extraction process is challenging. The amplification of CO1 rRNA and 16S rRNA target genes utilized the universal primer pair, CO1 Fish F1: 5'-ATCTTTGGTGCATGAGCAGGAATAGT-3' and the Fish R2 primer: 5'-ACTTCAGGGTGACCGAAGAATCAGAA-3' (Nuryanto et al. 2019). Similarly, amplify a fragment of 16S rRNA using primers 16Sar-L: 5'-CGCCTGTTTATCAAAAACAT-3' and 16Sbr-H: 5'-CCGGTCTGAACTCAGATCACGT-3' (Thomas et al. 2014). DNA was extracted from a small piece of ethanol-preserved muscle tissue using a slightly modified method based on the standard DNA extraction protocol for fish (Hellberg et al. 2014).

In this study, the amplicon is approximately 600 bp from the 5' region of the mitochondrial CO1 and 16S DNA genes. Polymerase Chain Reaction (PCR) amplification conditions were as follows: denaturation for 5 minutes in 35 cycles at temperatures of 95°C (30 seconds), 50°C (30 seconds), and 72°C (50 seconds). The PCR reaction used a total volume of 50 µL containing 1 µL of template DNA, 10 mm Tris-HCl (pH 9), 50 mm KCl, 2 mm MgCl2, 0.2 µm of each primer, 0.2 mm of each dNTP, and 1 U Taq polymerase. PCR products were migrated using a 1% agarose gel, visualized under UV light, and documented using gel photographs. The PCR products were then sent to PT Genetica Science Indonesia for sequencing. The results of DNA sequencing of the CO1 and 16S genes in the form of AB1 files were sent to researchers through email. Next, a match was conducted between the electropherogram and the DNA sequence obtained in this study. The sequences obtained from a pair of CO1 primers were used as forward and reverse primers, each with reverse complementation to be the reverse. The next step was to align the two sequences using the W cluster menu (Katoh et al. 2019).

Interpretation of CO1 and 16S gene DNA sequence data

The consensus sequences obtained from Sanger sequencing were analyzed based on chromatogram peak clarities with the help of Chromas Lite. Sequences were complemented using the Basic Local Alignment Search Tool (BLAST) search engine by the National Center for Biotechnology Information (NCBI) and the Barcode of Life Data Systems (BOLD) database, and reciprocal BLAST through NCBI for further confirmation. Next, the CO1 and 16S gene DNA sequence data were analyzed using MEGA 11 Software, edited, and aligned using Clustal W to assess nucleotide base diversity (Tamura et al. 2021). The sequence analyses used references from various species belonging to the Clupeidae family from the NCBI GenBank. Sequence alignments were also subjected to a BLAST nucleotide search to determine their identity. CO1 and 16S gene sequence data for all sardine species available in GenBank were included in subsequent analyses using MEGA 11 Software (Tamura et al. 2021). This study elucidates the consensus sequences in each specimen, and the sequence differences within and between species were analyzed and tested using bootstrap tests with 1000 replications.

Phylogenetic analysis

CO1 and 16S rRNA gene sequences were aligned, and the optimal substitution model used MEGA 11 (Tamura et al. 2021). Phylogenetic trees were constructed based on the concatenated data of the two gene sequences using Neighbor-Joining (NJ) tree methods with 1000 bootstrap replicates. Genetic distance matrix calculations used the Kimura-2 parameter model implemented in pairwise distance calculation in MEGA 11 Software (Tamura et al. 2021).

Genetic variation analyses

Median-joining haplotype networks of CO1 and 16S rRNA sequences of 671 and 625 bp were constructed using DnaSP v6 (Rozas et al. 2017) for sardine specimens. Analysis of molecular variance (AMOVA) used Arlequin v3.5 (Azevedo et al. 2017). To assess genetic differentiation among several groups based on molecular characteristics. Specifically, grouping the specimens were clustered into four clades.

RESULTS AND DISCUSSION

Characteristics of sardines from Lombok Strait

Sardines are one of the species of Actinopterygii fish from the Clupeidae family (Figure 2). Morphological characteristics belonging to the sardine samples were a body that was round and elongated with a color that tended to be silver on the stomach and blackish on the dorsal part, a faint yellow line that extends from the posterior operculum to the base of the tail, a forked-shaped tail fin. They are found in Indonesia and often consist of *S. lemuru*, *S. atricauda*, *S. longiceps*, *S. firm*, *S. clupeoid*, and *S. leiogaster* (Birge et al. 2021; Hunnam et al. 2021). *S. lemuru* is a common and recognized name In Indonesia.

Extraction and amplification of CO1 and 16S rRNA gene fragments

DNA isolated from sardine tissue using a commercial kit (DNeasy Blood and Tissue Kit, Qiagen Cat. No. 69504) successfully indicated by the qualitative test results of 1% agarose gel electrophoresis. The qualitative DNA isolation test showed a thin band on the agarose gel. This success is similar to the isolation results in marine fish tissues reported by Pereira et al. (2016). A pair of universal CO1 primers, LCO-1490 and HCO-2198, were used to amplify the CO1 gene fragment from sardines originating from the Lombok Strait, while a pair of primers, 16Sar-L, and 16Sbr-L, were used to amplify the 16S rRNA gene. The CO1 and 16S rRNA gene fragments were successfully amplified, with amplicon lengths of approximately 671 bp and 625 bp, respectively (Figure 3). The size of the amplified CO1 and 16S rRNA gene amplicons is consistent with previous studies (Pereira et al. 2016). Amplicons of 671 and 625 bp can exhibit polymorphisms due to the possibility of intraspecies variation when using universal primers (Yang et al. 2014). The CO1 and 16S rRNA genes have the advantage that their universal primers are very robust, allowing them to recognize the 5' end of most animal groups, although not all. However, they represent most of the phylum Animalia (Gong et al. 2018).

Species identification runs online using GenBank data at NCBI (http://blast.ncbi.nlm.nih.gov/Blast.cgi) with various access codes using the BLAST method (Basic Local Alignment Search Tool). Species identification results based on CO1 and 16S rRNA gene sequences in GenBank included 12 individuals from sardine, family Clupeidae, consisting of 4 genera obtained from GenBank and one sample fish from Lombok Straits, as presented in Table 1. Next, DNA analysis was carried out by calculating the genetic distance between species and analyzing the phylogenetic tree of marine invertebrates using the NJ method with a 2-parameter Kimura model, bootstrap value 1000× (Tamura et al. 2021).

