Molecular and phylogenetic analysis of *Sardinella lemuru* Bleeker 1835 at fishing ground Canggu-Bali inferred D-loop mutations of mtDNA

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Abstract. *Sardinella lemuru* resources are abundant, nutritious, high omega-3 and have high economic value in artisanal fisheries. Its production volume landed for over a decade, but the most significant increase was peak southeast monsoon 2019 at Canggu fishing ground. However, prior-conventional kinship assessments genetically need to be confirmed. These research objectives encompass both molecular identification and genetic relationship of Canggu *S. lemuru* with the others. Similar morphological samples had collected of ten individuals. Chelex 10% was used for genomic DNA extraction, CRK-CRE primer with Hotstart-50 PCR thermal profile had applied for amplification. 1% agarose electrophoresis and Sanger sequencing had examined for both quantity and quality of D-loop amplicon. Identification using the algorithm of BLASTn. Phylogenetic tree analysis uses the neighbor-joining method. The genetic identity of 10 D-loop Canggu samples was high (97.46%-98.47%) as Philippines *S. lemuru* (MK579633.1-MK579742.1). The phylogenetic significantly shows low barrier inter-waters from low to moderate genetic distance. Inter-waters samples are very close in intraclade, but it still has a different mutation rate in another clade. Means suggested that Canggu *S. lemuru* is more diverse in nucleotide base substitutions encouraging high variation. These study results provide needed information that southern translocation occurred among waters, its dispersal connection.

1. Introduction

*S. lemuru* resources were found suddenly abundant in the 2019 east monsoon (SE monsoon: JJA) on the East Coast of Bali, especially at Batu Bolong Beach, Canggu [1], after more than a decade of disappearance in the Bali Strait waters. Here is a unique event that has been occurred on this beach only since 2010, then becomes a sampling location. The production volume can reach 150 kg per
fisherman per night in 2019 [2]. However, the phenomenon of the abundance of *S. lemuru* on this beach had not occurred again in 2020 until now [2]. The abundant *S. lemuru* in 2019 had influenced by fluctuations in chlorophyll-a in the surface layer, which had a significant correlation with the positive IOD phase [3]. The impact of this period on the intensity of strong upwelling so that it is more intensive to encourage the production of small pelagic fish (sardine) [4] due to the availability of phytoplankton and zooplankton biomass. That high production of *S. lemura* happens after the time lag phase with marked energy transfer at the food trophic level about 2-3 months later [5].

The status of *S. lemuru*, nutrient-rich and omega-3-rich in artisanal fishery of Bali, was exploited at a high rate (65%) [6–8] that encourages various assessment efforts for stock management that prevent the shortage of the typical *S. lemuru* variant. One of them is stock kinship assessment based on conventional approaches, such as morphometric-meristic characteristics that analysed the stock of *S. lemuru* in Bali is closely related (± 96%) with *S. lemuru* in South Malang waters [9]. Previously, this finding is still in line with the stock assessment of *Sardinella* spp. with the pattern of reproductive activity and body length during the year of 1st to 3rd [10]. However, this approach has limitations in detecting intraspecies variation of fish because there is a susceptibility to morphological variation that is generally greater than that of other vertebrates [11].

These varied characters have been detected again by using mtDNA markers, especially DNA sequencing techniques that have been used widely for the relating study of fish stocks in phylogenetic trees [12]. mtDNA markers can provide a source of high variation at the nucleotide level [13]. In addition, a genomic abundance of mtDNA is still high in cells. The mutation rate is 5-10 fold faster than nuclear DNA [14]. The benefits of the high mutation rate, namely: the occurrence of intraspecific divergence in a relatively short evolutionary time, then become a potential indicator that is more sensitive to genetic drift between stocks [15].

Haploid polymorphisms have been high found at the displacement loop (D-loop) locus. The evidence is the high amount of variations in the replication of the mtDNA genome in vertebrate species [13]. This D-loop locus in mtDNA has been targeted further by the availability of specific primers, such as CRK-CRE [16]. The use of this locus can also analyze very close species relationships for the genus Sardinella. Moreover, these loci can further alleviate direct or purified selection bias [17].

The previous studies, genetic *S. lemuru*, have been conducted with various other mtDNA loci in Philippine waters. Although at the D-loop locus, haplotype diversity (Hd: 0.97-0.99) and nucleotide diversity (π: 0.041) tended to be high compared to COI markers (Hd: 0.7 and: 0.005) [16,18,19]. However, there has been no study of the genetic kinship of *S. lemuru* between Canggu (Bali) and Philippines waters with this D-loop polymorphic locus, because previously it was only confirmed with cytochrome b loci in Muncar Waters and the Philippines [20]. Therefore, molecular identification and phylogenetic study need to assess again. These research objectives are to confirm and analyze the genetic closeness of *S. lemuru* Canggu further, which was abundant again in 2019 with other *S. lemuru* of the Philippines, then to be able to update information on the movement of its stock genetically. Its connection base on the consideration of the potential of the D-loop locus, precisely as a more sensitive indicator of geographic genetic drift [21].

