

Molecular Characterization and Phylogenetic Relationships of Sardine (*Sardinella lemuru*) in the Coastal Waters of East Lombok

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ABSTRACT

Sardinella lemuru, a member of the herring family, is a commercially important fish species due to its rich omega-3 content. This study aims to investigate genetic diversity within and between *S. lemuru* populations along the coast of East Lombok using mitochondrial cytochrome c oxidase I (*MT-COI*) DNA sequences. For this purpose DNA extracted from 20 *S. lemuru* specimens was amplified for *COI* rRNA marker gene using a universal primer pair to establish a 650 bp fragment sequence. The *COI* genetic code analysis of *S. lemuru* was conducted using Molecular Evolutionary Genetics Analysis (MEGA) version 12 to validate data. Phylogenetic analysis using *COI* gene sequences revealed that *S. lemuru* along the eastern beaches of Lombok was determined by the *COI* gene from a set of 12. The genetic diversity of the 12 *S. lemuru* individuals was high (0.985), indicating that the *S. lemuru* sample has a better ability to adapt to environmental changes, disease, or ecological pressures.

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Authors' Contribution

Mahrus formulated the research design and wrote the initial manuscript for publication. LZ analyzed the research data and supervised the research process. AAI collected fish samples and conducted laboratory work. AS cross-checked the manuscript, edited the language and revised it. All authors read and approved the final manuscript.

Key words

COI, Phylogenetic analysis, *S. lemuru*, Sequence

INTRODUCTION

The *Sardinella lemuru* population in Indonesian waters is highly diverse and exhibits a low-to-moderate population structure, indicating significant gene flow and connectivity among different regional groups (Sartimbul *et al.*, 2023). Indonesian Throughflow (ITF), a major system of ocean currents, plays a critical role in facilitating this genetic connectivity by physically moving fish larvae and adults across southern Java and potentially assisting mixing with populations from the Philippines and Western Pacific. The geographic position of the Indonesian Archipelago on Earth links two seas, namely the Magellan Sea and

the Erythraean Sea, which impacts the exchange of water masses in Indonesia's waters. Compared to the Magellan Sea, the Erythraean Ocean has higher average water temperatures. Local weather and ocean currents cause temperatures to vary.

As Pacific waters transition into the Indian Ocean through the Indonesian archipelago, the resulting nutrient-rich upwellings create an ideal environment for thousands of fish species, assessing upwards of 33,000 extant fish species. Subsequently, the majority of the specimens collected were assigned to the families Pomacentridae (wrasses), Gobidae, Cardinalfishes, Butterflyfish, Groupers, Teuthididae, Snapper, Jacks, and Parrotfishes. Approximately 48.9% of all documented species are represented by these families. The local ichthyofauna is predominantly composed of reef-associated species, alongside pelagic and benthic fish found in offshore zones.

Lombok Island, situated to the east of Bali, serves as a crucial aquatic corridor linking the Java Sea to the Indian Ocean and is widely recognized as a dominant main water flow passage in the Indonesian Sea. Upwelling along the south Java-Sumatra Indian Ocean coastline and seasonal monsoon winds cause upward movement of cold, highly

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saline, and nourishing water masses in the southern waters of Lombok Island. This ecological event enhances water fertility and chlorophyll concentration, which support greater biofuel fish and make them plentiful (Hadibasyir and Wibowo, 2023; Dabuleviciene *et al.*, 2023).

The sea waters of East Lombok are primarily the Alas Strait, a narrow channel separating Lombok from Sumbawa to the east, while the larger Bali Sea lies to the north and the Indian Ocean to the south, featuring diverse waters, coral reefs, and small islands like Gili Maringkik, Gili Bidara, Gili Pasir, Gili Sulat, etc. known for pristine beaches and marine life (Susanto *et al.*, 2021; Karang *et al.*, 2025).

The Genus *Sardinella*, of the sardine family, which starts in the Middle Sea, is a tiny pelagic marine finfish with similar economic contexts (Hunnam, 2021). *Sardinella lemuru*, in Indonesian waters, is Indonesia's third-largest fishery commodity following shrimp and tuna, even though production volumes have become endangered over the past ten years by climate change, habitat degradation, pollution, and intense over-exploitation, similar to other exploited small pelagics like anchovies (*Spratelloides delicatulus*) in the marine south of Lombok, emphasizing the need for sustainable management of these economically crucial fish stocks (Mahrus *et al.*, 2022). The enormous *S. lemuru* region of Indonesia is in the Bali Channel, near the Lombok Channel, influenced by *S. lemuru* Bleeker 1853. It comprises five divisions, and fewer than 21 species are mysterious due to striking similarities that make it difficult to distinguish one species from another (Elyasigorji *et al.*, 2023).

The *S. lemuru* of Indonesia is known by several synonyms, including *Clupea nymphaea*, *Amblygaster posterus*, *S. lemuru* Bker 1853, *S. atricauda*, *S. longiceps*, *S. fimbriata*, and *S. clupeoides*, all of which are commonly referred to as lemuru in Indonesia (Hunnam, 2021; Mahrus *et al.*, 2025). *S. lemuru* shows slightly higher genetic diversity than other fish, despite having low variation within its own community; for example, *S. lemuru* Bker 1853 of the Bali Strait and northerly and southerly east Java, suggesting unique evolutionary patterns for this *S. lemuru* species, which researchers study for genetic insights (Winata *et al.*, 2022; Sartimbul *et al.*, 2023). Utilizing the mitochondrial *COI* gene has proven to be a powerful and efficient approach for the precise identification and characterization of animal life (Kowalczyk *et al.*, 2021; Elyasigorji *et al.*, 2023).