Nucleotide composition

The nucleotide composition found in the CO1 fragment of sardine from Lombok Strait is T=29.0%, C=31.1%, A=20.9%, and G=19.0%, with the nucleotide composition A+T=49.9% and G+C=50.1%. In comparison, the 16S rRNA fragment consists of T=22.8%, C=25.0%, A=28.7%, and G=23.5%, with the nucleotide composition A+T=51.5% and G+C=48.5%. The highest A+T nucleotide composition is in *H. dispilonotus* (52.7%) for the CO1 DNA fragment of the rRNA gene, while the highest G+C nucleotide composition is in *S. hualiensis* (53.2%). In contrast to the sardine 16S rRNA gene fragment, a high A+T nucleotide composition was found in *A. sirm* (53.1%), while the highest G+C nucleotide composition (52.6%) is in *S. fimbriata* (Table 2).

The fragment length of the CO1 and 16S rRNA of sardine ranged from 436 to 675 bp and 554 to 625 bp, respectively. A total of 12 individuals collected from the Lombok Strait were *Sardinella* spp.. The mean nucleotide composition among the sequences of 35 *Sardinella* spp. individuals was estimated as T=28.4±0.01%, C=30.7±1.07%, A=21.7±0.17%, and G=19.6±0.99%. The mean GC content is 50.4±1.4%. Nucleotide composition can support fish identification because differences in nucleotide composition indicate variations in nucleotide sites. These varied sites become specific characteristics that can differentiate fish species. According to Kombong and Arisuryanti (2018), differences in the nucleotide composition of the mitochondrial CO1 and 16S genes indicate genetic variation.

Figure 2. Sardine from Lombok Strait, Indonesia

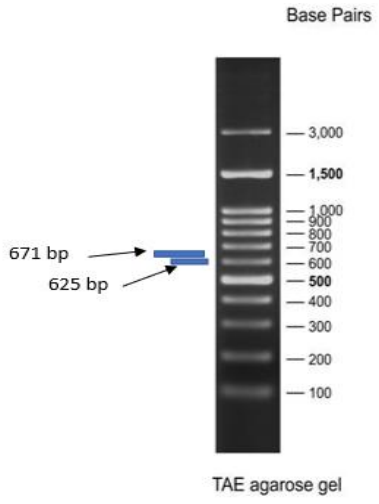

Figure 3. Qualitative test results from amplification on 1% agarose gel. M: Marker; COI gene: 671 bp; and 16S rRNA gene: 625 bp

Table 1. A total of twelve mitochondrial CO1 and 16S rRNA genes from sardine, family Clupeidae, consisting of four genera obtained from GenBank and one sample fish from Lombok Straits

Accession No.	Species name	Sequence length (bp)	Authors	Country
CO1 genes of twelve				
OQ387818.1	*Amblygaster sirm*	655	Bemis et al. 2023	Philippines
MW425682.1	*Escualosa thoracatax*	655	Khandan-Baraniand Alavi -Yeganeh 2022	Iran
KP001108.1	*Sardinella longiceps*	549	Sukumaran et al. 2016	India
MT293977.1	*Sardinella lemuru*	634	Labrador et al. 2021	Philippines
MN392930.1	*Sardinella gibbosa*	675	Devanand et al. 2018	India
OQ459661.1	*Sardinella aurita*	436	Turan et al. 2023	Northeastern Mediterranean
HQ231357.1	*Sardinella fimbriata*	650	Quilang et al. 2011	Philippines
OL409547.1	*Herklotsichthys quadrimaculatus*	633	Jaonalison et al. 2022	The eastern coast of Africa
OQ387855.1	*Herklotsichthys dispilonotus*	655	Bemis et al. 2023	Philippines
MT272807.1	*Sardinella maderensis*	645	Devanand et al. 2018	India
MK585640.1	*Sardinella hualiensis*	636	Chan et al. 2022	Philippines
	Sardine sample*	671	Present Study	Indonesia
16S rRNA gene of twelve				
KM518947.1	*Amblygaster sirm*	557	Thomas et al. 2014	Philippines
KM518949.1	*Escualosa thoracatax*	562	Thomas et al. 2014	Philippines
KX590730.1	*Sardinella longiceps*	564	Devanand et al. 2020b	India
KM518954.1	*Sardinella lemuru*	562	Thomas et al. 2014	India
KT323969.1	*Sardinella gibbosa*	616	Devanand et al. 2020a	India
KR824529.1	*Sardinella aurita*	578	Durand et al. 2010	Saudi Arabia
KM518950.1	*Sardinella fimbriata*	560	Thomas et al. 2014	Philippines
KM518959.1	*Herklotsichthys quadrimaculatus*	590	Thomas et al. 2014	Philippines
KM518957.1	*Herklotsichthys dispilonotus*	556	Thomas et al. 2014	Philippines
AM911205.1	*Sardinella maderensis*	568	Lavoué et al. 2007	France
KM518952.1	*Sardinella hualiensis*	554	Thomas et al. 2014	Philippines
	Sardine sample*	625	Present Study	Indonesia

Notes: *Fish sample from Lombok Strait of Indonesia

Table 2. Nucleotide composition (%) of CO1 and 16S rRNA gene fragments of twelve individuals from the Family Clupeidae under the Kimura 2-Parameter Model