2. Material and Methods

2.1 Sample collections

The *S. lemuru* specimens in this study were caught directly at the fishing ground in Canggu, Bali (Figure 1) using a beach trawl. The operated fishing gear by one person. Its specifications, namely: length 3 m, width 5 m, weight 5 kg and mesh size 1.25 cm. *S. lemuru* catches was sampled in the east monsoon (SE monsoon) and transition II in 2019: July (10 individuals), August (5 individuals) and September (5 individuals). This research specimen collection should pass a morphological identification process and measurement of 23 morphometric characters and ten meristic characters [9]. Quantitative traits, these various characters, are used to ensure the similarity between individual fish in the hierarchical cluster analysis of NTSYs 2.02 with the SAHN module [22].
Before the DNA extraction step in the laboratory, preserved individual tissue into specific tubes that consist of muscle and fin of caudal and pectoral. This method works under sterile conditions in a concentration of 96% ethanol (C2H5OH) [23]. On the other hand, interviews were conducted with local fishers to confirm the previous artisanal fishery conditions of S. lemuru, the timing of catching and types of fishing gear.

Figure 1. Sardinella lemuru sampling location at the Batu Bolong Coast, Canggu, Bali

2.2 Molecular analysis
Molecular analysis is taken place at the Bali Biodiversity Foundation Laboratory, Indonesia. Genomic DNA derived from muscle S. lemuru specimens extracted aseptically using Chelex 10%. This step can avoid the risk of cross-contamination [24]. The amplified target D-loop fragment locus in this DNA genome happen by the Polymerase Chain Reaction (PCR) method. This PCR method uses a forward primer (CRK: 5’- AGC TCA GCG CCA GAG CGC CGG TCT TGT AAA - 3’) [25], followed by a reverse primer (CRE: 5’- CCT GAA GTA GGA ACC AGA TG - 3’) [26].

Each PCR reaction used 25 µl of mixed reagents, namely: 1.25 µl per CRK-CRE primer, 2.5 µl dNTPs, 0.125 µl Amplitaq DNA Polymerase (PE), 14.5 µl ddH2O, 2 µl MgCl2, 2.5 µl PCR Buffer (PE-II) and 1-2 µl of S. lemuru genomic DNA (template). Each microtube was vortexed for 15-30 seconds to achieve homogeneous conditions. The PCR reactions are run and included their temperature cycle profiles: initial denaturation of 94°C for 15 seconds, followed by 38 cycles, each consisting of denaturation of 94°C for 30 seconds, annealing of 50°C for 30 seconds, extension 72 °C for 45 seconds. It was then terminated at a final extension of 72°C for 5 min after cycle 38 [25]. Visualized PCR products test with 1% agarose gel and luminescent Biotium ® gel red stain. There were ten specimen D-loop amplicons sent to the Sanger facility sequencing with Bigdye Chain Termination.

2.3 Data analysis
There are 10 D-loop sequences of the 2019 Canggu-Bali specimen that tested for the quality of their nucleotide base chromatograms with preGap4. The combined results, two-way readings of each nucleotide sequence, were obtained with Gap4 [27]. The algorithm in the BLASTn search engine can identify the similarity of nucleotide base sequences between specimen sequences and D-loop sequences in the NCBI (National Center for Biotechnology Information) gene bank database [28]. The CLUSTAL W algorithm ran so that the nucleotide sequence of the same sites can be aligned.
eliminated ambiguous nucleotides (insertions and deletions (indels)) on the sample nucleotide base sequences ran with MEGA X [29]. The obtained kinship topology of *S. lemuru* between waters using the Neighbor-Joining (NJ) method [30], then its significance was tested using the 10,000x bootstrap test [31]. The D-loop sequence *Amblygaster sirm* Lombok 2017 was chosen (unpublished data) as the outgroup.

2.4. Ocean circulation data and its analysis
Current data is used as secondary data to provide an overview of current circulation conditions thought to play a role in transporting larvae or fish of *S. lemuru* from one sea area to the others sea area. Daily current data for the eastward and northward components, during July–September 2019, at a depth of 10 – 318 metres from the Copernicus Marine Services (CMEMS) Archive with a spatial resolution of 1/12° arc degree were used in this study. Ocean Data View [32] tools are served for the visualization and its analysis.