The mitochondrial *COI* gene exhibits minimal variation within a single species (intraspecific) yet demonstrates substantial differences between distinct species (interspecific), making it particularly useful for distinguishing between closely related groups (Choi *et al.*, 2020).

Indonesia's fauna DNA barcode collection, managed by the National Research and Innovation Agency, includes Mammalian, avian species, *Varanus komodoensis*, and pest insects, though the number of Universal Product Codes is still low, showing that scientists are still testing how artificial intelligence can help study the biodiversity of water life. The molecular technique using DNA barcoding, the mitochondrial *COI* gene, is another way to study hard-to-tell species and provides a new option for tricky species; it works as a backup plan for hidden species (Hadebe *et al.*, 2023; Laojun *et al.*, 2023).

Molecular studies applying mitochondrial *COI* or additional genes are needed to clarify the exact number and identities of *S. lemuru* kinds present in Lombok, leaving their taxonomy ambiguous. Due to taxonomic uncertainty, this research aims to validate *S. lemuru* and determine genetic diversity within and between *S. lemuru* populations along the coast of East Lombok, employing the mitochondrial *COI* gene sequence. It is located along the east Lombok shoreline, province of West Nusa Tenggara, and has an area close to 222 km², with 220 km of shoreline and an ocean zone of 1,074.33 km² (Qur'ani *et al.*, 2019). Small-scale fisheries and aquaculture make critical contributions to employment, with over 41 million people worldwide utilizing fish resources optimally.

In the future, fish genetic diversity is crucial for sardine population survival and fisheries management, including environmental adaptation and disease resistance. Overseeing the genetic diversity of the sardine is important; hence, this study is vital to be conducted. This study aims to evaluate the genetic population diversity of *S. lemuru* along the coast of East Lombok using the mitochondrial *COI* gene. These findings provide baseline information to support future monitoring, conservation, and management of *S. lemuru* fisheries ecosystems along the East Lombok coast.

MATERIALS AND METHODS

Fish collection and morphological identification

The study took place from April to December 2025. Samples of fish were collected from the waters off the coast of East Lombok, which were divided into three sites: *¹Labuhan Lombok (116°678' West Longitude, -8°499' South Latitude), *²Labuhan Haji (116°575' West Longitude, -8°695' South Latitude), and *³Tanjung Luar (116°5' West Longitude, -8°778' South Latitude).

Fishermen collected the fish samples from the waters surrounding East Lombok. The fish were first identified in the Biology Laboratory of the Education Faculty of the University of Mataram. Next, the fish were categorized according to their morphological traits. About 100

specimens were gathered over a period of four months in 2025, encompassing the southeast monsoon (June to August (n= 50)) and the inter-monsoon period, from September to October (n=50).

The morphological characteristics that were measured included total length (TL), branching length (FL), standard length (SL), preanal length (PAL), predorsal length (PDL), preventral length (PVL), pre-pectoral length (PPL), body depth (BD), head length (HL), eye diameter (EyeL), and pre-orbital length (pre-OrbL). Additionally, meristic traits and additional supportive morphological markers were identified, such as the number of fin rays, caudal fin ratio, and fin spines (Kowalczyk *et al.*, 2021; Elyasigorji *et al.*, 2023). A similarity index based on a number of morphometric and meristic ratios was used to conduct morphological identification. Using a clustering method with the Braycurtis similarity coefficient and a bootstrap cycle of 1,000 iterations, similarity index analysis was carried out using PAST 3.1 software (Hadebe *et al.*, 2023; Laojun *et al.*, 2023).

PCR amplification and Sequencing of mitochondrial COI gene

To identify the *S. lemuru*, muscle tissues were preserved in 95% ethanol within 1.6 mL Eppendorf tubes. DNA was extracted using a 10% Chelex method (Gautam, 2022). Subsequently, a 655 bp fragment of the *COI* gene was amplified via PCR using universal primers (Nuryanto *et al.*, 2019; Sartimbul *et al.*, 2023; Jafar *et al.*, 2024), followed by gel electrophoresis and sequencing:

F: 5'-TCAACCAACCACAAAGACATTGGCAC-3', and
R: 5'-TAGACTTCTGGGTGGCCAAAGAATCA-3'

The PCR protocol was adjusted to utilize 2 µL of DNA template (Gautam, 2022).

DNAzure® Blue Nucleic Acid Gel Stain (Biotium, USA) was used to visualize amplified products on a 1% agarose gel (Promega, USA). Subsequently, the PCR products underwent Sanger sequencing at the DNA Sequencing Facility, Genetika Science Indonesia, Jakarta.

Data analysis

Molecular characterization was conducted on the mitochondrial *COI* gene sequencing data through the MEGA12 platform (Kumar *et al.*, 2024). Sequence comparison of the mitochondrial *COI* gene was performed using NCBI's BLASTn tool, matching specimens to the public nucleotide database. Phylogenetic reconstruction was performed using the maximum likelihood (ML) method (Kulikov *et al.*, 2024). Two configuration options, the Kimura-2 parameter substitution model and pairwise deletion, were selected for phylogenetic and genetic

distance analyses (Mahadani *et al.*, 2022). Goloboff *et al.* (2022) showed that only above branches with a probability value of 50% show the percentage of replicate trees that clustered together related taxa in a bootstrap test (10.000 replicates).