Accession no.	Species name	T(U)	C	A	G	A+T	G+C
CO1 gene fragment							
OQ387818.1	*A. sirm*	27.6	31.1	22.8	18.5	50.4	49.6
MW425682.1	*E. thoracatax*	29.0	29.9	19.7	21.4	48.7	51.3
KP001108.1	*S. longiceps*	28.7	31.1	20.9	19.2	49.6	50.3
MT293977.1	*S. lemuru*	29.2	30.9	21.1	18.8	50.3	49.7
MN392930.1	*S. gibbosa*	26.1	31.8	22.3	19.7	48.4	51.5
OQ459661.1	*S. aurita*	29.5	31.4	20.2	19.0	49.7	50.4
HQ231357.1	*S. fimbriata*	28.5	29.2	22.1	20.2	50.6	49.4
OL409547.1	*H. quadrimaculatus*	28.3	31.1	19.7	20.9	48	52
OQ387855.1	*H. dispilonotus*	29.9	28.3	22.8	19.0	52.7	47.3
MT272807.1	*S. maderensis*	27.8	31.1	22.1	19.0	49.9	50.1
MK585640.1	*S. hualiensis*	27.3	31.8	19.5	21.4	46.8	53.2
	Sardine sample*	29.0	31.1	20.9	19.0	49.9	50.1
		28.4	30.7	21.2	19.6	49.5	50.4
16S rRNA gene fragment							
KM518947.1	*A. sirm*	23.5	23.3	29.6	23.7	53.1	47
KM518949.1	*E. thoracatax*	22.3	25.5	26.8	25.5	49.1	51
KX590730.1	*S. longiceps*	21.5	25.4	29.3	23.9	50.8	49.3
KM518954.1	*S. lemuru*	21.3	25.4	29.7	23.7	51	49.1
KT323969.1	*S. gibbosa*	21.0	26.2	28.1	24.7	49.1	50.9
KR824529.1	*S. aurita*	21.6	25.2	29.3	23.9	50.9	49.1
KM518950.1	*S. fimbriata*	20.6	26.6	26.8	26.0	47.4	52.6
KM518959.1	*H. quadrimaculatus*	21.2	26.4	28.3	24.2	49.5	50.6
KM518957.1	*H. dispilonotus*	19.9	26.6	29.6	24.0	49.5	50.6
AM911205.1	*S. maderensis*	22.8	25.2	27.5	24.5	50.3	49.7
KM518952.1	*S. hualiensis*	21.0	25.5	27.9	25.7	48.9	51.2
	Sardine sample*	22.8	25.0	28.7	23.5	51.5	48.5
		21.6	25.5	28.4	24.4	50.0	49.9

Note: *Fish sample from Lombok Strait of Indonesia

The genomes of fish and amphibians are GC homogeneous, whereas birds and mammals are AT/GC heterogeneous, and the exact reason for this phenomenon remains controversial. Data on GC/AT in the CO1 and 16S rRNA gene fragments is homogeneous, as shown in Figure 4, i.e., 50.4-49.9% and 49.5- 50%, respectively. These results are supported and compatible with previous research on teleost fish genomes (Symonová and Suh 2019).

Genetic distance

The genetic distance ranges from 0.01 to 0.33 in CO1 and 0.00 to 0.38 in the 16S rRNA gene (Table 3). The highest intergeneric divergence (different genera) was between *A. sirm* and S. *aurita* and sardine sample* and between sardine sample* and *H. quadrimaculatus* (0.32) in the CO1 gene. In contrast, the highest intergeneric divergence in the 16S rRNA fragment (0.33) was between *A. sirm* and sardine sample*. The lowest intergeneric divergence in CO1 was between *S. lemuru* and *H. dispilonotus* (0.19), while in 16S, it exists between *S. gibbosa* and *H. dispilonotus* (0.03). In contrast, the highest intrageneric distance (within a genus) is between sardine sample* and *S. maderensis* (0.38) exists in the 16S rRNA gene.

Table 3. Estimates of Pairwise Genetic Distances and Standard Error of twelve individuals from the Clupeidae family under the Kimura 2-Parameter Model in CO1 and 16S rRNA genes

Accession no.	1	2	3	4	5	6	7	8	9	10	11	12
CO1 gene												
A. sirm												
E. thoracatax	0.26											
S. longiceps	0.26	0.23										
S. lemuru	0.26	0.21	0.01									
S. gibbosa	0.21	0.25	0.17	0.18								
S. aurita	0.32	0.31	0.27	0.26	0.27							
S. fimbriata	0.24	0.30	0.23	0.22	0.20	0.33						
H. quadrimaculatus	0.30	0.29	0.26	0.25	0.25	0.28	0.27					
H. dispilonotus	0.28	0.28	0.21	0.19	0.25	0.30	0.26	0.26				
S. maderensis	0.22	0.24	0.18	0.17	0.12	0.28	0.19	0.23	0.23			
S. hualiensis	0.31	0.27	0.22	0.21	0.21	0.28	0.23	0.24	0.25	0.18		
Sardine sample*	0.32	0.31	0.26	0.25	0.27	0.26	0.29	0.32	0.30	0.29	0.30	
16S rRNA												
A. sirm												
E. thoracatax	0.14											
S. longiceps	0.19	0.15										
S. lemuru	0.19	0.15	0.00									
S. gibbose	0.19	0.15	0.07	0.08								
S. aurita	0.18	0.15	0.01	0.01	0.07							
S. fimbriata	0.21	0.17	0.12	0.13	0.12	0.13						
H. quadrimaculatus	0.17	0.13	0.05	0.05	0.03	0.05	0.10					
H. dispilonotus	0.19	0.13	0.08	0.08	0.06	0.08	0.11	0.05				
S. maderensis	0.24	0.20	0.19	0.18	0.21	0.18	0.21	0.18	0.18			
S. hualiensis	0.21	0.16	0.09	0.09	0.10	0.09	0.09	0.08	0.11	0.21		
Sardine sample*	0.33	0.29	0.27	0.27	0.26	0.26	0.28	0.26	0.27	0.38	0.29	

*Fish sample from Lombok Strait of Indonesia

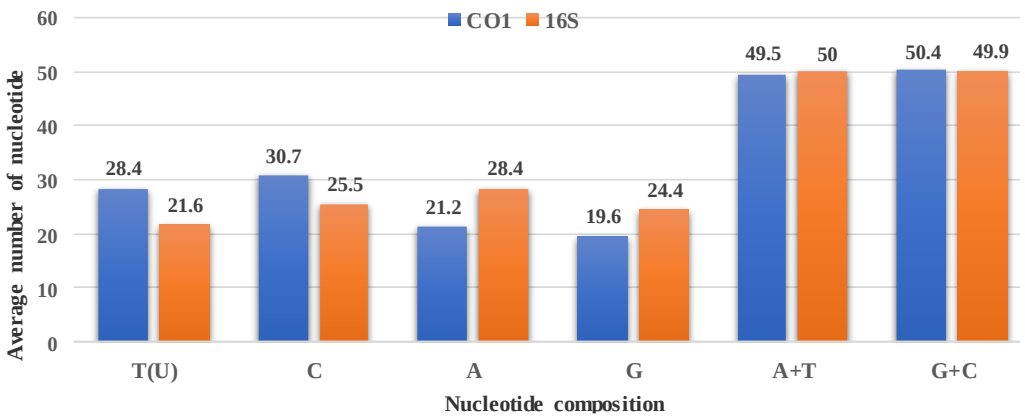

Figure 4. Nucleotide Composition of 12 individuals of *Sardine* based on CO1 and 16S rRNA gene sequences