3. Results and Discussion
3.1 Morphometric characters, meristics and their proximity dendogram
Based on variations in the morphometric characters of *S. lemuru* Canggu 2019, then it can be classified as a "protolan" group because the average total length (TL) is about 12.33 cm (Table 1). This local term had often been used for the *S. lemuru* size 11–15 cm [33]. This site is in line with the average TL of *S. lemuru* from Banyuwangi 2017 (Table 1).

| Morphometric (cm)a | Bali Strait |
|-------------------|------------|
|                   | Canggu 2019 | Banyuwangi 2017 [9] |
| TL                | 12.33 ± 1.26 | 11.89 ± 1.75 |
| FL                | 11.18 ± 1.03 | 10.64 ± 1.67 |
| SL                | 10.47 ± 0.94 | 10.01 ± 1.43 |
| PreDL             | 4.80 ± 0.46  | 4.44 ± 0.58  |
| OrbL              | 0.67 ± 0.07  | 0.68 ± 0.10  |
| EyeL              | 0.32 ± 0.04  | 0.34 ± 0.05  |
| CpedL             | 1.10 ± 0.16  | 0.80 ± 0.23  |
| HdL               | 2.99 ± 0.29  | 2.42 ± 0.36  |
| SnrL              | 0.73 ± 0.11  | 0.80 ± 0.05  |
| POL               | 1.51 ± 0.12  | 0.94 ± 0.26  |
| HH                | 2.08 ± 0.24  | 1.75 ± 0.27  |
| BH                | 2.40 ± 0.41  | 2.39 ± 0.46  |
| CH                | 0.17 ± 0.03  | 0.76 ± 0.13  |
| UEH               | 0.12 ± 0.06  | 0.30 ± 0.11  |
| DBL               | 1.33 ± 0.23  | 1.37 ± 0.24  |
| ABL               | 1.10 ± 0.14  | 1.32 ± 0.29  |
| DFH               | 1.33 ± 0.17  | 1.21 ± 0.15  |
| AFH               | 0.30 ± 0.00  | 0.36 ± 0.08  |
| PFL               | 1.53 ± 0.26  | 1.48 ± 0.23  |
| VFL               | 0.72 ± 0.05  | 0.88 ± 0.11  |
| MXBL              | 0.14 ± 0.09  | 0.59 ± 0.12  |
| MNBL              | 0.25 ± 0.12  | 0.47 ± 0.08  |

Meristic featuresb

|               | Canggu 2019 | Banyuwangi 2017 |
|---------------|-------------|------------------|
| D-hard        | 3.00 ± 1.56 | 2.00 ± 0.90     |
| D-soft        | 10.30 ± 1.42| 14.40 ± 0.68    |
| P-hard        | 2.30 ± 0.48 | 2.00 ± 0.00     |
| P-soft        | 11.90 ± 2.47| 13.95 ± 1.43    |
| A-soft        | 13.00 ± 2.05| 13.65 ± 2.06    |
| V-hard        | 2.00 ± 0.00 | 1.00 ± 0.00     |
The measurement results of its morphometric and meristic characters inter-waters (Table 1) tend to be similar, especially for the sizes of SL, OrbL, EyeL, else, had been supported the results of the paired t-test. The results showed that there were no significant differences in 31 morphological characters of *S. lemuru* across waters. It means that the characteristics of *S. lemuru* caught in the Canggu Coast are identical to those of *S. lemuru* Banyuwangi 2017. Even all specimen of Canggu show a highly heterogeneous distribution during the east monsoon (SE monsoon) because supported by the Euclidean similarity model. These heterogeneous clusters are constructed (Figure 2) based on 23 morphometric characters from Table 1. The bar scale size indicates the closeness between individuals. Twenty specimens of *S. lemuru* Canggu with inter-monthly representation during the SE monsoon to transition II 2019 are consisted: July (CG1-5), August (CG6-15), and September (CG16-20).

![Figure 2. Constructed cladogram of *S. lemuru* morphometric characters has formed from the similarity matrix with the SAHN module](image)

Even though the other morphometrics, such as PreDL, SntL, EyeL, POL, else, tended to be the same across these waters. These same variations could be due to its morphological characteristics widely were distributed inter-waters. However, separated clusters of morphological measurement of *S. lemuru* between the Iloilo waters of the Philippines and Bali Strait were known [20].

### 3.2 Molecular identification based on *d*-loop sequence

After the conducted alignment process on these primary readings of two directions, then the optimal D-loop consensus sequence in each of the specimens can be obtained, then pairing with the most identical reference sequence that strengthened with various parameters such as expected value (E), % Query Cover (QC), and the identity percentage in Table 2. The significance of nucleotide base identity in these ten specimens is observed still high (> 96%) against Philippine *S. lemuru* (MK579633.1-
MK579742.1). In addition, the E-value is very good at explaining the level of homology that exists between specimens with previous gene bank reference sequences. However, the QC value is relatively low (Table 2) because the number of D-loop base sites compared is not commensurate with the D-loop reference sequence length (387 bp) on Philippine S. lemuru.