The median haplotype network of the mitochondrial *COI* gene sequences is approximately 650 bp. It was reconstructed using DNASP V6 for *S. lemuru* specimens (Rozas *et al.*, 2017). Analysis of molecular variance (AMOVA) was done using Arlequin V3.5 (Huang *et al.*, 2021) to evaluate genetic differences among multiple groups using molecular traits. Several reference genetic diversity datasets were collected from various species within the genus *Sardinella* in other waters, primarily from scientific Journal articles, and compared with *S. lemuru* along the coast of East Lombok, West Nusa Tenggara.

RESULTS AND DISCUSSION

Morphological and molecular characteristics of *S. lemuru*

S. lemuru, known as a little pelagic angle that lives in groups in tropical and subtropical oceans, has imperative financial esteem commercially, characterized by a slim shimmering body with unmistakable scales and a terminal mouth, and is broadly utilized within the canning and utilization industries. The morphological characteristics of *S. lemuru* from along the coast of East Lombok in Figure 1A are the same as those of sardines found in Timor-Leste and in Indo-West Pacific fisheries. They had a terminal mouth and a slender, shimmering body with distinctive scales (Hunnam, 2021, 2022). Figure 1B shows the amplicon of the mitochondrial *COI* gene (650 base pairs).

The research sample from the coast of East Lombok has a sequence that is 99.3% identical to the DNA barcode segment of *S. aurita*, according to an analysis of sequence similarity using the GenBank database via NCBI and the BOLD System. The results of this analysis are similar to the results of the analysis reported in the study of *S. lemuru* at the Tanjung Luar Fish Landing Center, East Lombok (Mahrus *et al.*, 2025).

The mitochondrial DNA *COI* gene is a marker for population determination. In the middle of the subjective *COI* quality mitochondrial DNA segregation test, a lean band was discovered on the 1% agarose gel. Twelve *S. lemuru* species within the Standardized Identification of Life Data Systems (Strong) databases were followed for mitochondrial *COI* gene quality arrangement; this shows that not all Fundamental Nearby Arrangement Look Instrument (Impact) arrangements are DNA of the same length in the test groups (Table I).

Table I. Analysis of BLAST to 12 *COI* gene mitochondrial DNA species from the Clupeidae Family, the genus *Sardinella*, obtained from the NCBI.

No.	Registration number	Species name	Chain size	Authors	Country
1	MN392930.1	<i>S. gibbosa</i>	675 bp	Bhaskar 2019.	India
2	OQ387818.1	<i>A. sirm</i>	655 bp	Bemis <i>et al.</i> , 2023	USA
3	HQ231357.1	<i>S. fimbriata</i>	650 bp	Quilang <i>et al.</i> , 2010	Philippine
4	MN586892.1	<i>S. sindensis</i>	570 bp	Kumar <i>et al.</i> , 2019	India
5	OQ552973.1	<i>S. jussieu</i>	655 bp	Jiang <i>et al.</i> , 2023	China
6	KP001108.1	<i>S. longiceps</i>	549 bp	Sukumaran <i>et al.</i> , 2014	India
7	MT293977.1	<i>S. lemuru</i>	634 bp	Labrador <i>et al.</i> , 2021	Philippine
8	LOTIM2025	<i>S. sample*</i>	650 bp	Present Study	Indonesia
9	OR145142.1	<i>S. melanura</i>	652 bp	Kongchum, 2023	Thailand
10	JQ365537.1	<i>Sardinella</i> sp.	652 bp	Ribeiro <i>et al.</i> , 2012	Brazil
11	OQ459661.1	<i>S. aurita</i>	436 bp	Uyan <i>et al.</i> , 2023	Mediterranean
12	OK445670.1	<i>S. albella</i>	652 bp	Afrand <i>et al.</i> , 2021	Iran

The asterisk (*) indicates Sardine samples from the coast of East Lombok

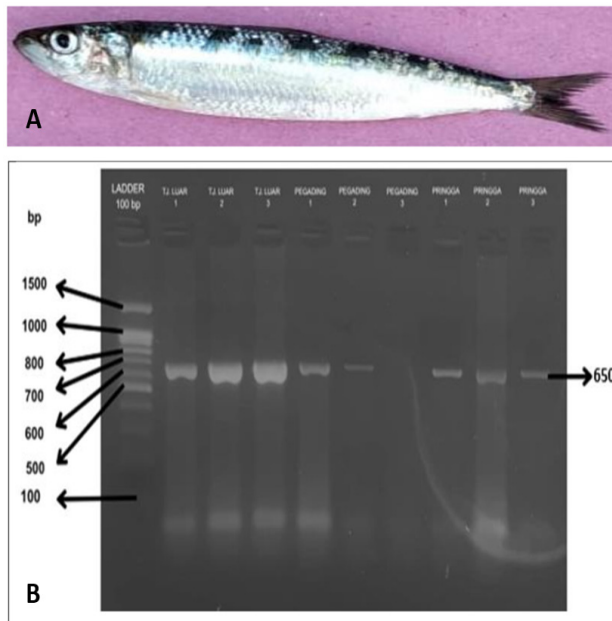


Fig. 1. *S. lemuru* from the coast of East Lombok (A) and visualization of *COI* gene PCR amplification of *S. lemuru* collected from the coast of East Lombok (B).