Genetic distance measures the evolutionary divergence between copies of homologous genes that share a common ancestor (Dogan and Dogan 2016; Shirk et al. 2017). The results of genetic distance analysis found that the lowest genetic distance (0.01) based on CO1 fragments occurred in *S. lemuru* and *S. longiceps*. The same applies to the 16S rRNA fragment, with a genetic distance of 0.00. According to Shirk et al. (2017), the two sardine species are closely related or the same kind. Apart from that, the genetic distance of all individuals ranges between 0.17 and 0.32 in the CO1 fragment, and the 16S rRNA fragment has a genetic distance ranging between 0.01 and 0.38. The highest genetic distance in the CO1 fragment occurred between sardine (fish from Lombok Strait) and *A. sirm* (0.32), while in the 16S rRNA fragment, it occurred between sardine (fish from Lombok Strait) and *S. maderensis* (0.38). Additionally, sardines (fish from Lombok Strait) in the 16S rRNA fragment are outside the Clupeidae family group. Genetic distance lower than 3% in a phylogenetic relationship can vary species or other sets of sister lineages (Rosselló-Móra and Amann 2015; Mallik et al. 2019).

The maximum and minimum genetic distances of the sardines family were recorded as 0.38 to 0.00 in the Clupeidae; the CO1 fragment has a genetic distance from 0.33 to 0.01 and 0.38 to 0.00 in the 16S fragment. This study differed from preliminary research reporting that average heterozygosity ranged from 0.3009 to 0.3744 (Mahboob et al. 2019). This information on genetic polymorphism is helpful for authorities developing strategies to conserve the diversity of tilapia in the country.

Phylogenetic construction

The NJ tree revealed distinct clades separated based on the genus of *Sardinella* family Clupeidae. The clades showed high bootstrap values ranging from 45 to 97%, a strong indication of the accuracy of our research. Most of these sequences matched reference sequences on GenBank or BOLD databases with more than 99% identity, further validating our findings. The overall mean distance of the sardine sequences was 25% for fragment CO1 and 16% for fragment 16S rRNA. The intraspecific K2P divergences ranged from 1 to 32% for fragment gen CO1 rRNA, while for the 16S rRNA fragment gene, it ranged from 0.00 to 38%. It is in the same clade as *H. dispilonotus*, *S. longiceps*, and *S. lemuru*, not found in the results of phylogenetic analysis using the 16S gene sequence. Analysis based on the phylogenetic tree shows that all sardine members of the family Clupeidae are monophyletic and grouped into four genera, namely *Sardinella*, *Amblygaster*, *Escualosa*, and *Herklotsichthys*. The preliminary study is the same as *Cardiidae*, *Tridacninae*, and *Hippopus* reported by researchers (Findra et al. 2017). The reconstruction of the phylogenetic tree in Figure 5(a) confirms that sardine (from Lombok Straits) and *S. aurita* are the same species based on the CO1 gene sequence, whereas, based on the 16S rRNA gene in Figure 5.B, sardine is outside the group. The results of nucleotide alignment along the 671 bp mitochondrial DNA CO1 gene fragment support this identification result. The twelve individual fish in the Clupeidae family are shown in Figures 5.A and Figure 5.B consisted of four clades based on the CO1 and 16S rRNA gene sequences.

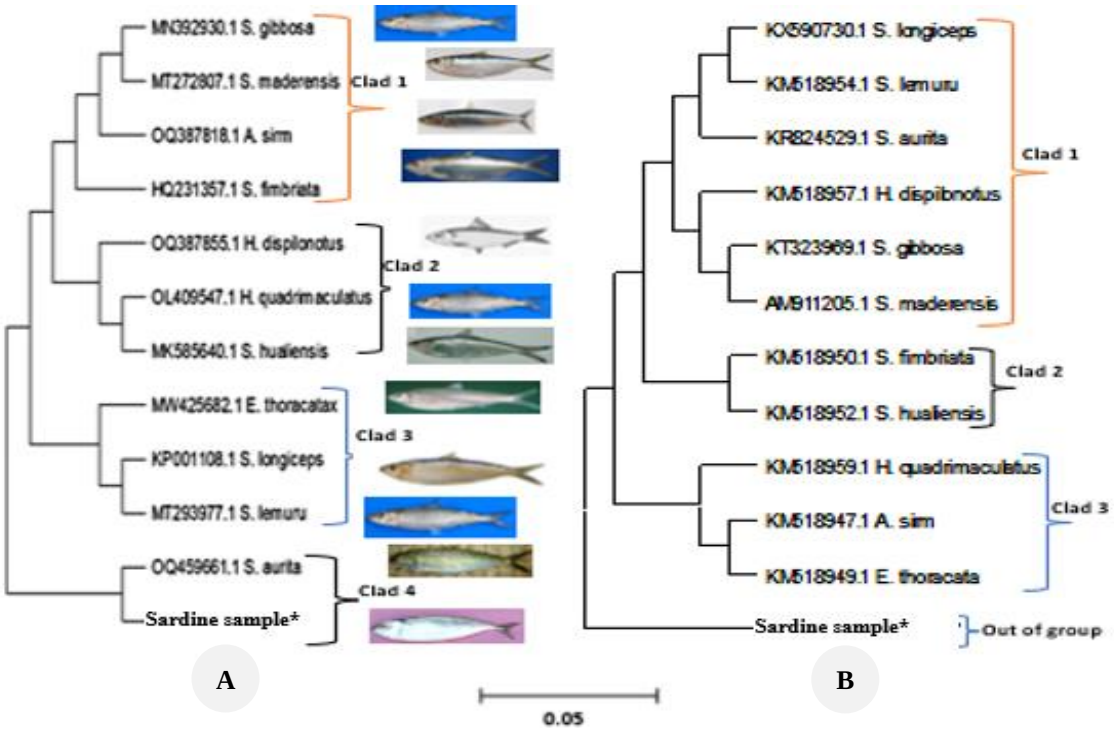

Figure 5. Phylogenetic consensus tree of 12 sardine species constructed based on CO1 and 16S rRNA genes NJ Method. A. NJ tree of mtDNA CO1 rRNA; B. NJ tree of mtDNA 16S rRNA. *Fish samples from Lombok Strait and others from various countries acceded from GenBank