Analysis of the *Sardinella* sample collected along the coast of East Lombok, using the NCBI database and the barcode of life data systems (BOLD Systems), revealed sequence homology. The sample's sequence showed similarity with the mitochondrial *COI* gene of *S. aurita*. According to NCBI and BOLD, the similarity

between 95.00% and 100.00% and 95.00% and 99.00%, respectively. Sequences from the BOLD structure exhibit 100% correctness and 99% reliability for *Sardinella* spp. compared to GenBank. The research sample from the coast of East Lombok has a sequence that is 99.5% identical to the mitochondrial *COI* gene of *S. aurita*, according to an analysis of sequence homology of the GenBank-NCBI and the BOLD system. The 650 bp DNA sequence guidelines for 216 alpha-amino acids, with the enlarged area situated at specific gene positions.

Phylogenetic analysis

A phylogenetic tree based on maximum likelihood estimation using K2P distances illustrated mitochondrial *COI* gene-based genetic divergence among intra- and interspecific hierarchical units. All species were determined using the *COI* gene. Bootstrap analysis: Consensus trees with 10.000 were replicates obtained from maximum likelihood (ML) in Figure 2. All lemuru species in this phylogenetic tree form 4 clades in Figure 3, as follows: Clade A includes *S. melanura*, *S. albella*, and *S. fimbriata*; Clade B includes *S. jussieu*, *S. gibbosa*, and *S. sindensis*; Clade C includes *Sardinella* sample, and *S. aurita*; and Clade D includes *S. longiceps*, *S. lemuru*, and *Sardinella* sp. from the long coast of East Lombok.

As shown in Figure 2, the phylogenetic tree reveals four distinct clades for the lemuru species: Clade A consists of *S. melanura*, *S. albella*, and *S. fimbriata*; Clade B contains *S. jussieu*, *S. gibbosa*, and *S. sindensis*; Clade C is composed of the *Sardinella* sample and *S. aurita*; and

Clade D comprises *S. longiceps*, *S. lemuru*, and *Sardinella* sp. from along the coast of East Lombok. In the same case, the *COI* fragments from the long coat of East Lombok are in the same clade as *S. aurita* in the Lombok Strait, indicating a close relationship (Mahrus *et al.*, 2024).

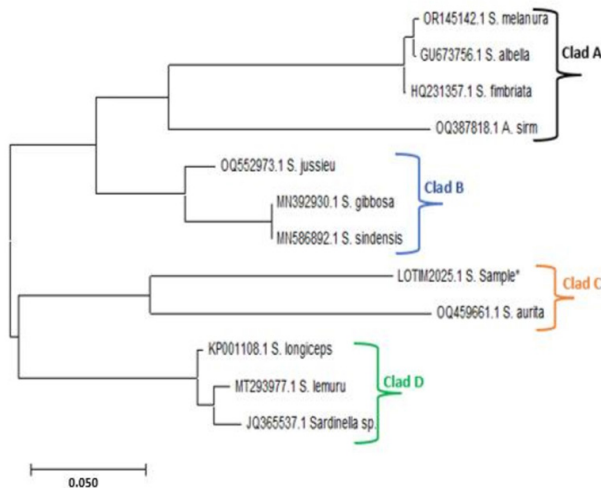


Fig. 2. Maximum-likelihood (ML) phylogenetic tree of 12 *COI* gene sequences of *S. lemuru* using the Kimura-2 model, with a bootstrap value of 10,000 replicates. The asterisk (*) indicates Sardine samples from the coast of East Lombok.

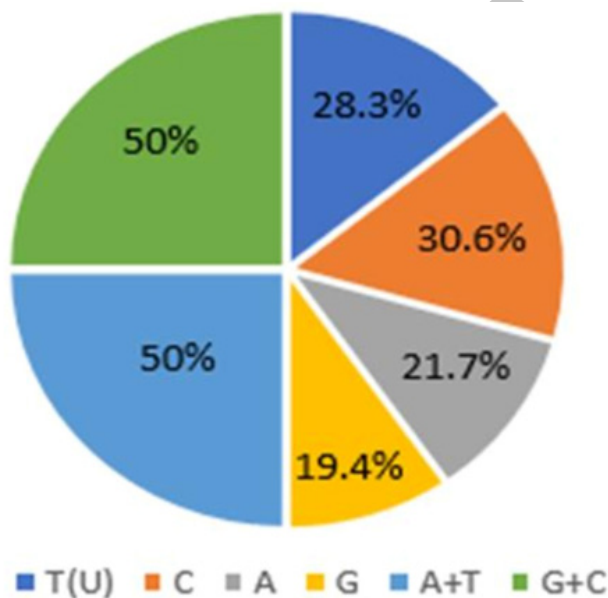


Fig. 3. *COI* gene arrangement of 12 species of *S. lemuru*.

The evolutionary tree reconstruction in Figure 2 verifies that *S. lemuru* along the coast of East Lombok and

Sardinella spp. are in the same clade as *S. aurita* and have a very close relationship. Similar results from a previous study in the Lombok Strait using a different gene fragment, the *12S* gene, found that the lemuru (*Sardinella* samples) and *S. aurita* are very closely related (Mahrus *et al.*, 2012).

There is no overlap in the linked set of alleles. The mean of allele combination variety, as determined by DNAsp, is 0.9743, indicating high genetic variety in *S. lemuru*. However, population composition is small ($F_{st} = 0.37489$). Phylogeny using the *COI*-linked set of alleles can clearly distinguish all 14 wild species in seawater. Population composition has a role in analyzing gene expression within populations (Fachrul *et al.*, 2023). Molecular data evaluation is necessary to identify broad phylogenetic relationships and population composition studies. within a kind, and reconciling taxonomic conflicts, particularly in lemuru, and has proven to be crucial (Devanand *et al.*, 2020). It has been suggested that the *COI* gene, as a DNA barcoding tool, can help taxonomists. In addition to conventional taxonomy, it can help solve taxonomic ambiguities, particularly in lemuru.

The results showed a small population composition with a rich gene pool, analogous to that of another commercially important fish species (*Lutjanus jocu*), as stated by Tovar-Verba *et al.* (2023). Initial findings also indicated analogous occurrence of small population composition and gene diversity in Blasius's horseshoe bat (*Rhinolophus blasii*) in Europe (Jakab *et al.*, 2021). Aspects influencing low population composition include limited food availability, predation, competition with conspecifics or diverse species, weather, environmental destruction, and illness (Fisher *et al.*, 2021). Furthermore, fundamental differences and coloration in these fish were too negligible to indicate a new species. Furthermore, larval dispersal, the movement of juveniles, and the movement of actively moving mature fish can lead to genetic connections between species or populations in different areas.

The entire range of genes and alleles found in a species or population is known as genetic diversity, and it serves as the basis for adaptation, survival, and evolution. While low genetic diversity increases the risk of extinction due to illness or environmental changes, high genetic diversity promotes ecosystem resilience. It is crucial for food security and conservation. According to the data in Table II on haplotype diversity (H_d) using DNAsp, the H_d was 0.98485 based on the *COI* gene sequence, indicating high genetic diversity among sardines, and there was no haplotype sharing or overlapping.

The high haplotype diversity ($H_d = 0.98485$) observed in the *COI* gene sequence (Table II) indicates that the *S. lemuru* population possesses high genetic variation (Shirk *et al.*, 2017). *S. lemuru* in the waters along the coast of East

Lombok have the highest nucleotide diversity in clade C (*Sardinella* sample and *S. aurita*) of 0.19002; the lowest haplotype diversity (hd) was found in clade B (*S. jussieu*, *S. gibbosa*, *S. sindensis*) of 0.66667, while clades A, C, and D have the same haplotype diversity of 1.00000. The highest number of haplotypes found in the waters along the coast of East Lombok was 4 haplotypes in clade 1 (*S. melanura*, *S. albella*, *S. fimbriata*, and *A. sirm*). The genetic diversity of the lemuru fish is similar to the genetic diversity of the Bali *Sardinella* (*Sardinella lemuru*) in the Bali Strait (Sartimbul *et al.*, 2025).

Table II. Genetic diversity of *S. lemuru* from the beach of East Lombok on the basis of the *COI* gene.

Clad	Genetic diversity			
	n	Hn	Hd	π
A	4	4	1.00000	0.09422
B	3	2	0.66667	0.03325
C	2	2	1.00000	0.19002
D	3	3	1.00000	0.01900
Total population	12	11	0.98485	0.15637

The genetic differentiation of *COI* gene varies between 0.00 and 0.29 (Table III). The overall average genetic diversity of 12 *S. lemuru* is 0.18. The *COI* gene showed the greatest intergeneric divergence (different genera) between *A. sirm* and *S. aurita* and the *Sardinella* sample, as well as between the *Sardinella* sample (0.25).

The evolutionary divergence between copies of homologous genes that have a shared origin is evaluated by genetic distance (Shirk *et al.*, 2017). *S. gibbosa* and *S. sindensis*, *S. melanura* and *S. fimbriata*, and *S. albella* and *S. fimbriata* had the lowest genetic distance (0.00), according to the genetic distance study. In the mitochondrial *COI* gene fragment, *Sardinella* sp. and *S. melanura* showed the most genetic distance (0.29). The maximum and minimum genetic distances for the Clupeidae family were found to be between 0.00 and 0.29, whereas the *COI* fragment has a genetic distance between 0.29 and 0.00. On the other hand, this study found that the mean genetic diversity ranged from 0.3009 to 0.3744 (Mahboob *et al.*, 2019). This genetic diversity information is very useful for creating plans to preserve genetic fish diversity.

Nucleotide composition

The fragment length of the *COI* of *S. lemuru* varies between limits of 436 and 688 bp. Comparison of DNA arrangement in 12 species of *S. lemuru* according to *COI* DNA arrangement is uniform (Table IV). The precise cause of this occurrence is still up for debate; the genomes of fish and amphibians are GC-homogeneous, while those of birds and mammals are AT/GC-heterogeneous. Figure 3 illustrates the homogeneity of the GC/AT data in the *COI* gene fragments, which are 50% and 50%, respectively.

The average nucleotide content of 12 distinct *S. lemuru* sequences was calculated to be T = 28.3%, C = 30.6%, A = 21.7%, and G = 19.4% (Table II). CG and AT had average contents of 50% and 50%, respectively. The cause behind this pattern is still debated; however, it aligns with findings

Table III. Pairwise genetic divergence and standard error of twelve individuals from the Clupeidae family under the K2P Model in *COI* genes

Species	1	2	3	4	5	6	7	8	9	10	11	12
<i>S. gibbosa</i>												
<i>A. sirm</i>	0.18											
<i>S. fimbriata</i>	0.18	0.20										
<i>S. sindensis</i>	0.00	0.18	0.18									
<i>S. jussieu</i>	0.05	0.16	0.16	0.05								
<i>S. longiceps</i>	0.15	0.21	0.20	0.15	0.15							
<i>S. lemuru</i>	0.16	0.21	0.19	0.16	0.16	0.01						
<i>S. sample*</i>	0.22	0.25	0.23	0.22	0.21	0.21	0.20					
<i>S. melanura</i>	0.21	0.20	0.00	0.00	0.16	0.21	0.20	0.24				
<i>Sardinella</i> sp.	0.16	0.21	0.20	0.16	0.16	0.02	0.01	0.22	0.29			
<i>S. aurita</i>	0.22	0.25	0.26	0.22	0.21	0.22	0.21	0.21	0.26	0.21		
<i>S. albella</i>	0.18	0.21	0.00	0.18	0.16	0.21	0.20	0.23	0.00	0.26	0.26	

The asterisk (*) indicates Sardine samples from the coast of East Lombok.