The four clades in Figure 5.A are as follows: Clade 1 includes *S. gibbosa*, *S. maderensis*, *S. fimbriata*, and *A. sirm*; Clade 2 includes *H. dispilonotus*, *S. longiceps*, and *S. lemuru*; Clade 3 includes *H. quadrimaculatus* and *S. hualensis*, and Clade 4 includes *S. aurita* and a sardine from Lombok Strait. In Figure 5(b), the clades are: Clade 1 includes *S. longiceps*, *S. lemuru*, *S. aurita*, *H. dispilonotus*, *S. gibbosa*, and *S. maderensis*; Clade 2 includes *S. fimbriata* and *S. hualiensis*; Clade 3 includes *H. quadrimaculatus*, *A. firm*, and *E. thoracic*; and Clade 4 includes only the sardine from the Lombok Strait. A notable difference appears in the 16S rRNA gene fragment in Figure 5.B, where the sardine from the Lombok Strait is outside the group. In contrast, the CO1 fragments from Lombok Strait are in the same clade as *S. aurita*, indicating a close relationship.

A phylogenetic NJ tree using K2P distances illustrated CO1-based genetic divergence among intra and inter-specific hierarchical units. All species were determined using the CO1 gene. Bootstrap analysis consensus trees with 1000 replicates obtained from NJ (Figure 5) and MP showed similar patterns in the genetic relationships among the sardine species studied. There was no haplotype sharing or overlapping. According to data on haplotype diversity (Hd) using DNAsp, the Hd was 0.95455 based on the CO1 gene sequence and 0.95238 for the 16S rRNA gene, indicating high genetic diversity among sardines (Shirk et al. 2017). The phylogenetic trees used the haplotypes for CO1 and 16S rRNA genes, which could unambiguously differentiate all twelve species of wild sardines in marine waters.

Population structure based on CO1 and 16S rRNA gene sequences can be used to control population structure in gene expression analysis (Fachrul et al. 2023). The results of this study, which show a low population structure with high genetic diversity, are similar to findings on an economically important fish species (*Lutjanus jocu* Bloch and Schneider 1801) reported by Tovar Verba et al. (2023). Preliminary research also reported similar cases of low population structure and high genetic diversity in *Rhinolophus blasii* Peters 1867 in Europe (Jakab et al. 2021). Factors influencing low population structure include the availability of limited food, predators, competition with other species or conspecifics, climate, habitat changes, and disease (Zulfikar et al. 2020; Tovar Verba et al. 2023).

In conclusion, CO1 and 16S rRNA gene fragments were successfully amplified from sardine species in the Lombok Strait, with amplicon lengths of approximately 671 bp and 625 bp, respectively. The study indicates that the mitochondrial DNA CO1 gene sequence can identify wild sardine species with elevated levels of genetic divergence (0.0009-1.15). In contrast, the 16S rRNA gene is less effective due to lower levels of genetic divergence (0.000-0.0018). The study also reveals strikingly high genetic diversity in sardines, with a Hd of 0.95455 based on the CO1 gene sequence and 0.95238 for the 16S rRNA gene, indicating high genetic diversity among sardines. However, the population structure is low for the CO1 and 16S rRNA genes, with values of 0.04549 and -0.09473, respectively. The results of this study indicate that CO1 is more effective than 16S rRNA for determining molecular characteristics, genetic diversity, and kinship in sardines from the Lombok Strait waters. The implications are as follows: (i) It can determine the complete name of sardine species based on the CO1 gene sequence; (ii) The survival of the sardine population because the high category of genetic diversity allows wild sardine populations to survive, reproduce, and adapt to environmental changes in the short and long term; (iii) Determine the policy for managing sardine resources to provide and maintain sustainable national food availability.

ACKNOWLEDGEMENTS

The authors are grateful to the rector of Universitas Mataram, Mataram, West Nusa Tenggara, Indonesia for the facilities and financial assistance. Special thanks to the dean of the Faculty of Sciences and the head of the Immunology Laboratory, particularly laboratory assistant Sitti Rosida, for her significant help in laboratory activities such as isolation, extraction, and PCR to obtain DNA CO1 and 16S rRNA genes.

REFERENCES

Azevedo RS, Bitencourt A, Silva DA, Amorim A, Mazzoni R, Carvalho EF, Amaral CR. 2017. Genetic diversity of *Geophagus brasiliensis* from the South American Atlantic Rainforest. Forensic Sci Intl Genet Suppl Ser 26: e433-e434. DOI: 10.1016/j.fsigss.2017.09.149.

Barrientos-Villalobos J, Schmitter-Soto JJ. 2019. Phylogeography of the Mayan cichlid *Mayaheros urophthalmus* (Teleostei: Cichlidae) in the Yucatan peninsula based on mitochondrial markers CYTB and COI. Environ Biol Fish 102: 1461-1472. DOI: 10.1007/s10641-019-00920-4.

Bemis KE, Girard MG, Santos MD, Carpenter KE, Deeds JR, Pitassy DE, Flores NA, Hunter ES, Driskell AC, Macdonald III KS, Weigt LA. 2023. Biodiversity of Philippine marine fishes: A DNA barcode reference library based on voucher specimens. Sci Data 10 (1): 411. DOI: 10.1038/s41597-023-02306-9.

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.