Table IV. Gene arrangement of 12 species of *S. lemuru*.

No.	Accession number	Species name	T	C	A	G	A+T	C+G
1	MN392930.1	<i>S. gibbosa</i>	26.1	31.8	22.3	19.7	48.4	51.5
2	OQ387818.1	<i>A. sirm</i>	27.6	31.1	22.8	18.5	50.4	49.6
3	HQ231357.1	<i>S. fimbriata</i>	28.5	29.2	22.1	20.2	50.6	49.4
4	MN586892.1	<i>S. sindensis</i>	26.1	31.8	22.3	19.7	48.4	51.5
5	OQ552973.1	<i>S. jussieu</i>	27.8	30.4	22.1	19.7	49.9	50.1
6	KP001108.1	<i>S. longiceps</i>	28.7	31.1	20.9	19.2	49.6	50.3
7	MT293977.1	<i>S. lemuru</i>	29.2	30.9	21.1	18.8	50.3	49.7
8	LOTIM2025	<i>S. sample*</i>	29.0	31.1	20.9	19.0	49.9	50.1
9	OR145142.1	<i>S. melanura</i>	29.0	28.7	22.3	20.0	51.3	48.7
10	JQ365537.1	<i>Sardinella</i> sp.	29.7	30.4	20.7	19.2	50.4	49.
11	OQ459661.1	<i>S. aurita</i>	29.5	31.4	20.2	19.0	49.7	50.4
12	OK445670.1	<i>S. albella</i>	29.0	28.7	22.1	20.2	51.1	48.9
Average			28.3	30.6	21.7	19.4	50.0	50.0

The asterisk (*) indicates Sardine samples from the coast of East Lombok.

that fish and amphibians possess homogeneous GC content, unlike the heterogeneous genomes of mammals and birds. [Figure 3](#) highlights this 50/50 AT/GC balance in the mitochondrial *COI* gene fragments, which is consistent with existing teleost genome studies ([Symonová and Suh, 2019](#)). With an average content of 50% AT and 50% GC, these results align with previous studies on Indian Marine Clupeidae, which showed a similar *COI* nucleotide composition of 50.1% AT and 49.9% GC ([Devanand et al., 2020](#)).

Fish can be identified by nitrogenous base structure distinctions, which display genetic variants and positions. These distinct sites are traits that they are able to be utilized to distinguish between various fish species. [Kombong and Arisuryanti \(2018\)](#) claim that changes in the nitrogenous base arrangement of mitochondrial DNA *COI* genes are indicators of genetic diversity. Fish and frogs have GC-rich genomes, while fowl and beasts have AT/GC divers genomes. There is ongoing discussion over the exact cause of this oddity. GC/AT values for the mitochondrial *COI* gene fragments are reliable. The results align with previous studies of teleost fish genomes ([Symonová and Suh, 2019](#)). Using a 650 bp amplicon, a study on *S. lemuru* discovered that the typical nucleotide content was T: 28.3%, C: 30.6%, A: 21.7%, and G: 19.4%. On the other hand, the mitochondrial *COI* gene segments from several lemuru species had an average primer composition of T: 29.0%, C: 31.1%, A: 20.9%, and G: 19.0% ([Lakra et al., 2016](#)). According to [Jamandre et al. \(2014\)](#), *Mugil*

cephalus with rich and deficient control sections had a G content of 5%.

CONCLUSION

S. lemuru sample, which have a mitochondrial DNA *COI* gene length of about 650 base pairs, was effectively amplified from fish along the East Lombok coast using the *COI* gene fragments. According to the study, wild *S. lemuru* populations with high genetic diversity can be identified using the mitochondrial DNA *COI* gene sequence. Furthermore, for a variety of marine fish species, the mitochondrial DNA *COI* gene order may be suitable as a hallmark.

DECLARATIONS

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Generative AI and AI assisted technology statement

The authors declare that no generative AI and AI

assisted technology was used in the creation of this manuscript.

Statement of conflict of interest

The authors have declared no conflict of interest.