Birge TL, Ralph GM, Di Dario F, Munroe TA, Bullock RW, Maxwell SM, Santos MD, Hata H, Carpenter KE. 2021. Global conservation status of the world's most prominent forage fishes (Teleostei: Clupeiformes). Biol Conserv 253: 108903-2021-01. DOI: 1016/j.biocon.2020.108903.

Buckwalter J, Angermeier PL, Argentina J, Wolf S, Floyd S, Hallerman EM. 2019. The drift of larval darters (Family Percidae) in the upper Roanoke River basin, USA, characterized using phenotypic and DNA barcoding markers. Fishes 4 (4): 59. DOI: 10.3390/fishes4040059.

Chan KO, Hertwig ST, Neokleous DN, Flury JM, Brown RM. 2022. Widely used, short 16S rRNA mitochondrial gene fragments yield poor and erratic results in phylogenetic estimation and species delimitation of amphibians. BMC Ecol Evol 22: 37. DOI: 10.1186/s12862-022-01994-y.

Chen C, Ding Y, Jiang Z, Jiang H, Lu C, Zhang L, Chen Z, Zhu C. 2021. DNA barcoding of yellow croakers (*Larimichthys* spp.) and morphologically similar fish species for authentication. Food Control 127: 108087. DOI: 10.1016/j.foodcont.2021.108087.

Choi EH, Kim G, Cha SH, Lee JS, Ryu SH, Suk HY, Lee YS, Baek SY, Hwang UW. 2020. Molecular phylogenetic, population genetic, and demographic studies of *Nodularia douglasiae* and *Nodularia breviconcha* based on CO1 and 16S rRNA. Sci Rep 10 (1): 16572. DOI: 10.1038/s41598-020-72015-5.

Devanand TN, Anjanayappa HN, Kumar G, Naveen Kumar BT, Gopan A, Panda K. 2020a. A comparative study of COI and 16S rRNA genes

for DNA barcoding of Indian marine clupeids. J Exp Zool India 23 (2): 1439-1444.
Devanand TN, Kumar G, Anjanayappa HN, Naveen Kumar BT, Soman C, Panda K. 2020b. Identification of *Coilia dussumieri* and *Sardinella gibbosa* from Mangaluru waters using 16S rRNA gene. J Exp Zool India 23 (2): 1355-1360.
Devanand TN, Naveenkumar BT, Soman C, Anjanayappa HN, Naik M, Sathish RP, Kagalgomb A. 2018. DNA barcoding of *Coilia dussumieri* and *Sardinella gibbosa* using Cytochrome C oxidase sub unit I (CO1): A mitochondrial gene. J Exp Zool India 21: 1207-1211.
Dogan İ, Dogan N. 2016. Genetic distance measures. Turkiye Klinikleri J Biostat 8 (1): 87-93. DOI: 10.5336/biostatic.2015-49517.
Dong D, Gan Z, Li X. 2021. Descriptions of eleven new species of squat lobsters (Crustacea: Anomura) from seamounts around the Yap and Mariana Trenches with notes on DNA barcodes and phylogeny. Zool J Linn Soc 192 (2): 306-355. DOI: 10.1093/zoolinnean/zlab003.
Durand JD, Diatta MA, Diop K, Trape S. 2010. Multiplex 16S rRNA haplotype-specific PCR, a rapid and convenient method for fish species identification: An application to West African Clupeiform larvae. Mol Ecol Resour 10 (3): 568-72. DOI: 10.1111/j.1755-0998.2009.02776.x.
Eisele MH, Madrigal-Mora SM. 2021. Drivers of reef fish assemblages in an upwelling region from the Eastern Tropical Pacific Ocean. J Fish Biol 98 (4): 1074-1090. DOI: 10.1111/jfb.14639.
Eviasta IW, Boer M, Butet NA. 2018. Kajian stok ikan teri (*Stolephorus commersonnii* Lacepede, 1803) di Teluk Palabuhanratu, Sukabumi, Jawa Barat. J Trop Fish Manag 2 (2): 61. DOI: 10.29244/jppt.v2i2.26322. [Indonesian]
Fachrul M, Karkey A, Shakya M, Judd LM, Harshegyi T, Sim KS, Tonks S, Dongol S, Shrestha R, Salim A. 2023. Direct inference and control of genetic population structure from RNA sequencing data. Commun Biol 6 (1): 804. DOI: 10.1038/s42003-023-05171-9.
Findra MN, Setyobudiandi I, Butet NA, Solihin DD. 2017. Genetic profile assessment of giant clam genus *Tridacna* as a basis for resource management at Wakatobi National Park Waters. Ilmu Kelautan: Indones J Mar Sci 22 (2): 67-74. DOI: 10.14710/ik.ijms.22.2.67-74.
Gong S, Ding Y, Wang Y, Jiang G, Zhu C. 2018. Advances in DNA barcoding of toxic marine organisms. Intl J Mol Sci 19 (10): 2931. DOI: 10.3390/ijms19102931.
Hellberg RS, Kawalek MD, Van KT, Shen Y, Williams-Hill DM. 2014. Comparison of DNA extraction and PCR setup methods for use in high-throughput DNA barcoding of fish species. Food Anal Methods 7: 1950-1959. DOI: 10.1007/s12161-014-9865-z.
Hendiari IG, Sartimbul A, Arthana IW, Kartika GR. 2020. Keragaman genetik ikan *lemuru* (*Sardinella lemuru*) di wilayah perairan Indonesia. Acta Aquatica: Aquat Sci 7: 28-36. DOI: 10.29103/aa.v7i1.2405. [Indonesian]
Hunnam K, Carlos I, Hammer MP, Dos Reis Lopes J, Mills DJ, Stacey N. 2021. Untangling tales of tropical sardines: Local knowledge from fisheries in Timor-Leste. Front Mar Sci 8: 673173. DOI: 10.3389/fmars.2021.673173.
Hunnam K. 2021. The biology and ecology of tropical marine sardines and herrings in Indo-West Pacific fisheries: A review. Rev Fish Biol Fish 31 (3): 449-84. DOI: 10.1007/s11160-021-09649-9.
Jakab E, Bücs S, Jére C, Csösz I, Jakab RI, Szodoray-Parádi F, Popescu O. 2021. Low population structure and genetic diversity in *Rhinolophus blasii* at the northern limit of its European range: Are there undiscovered colonies?. Acta Chiropterol 23 (2): 301-311. DOI: 10.3161/15081109ACC2021.23.2.003.
Jaonalison H, Durand JD, Mahafina J, Valade P, Collet A, Cerqueira F, Ponton D. 2022. Application of DNA barcoding for monitoring Madagascar fish biodiversity in coastal areas. Diversity 14 (5): 377. DOI: 10.3390/d14050377.
Jaya I, Satria F, Nugroho D, Sadiyah L, Buchary EA, White AT, Franklin EC, Courtney CA, Green G, Green SJ. 2022. Are the working principles of fisheries management at work in Indonesia?. Mar Pol 140 (2-3): 105047. DOI: 10.1016/j.marpol.2022.105047.
Kartika GR, Sartimbul A, Widodo W. 2017. Varian genetik *Sardinella lemuru* di perairan Selat Bali. Jurnal Kelautan: Indones J Mar Sci 10 (1): 21-28. DOI: 10.21107/jk.v10i1.1615.
Katoh K, Rozewicki J, Yamada KD. 2019. MAFFT online service: Multiple sequence alignment, interactive sequence choice and visualization. Brief Bioinform 20 (4): 1160-1166. DOI: 10.1093/bib/bbx108.
Keat-Chuan Ng C, Aun-Chuan Ooi P, Wong WL, Khoo G. 2017. A review of fish taxonomy conventions and species identification techniques. J Surv Fish Sci 4 (1): 54-93. DOI: 10.17762/sfs.v4i1.140.
Khandan-Barani H, Alavi-Yeganeh MS. 2022. First record of white sardine *Escualosa thoracata* (Valenciennes, 1847) (Clupeiformes: Clupeidae) from the Oman Sea. Acta Zool Bulg 74 (1): 147-150.
Kombong CB, Arisuryanti T. 2018. The 16S and COI mitochondrial DNA nucleotide composition of stripped snakehead (*Channa striata* Bloch, 1793) from Lake Sentani, Jayapura, Papua. Jurnal Perikanan Universitas Gadjah Mada 20 (2): 57-62. DOI: 10.22146/jfs.35551.
Labrador K, Agmata A, Palermo JD, Ravago-Gotanco R, Pante MJ. 2021. Mitochondrial DNA reveals genetically structured haplogroups of Bali sardinella (*Sardinella lemuru*) in Philippine waters. Reg Stud Mar Sci 41: 101588. DOI: 10.1016/j.rsma.2020.101588.
Lavoué S, Miya M, Saitoh K, Ishiguro NB, Nishida M. 2007. Phylogenetic relationships among anchovies, sardines, herrings and their relatives (Clupeiformes), inferred from whole mitogenome sequences. Mol Phylogenet Evol 43 (3): 1096-105. DOI: 10.1016/j.ympev.2006.09.018.
Mahboob S, Al-Ghanim KA, Al-Misned F, Al-Balawi HA, Ashraf A, Al-Mulhim NM. 2019. Genetic diversity in tilapia populations in a freshwater reservoir assayed by randomly amplified polymorphic DNA markers. Saudi J Biol Sci 26 (2): 363-367. DOI: 10.1016/j.sjbs.2018.11.015.
Mahrus H, Al Idrus AG, Zulkifli L. 2022. Molecular phylogeny of anchovy (Clupeiformes: Clupeidae) from southern waters of Lombok using mitochondrial DNA CO1 gene sequences. Biodiversitas 23 (5): 2433-2443. DOI: 10.13057/biodiv/d230522.
Mahrus, Sumitro SB, Utomo DH, Sartimbul A, Toha AH, Widodo N. 2012. Genetic relationship of *Sardinella lemuru* from Lombok Strait with fish rich in omega-3 fatty acid. Bioinformation 8 (25): 1271-1276. DOI: 10.6026/97320630081271.
Mallik AK, Achyuthan NS, Ganesh SR, Pal SP, Vijayakumar SP, Shanker K. 2019. Discovery of a deeply divergent new lineage of vine snake (Colubridae: Ahaetuliinae: *Proahaetulla* gen. nov.) from the southern Western Ghats of Peninsular India with a revised key for Ahaetuliinae. PLoS One 14 (7): e0218851. DOI: 10.1371/journal.pone.0218851.
Martínez-Huitle CA, Panizza M. 2018. Electrochemical oxidation of organic pollutants for wastewater treatment. Curr Opin Electrochem 11: 62-71. DOI: 10.1016/j.coelec.2018.07.010.
Miya S, Takahashi H, Nakagawa M, Kuda T, Igimi S, Kimura B. 2015. Genetic characteristics of Japanese clinical *Listeria monocytogenes* isolates. PLoS One 10: e0122902. DOI: 10.1371/journal.pone.0122902.
Mohanty M, Jayasankar P, Sahoo L, Das P. 2015. A comparative study of COI and 16 S rRNA genes for DNA barcoding of cultivable carps in India. Mitochondrial DNA 26 (1): 79-87. DOI: 10.3109/19401736.2013.823172.
Nuryanto A, Dewi RE, Pramono H. 2019. Genetic homogeneity of commerson's anchovy (*Stolephorus commersonnii*) in Segara Anakan Cilacap Central Java Inferred from PCR-RFLP Marker. Biogenesis 7 (1): 14-23. DOI: 10.24252/bio.v7i1.6352.
Pereira LL, Damasceno JD, Leandro ED. 2016. Comparison of DNA extraction protocols for different marine fish tissues. Pan-Am J Aquat Sci 11 (2): 135-142.
Phillips JD, Gillis DJ, Hanner RH. 2019. Incomplete estimates of genetic diversity within species: Implications for DNA barcoding. Ecol Evol 9 (5): 2996-3010. DOI: 10.1002/ece3.4757.
Queiroz CD, Souza RF, Silva SS, de Mattos Dias CA, Fecury AA, de Oliveira E, da Cunha DB, Schneider H, Sampaio I. 2020. Molecular phylogeny of Clupeiformes and the placement of some Western Atlantic and Amazonian taxa. Biota Amazônia 10 (2): 14-19. DOI: 10.18561/2179-5746/biotaamazonia.v10n2p14-19.
Quilang JP, Santos BS, Ong PS, Basiao ZU, Fontanilla IK, Cao EP. 2011. DNA barcoding of the Philippine endemic freshwater sardine *Sardinella tawilis* (Clupeiformes: Clupeidae) and its marine relatives. Philipp Agric Sci 94 (3): 248-257.
Rahayu DA, Jannah M. 2019. DNA barcode hewan dan tumbuhan Indonesia. Yayasan Inspirasi Ide Berdaya. DOI: 10.17605/OSF.IO/TY7E5. [Indonesian]
Rosselló-Móra R, Amann R. 2015. Past and future species definitions for Bacteria and Archaea. Syst Appl Microbiol 38 (4): 209-216. DOI: 10.1016/j.syapm.2015.02.001.
Rozas J, Ferrer-Mata A, Sánchez-DelBarrio JC, Guirao-Rico S, Librado P, Ramos-Onsins SE, Sánchez-Gracia A. 2017. DnaSP 6: DNA sequence polymorphism analysis of large data sets. Mol Evol 34 (12): 3299-302. DOI: 10.1093/molbev/msx248.