REFERENCES

- Choi, E.H., Kim, G., Cha, S.H., Lee, J.S., Ryu, S.H., Suk, H.Y. and Hwang, U.W., 2020. Molecular phylogenetic, population genetic and demographic studies of *Nodularia douglasiae* and *Nodularia breviconcha* based on *COI* and *16S rRNA*. *Sci. Rep.*, **10**: 16572. <https://doi.org/10.1038/s41598-020-72015-5>
- Dabuleviciene, T., Jucevicius, D., Zolubas, T., Vaiciute, D. and Nika, N., 2023. The effect of short-term upwelling events on fish assemblages at the south-eastern coast of the Baltic sea. *Water*, **2023**: 452. <https://doi.org/10.3390/w15030452>
- Devanand, T.N., Anjanayappa, H.N., Kumar, G., Naveen Kumar, B.T., Gopan, A. and Panda, K., 2020. A comparative study of *COI* and *16S rRNA* genes for DNA barcoding of Indian marine clupeids. *J. exp. Zool. India*, **23**: 1439-1444.
- Elyasigorji, Z., Izadpanah, M., Hadi, F. and Zare, M., 2023. Mitochondrial genes as strong molecular markers for species identification. *Nucleus*, **66**: 81-93. <https://doi.org/10.1007/s13237-022-00393-4>
- Fachrul, M., Karkey, A., Shakya, M., Judd, L.M., Harshegyi, T., Sim, K.S. and Inouye, M., 2023. Direct inference and control of genetic population structure from RNA sequencing data. *Commun. Biol.*, **6**: 804. <https://doi.org/10.1038/s42003-023-05171-9>
- Fisher, D.N., Kilgour, R.J., Siracusa, E.R., Foote, J.R., Hobson, E.A., Montiglio, P.O. and Wice, E.W., 2021. Anticipated effects of abiotic environmental change on intraspecific social interactions. *Biol. Rev.*, **96**: 2661-2693. <https://doi.org/10.1111/brv.12772>
- Gautam, A., 2022. DNA isolation by Chelex method. In: *DNA and RNA isolation techniques for non-experts*. Cham: Springer International Publishing. pp. 79-84. https://doi.org/10.1007/978-3-030-94230-4_10
- Goloboff, P.A., Catalano, S.A. and Torres, A., 2022. Parsimony analysis of phylogenomic datasets (II): Evaluation of PAUP*, MEGA and MPBoot. *Cladistics*, **38**: 126-146. <https://doi.org/10.1111/cla.12476>
- Hadebe, M.T., Malgwi, S.A. and Okpeku, M., 2023. Revolutionizing malaria vector control: The importance of accurate species identification through enhanced molecular capacity. *Microorganisms*, **12**: 82. <https://doi.org/10.3390/microorganisms12010082>
- Hadibasyir, H.Z. and Wibowo, A.A., 2023. Analysis of fish catches of fishermen in the southern waters of Java Island in 2018 and 2020 based on chlorophyll-a fertility and sea surface temperature. In: *International conference of geography and disaster management (ICGDM 2022)*. Atlantis Press. pp. 216-232. https://doi.org/10.2991/978-2-38476-066-4_14
- Huang, K., Wang, T., Dunn, D.W., Zhang, P., Sun, H. and Li, B., 2021. A generalized framework for AMOVA with multiple hierarchies and ploidies. *Integr. Zool.*, **16**: 33-52. <https://doi.org/10.1111/1749-4877.12460>
- 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**: 449-484. <https://doi.org/10.1007/s11160-021-09649-9>
- Jafar, S., Anjum, K.M., Zahoor, M.Y. and Shehzad, W., 2024. Development and validation of a universal primer pair for the taxonomic and phylogenetic studies of vertebrates. *Mol. Biol. Rep.*, **51**: 332. <https://doi.org/10.1007/s11033-023-09175-w>
- Jakab, E., Bücs, S., Jéré, C., Csösz, I., Jakab, R.I., Szodoray-Parádi, F. and 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**: 301-311. <https://doi.org/10.3161/15081109ACC2021.23.2.003>
- Jamandre, B.W., Durand, J.D. and Tzeng, W.N., 2014. High sequence variations in mitochondrial DNA control region among worldwide populations of flathead mullet *Mugil cephalus*. *Int. J. Zool.*, **2014**: 564105. <https://doi.org/10.1155/2014/564105>
- Karang, I.W.G.A. and Pratama, R.T., 2025. Statistical analyses of ocean eddies in the Lombok Strait and adjacent waters based on Synthetic Aperture Radar imagery. *Adv. Space Res.*, **76**: 3201-3219. <https://doi.org/10.1016/j.asr.2025.06.067>
- Kombong, C.B.S. and Arisuryanti, T., 2018. The 16S and *COI* mitochondrial DNA nucleotide composition of striped snakehead (*Channa striata* Bloch, 1793) from Lake Sentani, Jayapura, Papua. *J. Perikan. Univ. Gadjah Mada*, **20**: 57-62. <https://doi.org/10.22146/jfs.35551>