Saher NU, Niazi RM, Qureshi NA. 2018. Occurrence and abundance of commercially important fish species of family Clupeidae and Engraulidae in the MianiHor, Sonmiani Bay, Balochistan. Indian J Geo-Mar Sci 47 (03): 702-711.

Sartimbul A, Nakata H, Herawati EY, Rohadi E, Yona D, Harlyan LI, Putri AD, Winata VA, Khasanah RI, Arifin Z, Susanto RD. 2023. Monsoonal variation and its impact on the feeding habit of Bali Sardinella (*S. lemuru* Bleeker, 1853) in Bali Strait. Deep-Sea Res PT II 211: 105317. DOI: 10.1016/j.dsr2.2023.105317.

Sartimbul A, Rohadi E, Ikhsani SN, Listiyaningsih D. 2018. Morphometric and meristic variations among five populations of *Sardinella lemuru* Bleeker, 1853 from waters of Bali Strait, northern and southern-east Java and their relation to the environment. AACL Bioflux 11 (3): 744-752.

Shirk AJ, Landguth EL, Cushman SA. 2017. A comparison of individual-based genetic distance metrics for landscape genetics. Mol Ecol Resour 17 (6): 1308-1317. DOI: 10.1111/1755-0998.12684.

Sidiq M, Ahmed I, Bakhtiyar Y. 2021. Length-weight relationship, morphometric characters, and meristic counts of the coldwater fish (Heckel) from Dal Lake. Fish Aquat Life 29 (1): 29-34. DOI: 10.2478/aopf-2021-0003.

Suherman AS, Boesono H, Kurohman F, Muzakir AK. 2020. Kinerja Pelabuhan Perikanan Nusantara (PPN) Pengambengan Jembrana Bali. Indones J Fish Sci Technol 16 (2): 123-131. DOI: 10.14710/ijfst.16.2.123-131. [Indonesian]

Sukumaran S, Sebastian W, Gopalakrishnan A. 2016. Population genetic structure of Indian oil sardine, *Sardinella longiceps* along Indian coast. Gene 576 (1): 372-378. DOI: 10.1016/j.gene.2015.10.043.

Symonová R, Suh A. 2019. Nucleotide composition of transposable elements likely contributes to AT/GC compositional homogeneity of teleost fish genomes. Mobile DNA 10: 1-8. DOI: 10.1186/s13100-019-0195-y.

Tamura K, Stecher G, Kumar S. 2021. MEGA11: Molecular Evolutionary Genetics Analysis version 11. Mol Biol Evol 38 (7): 3022-3027. DOI: 10.1093/molbev/msab120.

Thomas Jr RC, Willette DA, Carpenter KE, Santos MD. 2014. Hidden diversity in sardines: Genetic and morphological evidence for cryptic species in the goldstripe sardinella, *Sardinella gibbosa* (Bleeker, 1849). PLoS One 9: e84719. DOI: 10.1371/journal.pone.0084719.

Tinungki GM, Sirajang N. 2019. The dynamic system analysis of *lemuru* fishery in Bali strait by using biological parameter yield of some surplus production models. IOP Conf Ser: Earth Environ Sci 279 (1): 012054. DOI: 10.1088/1755-1315/279/1/012054.

Tovar Verba J, Stow A, Bein B, Pennino MG, Lopes PF, Ferreira BP, Mortier M, Maia Queiroz Lima S, Pereira RJ. 2023. Low population genetic structure is consistent with high habitat connectivity in a commercially important fish species (*Lutjanus jocu*). Mar Biol 170: 5. DOI: 10.1007/s00227-022-04149-1.

Turan C, Uyan A, Atalay MA, Dogdu SA, Ayas D, Erguden D, Turan F, Gokoglu M, Gurlek M. 2023. Genetic differentiation of round sardinella *Sardinella aurita* (Clupeidae) populations from the Northeastern Mediterranean. J Ichthyol 63 (5): 962-968. DOI: 10.1134/S0032945223050119.

Wang ZL, Yang XQ, Wang T Z, Yu X. 2018. Assessing the effectiveness of mitochondrial COI and 16S rRNA genes for DNA barcoding of farmland spiders in China. Mitochondrial DNA Part A 29 (5): 695-702. DOI: 10.1080/24701394.2017.1350949.

Yang CH, Wang TW, Ng PK, Chan TY, Lin YY, Chan BK. 2022. Population genetic differentiation on the hydrothermal vent crabs *Xenograpsus testudinatus* along depth and geographical gradients in the Western pacific. Diversity 14 (3): 162. DOI: 10.3390/d14030162.

Yang L, Tan Z, Wang D, Xue L, Guan MX, Huang T, Li R. 2014. Species identification through mitochondrial rRNA genetic analysis. Sci Rep 4 (1): 4089. DOI: 10.1038/srep04089.

Zulfikar, Arisoesilaningsih E, Indriani S, Fernandes AAR. 2020. Community structure of fruit tree species on successful marginal land conservation in Jombang Regency, East Java Province, Indonesia. J Phys Conf Ser 1563: 012025. DOI: 10.1088/1742-6596/1563/1/012025.