- Kowalczyk, M., Staniszewski, A., Kamińska, K., Domaradzki, P. and Horecka, B., 2021. Advantages, possibilities, and limitations of mitochondrial DNA analysis in molecular identification. *Folia Biol. (Kraków)*, **69**: 101-111.
- Kulikov, N., Derakhshandeh, F. and Mayer, C., 2024. Machine learning can be as good as maximum likelihood when reconstructing phylogenetic trees and determining the best evolutionary model on four taxon alignments. *Mol. Phylogenet. Evol.*, **200**: 108181. <https://doi.org/10.1016/j.ympev.2024.108181>
- Kumar, S., Stecher, G., Suleski, M., Sanderford, M., Sharma, S. and Tamura, K., 2024. MEGA12: molecular evolutionary genetic analysis version 12 for adaptive and green computing. *Mol. Biol. Evol.*, **41**: msae263. <https://doi.org/10.1093/molbev/msae263>
- Lakra, W.S., Singh, M., Goswami, M., Gopalakrishnan, A., Lal, K.K., Mohindra, V. and Ayyappan, S., 2016. DNA barcoding Indian freshwater fishes. *Mitochond. DNA Part A*, **27**: 4510-4517. <https://doi.org/10.3109/19401736.2015.1101540>
- Laojun, S., Changbunjong, T. and Chaiphongpachara, T., 2023. Evaluation of modern techniques for species identification of Lutzia mosquitoes (Diptera: Culicidae) in Thailand: Geometric morphometrics and DNA barcoding. *Insects*, **14**: 78. <https://doi.org/10.3390/insects14010078>
- Mahadani, A.K., Awasthi, S., Sanyal, G., Bhattacharjee, P. and Pippal, S., 2022. Indel-K2P: A modified Kimura 2 Parameters (K2P) model to incorporate insertion and deletion (Indel) information in phylogenetic analysis. *Cyber-Phys. Syst.*, **8**: 32-44. <https://doi.org/10.1080/23335777.2021.1879274>
- Mahboob, S., Al-Ghanim, K.A., Al-Misned, F., Al-Balawi, H.A., Ashraf, A. and Al-Mulhim, N.M., 2019. Genetic diversity in tilapia populations in a freshwater reservoir assayed by randomly amplified polymorphic DNA markers. *Saudi J. Biol. Sci.*, **26**: 363-367. <https://doi.org/10.1016/j.sjbs.2018.11.015>
- Mahrus, H., Al-Idrus, A. and Syukur, A., 2024. Mitochondrial DNA of *COI* and *16S rRNA* genes for diversity and phylogenetic analysis of sardine (*Sardinella* spp.) from the Lombok Straits, Indonesia. *Biodiv. J. Biol. Divers.*, **25**: 3500-3509. <https://doi.org/10.13057/biodiv/d251012>
- Mahrus, H., Al-Idrus, A. and Zulkifli, L., 2022. Molecular phylogeny of anchovy (Clupeiformes: Clupeidae) from southern waters of Lombok using mitochondrial DNA *COI* gene sequences. *Biodiv. J. Biol. Divers.*, **23**. <https://doi.org/10.13057/biodiv/d230522>
- Mahrus, H.M., Karnan, K. and Sedijani, P., 2025. Molecular and phylogenetic analysis of Sardines at the fish landing center-Tanjung Luar-east Lombok using DNA sequences of the *COI* gene. *Fish. aquat. Sci.*, **28**: 152-162. <https://doi.org/10.47853/FAS.2025.e14>
- Mahrus, Sumitro, S.B., Utomo, D.H., Sartimbul, A., Toha, A.H. and Widodo, N., 2012. Genetic relationship of *Sardinella lemuru* from lombok strait with fish rich in omega-3 fatty acid. *Bioinformation*, **8**: 1271-1276. <https://doi.org/10.6026/97320630081271>
- Nuryanto, A., Dewi, R.E. and 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. <https://doi.org/10.24252/bio.v7i1.6352>
- Qur'ani, J.W., Awaluddin, M. and Amarrohman, F.J., 2019. Analisis batas pengelolaan wilayah laut antara kabupaten lombok utara, lombok barat, lombok tengah, dan lombok timur. *J. Geodesi Undip*, **8**: 40-49.
- Rozas, J., Ferrer-Mata, A., Sánchez-DelBarrio, J.C., Guirao-Rico, S., Librado, P., Ramos-Onsins, S.E. and Sánchez-Gracia, A., 2017. DnaSP 6: DNA sequence polymorphism analysis of large data sets. *Mol. Biol. Evol.*, **34**: 3299-3302. <https://doi.org/10.1093/molbev/msx248>
- Sartimbul, A., Diah Hendiari, I., Arthana, I.W., Kartika, G.R.A., Winata, V.A., Pranowo, W.S. and Iranawati, F., 2025. The Genetic Diversity of Bali *Sardinella* (*Sardinella lemuru*) caught at Bali strait and its possible relation to the ocean circulation. *Sci. J. Fish. Mar. J. Ilmiah Perikan. Kelautan*, **17**: 296. <https://doi.org/10.20473/jipk.v17i2.56670>
- Sartimbul, A., Winata, V.A., Kasitowati, R.D., Iranawati, F., Rohadi, E., Yona, D. and Lauro, F.M., 2023. Seasonal Indonesian Throughflow (ITF) across southern Java determines genetic connectivity of *Sardinella lemuru* (Bleeker, 1835). *Deep Sea Res. Part II: Topic. Stud. Oceanogr.*, **209**: 105295. <https://doi.org/10.1016/j.dsr2.2023.105295>
- Shirk, A.J., Landguth, E.L. and Cushman, S.A., 2017. A comparison of individual-based genetic distance metrics for landscape genetics. *Mol. Ecol. Resour.*, **17**: 1308-1317. <https://doi.org/10.1111/1755-0998.12684>
- Susanto, R.D., Waworuntu, J.M., Prayogo, W. and Setianto, A., 2021. Moored observations

- of current and temperature in the Alas Strait. *Oceanography*, **34**: 240-248.
- Symonová, R. and Suh, A., 2019. Nucleotide composition of transposable elements likely contributes to AT/GC compositional homogeneity of teleost fish genomes. *Mobile DNA*, **10**: 49. <https://doi.org/10.1186/s13100-019-0195-y>
- Tovar Verba, J., Stow, A., Bein, B., Pennino, M.G., Lopes, P.F., Ferreira, B.P. and Pereira, R.J., 2023. Low population genetic structure is consistent with high habitat connectivity in a commercially important fish species (*Lutjanus jocu*). *Mar. Biol.*, **170**: 5. <https://doi.org/10.1007/s00227-022-04149-1>
- Winata, V.A., Kasitowati, R.D., Iranawati, F., Pranowo, W.S. and Sartimbul, A., 2022. Molecular and phylogenetic analysis of *Sardinella lemuru* Bleeker 1835 at fishing ground Canggu-Bali inferred D-loop mutations of mtDNA. In: *IOP Conf. Ser. Earth Environ. Sci. IOP Publ.*, **1036**: 012065. <https://doi.org/10.1088/1755-1315/1036/1/012065>

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