Table 2. Molecular results in 2019 Canggu specimens. The d-loop base length of each specimen is considered optimal in fish mtDNA (> 500 bp)

| Sample Codes | Species            | % Query cover | % Identity | E-value | Length D-loop of samples |
|--------------|--------------------|---------------|------------|---------|-------------------------|
| SL2:CGU072019| Sardinella lemuru  | 65%           | 97.96%     | 0.0     | 602 bp                  |
| SL3:CGU072019| Sardinella lemuru  | 65%           | 97.70%     | 0.0     | 599 bp                  |
| SL6:CGU82019 | Sardinella lemuru  | 65%           | 98.21%     | 0.0     | 597 bp                  |
| SL7:CGU082019| Sardinella lemuru  | 68%           | 98.47%     | 0.0     | 574 bp                  |
| SL8:CGU082019| Sardinella lemuru  | 68%           | 98.47%     | 0.0     | 576 bp                  |
| SL11:CGU082019| Sardinella lemuru | 65%           | 97.96%     | 0.0     | 596 bp                  |
| SL13:CGU082019| Sardinella lemuru | 68%           | 98.21%     | 0.0     | 573 bp                  |
| SL14:CGU082019| Sardinella lemuru | 65%           | 98.47%     | 0.0     | 595 bp                  |
| SL15:CGU082019| Sardinella lemuru | 66%           | 97.46%     | 0.0     | 591 bp                  |
| SL18:CGU092019| Sardinella lemuru | 65%           | 97.96%     | 0.0     | 603 bp                  |

*The D-loop base length of each specimen is considered optimal in fish mtDNA (500-600 bp)

The identification results of Philippines S. lemuru are known well using D-loop markers or another marker (COI). It can represent its sensitivity in differentiating the maternal lineage in 223 D-loop sequences [19]. However, its length, D-loop sequence in S. lemuru, was only partially provided, so it is still insufficient to be used as a basis for further complete identification. In addition, a high degree of similarity (98-100%) [34] has also been found in the COI nucleotide base sequences of S. lemuru from the Bali Strait (Muncar-Kedonganan) compared to the megaBLASTn COI database from some sea areas of Indo-Pacific.

3.3 Inter-water phylogenetic tree of S. lemuru
This reconstructed phylogenetic tree is shown (Figure 3) with 17 D-loop nucleotide base sequences. The insertions, as well as deletions (indels), have been eliminated. These indels can occur between the nucleotide base sites of the specimen due to the point mutation process. Furthermore, the analyzed length of the tree nodes with each other its nodes has consisted of the final dataset with the number of sites of 318 nucleotide bases per specimen.
Figure 3. The evolutionary history is calculated of *S. lemuru* relatives by the NJ method. The optimal phylogenetic tree shown with % tree replication that contained taxa cluster was associated, then supported by bootstrap test (10,000x) next to the node. The scale of the branch length unit ≈ the relative distance because computed with p-distance in units of base difference per site [35]

This phylogenetic tree shows the low geographic barrier between Canggu and the Philippines Waters (Figure 3). It is characterized by relatively low genetic distance values in intraclade-1 (0.03 ± 0.01) and intraclade-2 (0.02 ± 0.00). On the other hand, the value of inter-clade genetic distance analyzed was relatively moderate (0.595 ± 0.023). Interestingly, there are very close inter-waters specimens, particularly between SL11:CGU082019 and MK579675.1 in clade 1. However, the mutation rate is different from that of other *S. lemuru* specimens in the 2nd clade. This kind of differentiation can be caused the average genetic distance to be classified as moderate (0.32 ± 0.01) in the analysis dataset. In addition, *Amblygaster sirm* is separated significantly as outgroup species (Figure 3).

The phylogenetic tree of the Philippines *S. lemuru* haplogroup also was constructed with two markers comparison, namely: D-loop and COI. The number of observed clades from each D-loop haplogroup to be relatively more varied than COI. The different amounts of haplogroup could be due to different mutation rates between markers [19]. Furthermore, this D-loop marker masked the weakness of the reconstruction in the previous COI phylogenetic tree of *S. lemuru*, as there was only weak inter-clade separation but remained clustered as terminal taxa [34]. The emergence of this single clade also occurred in the cytochrome b phylogenetic tree between the Philippines and Bali Strait *S. lemuru* specimens [20]. In addition, another similarity between the two types of phylogenetic trees is the significant separation of *S. lemuru* specimens (99-100% bootstrap) from *S. longiceps*.

The number finding of different clades was further found in species of highly migratory pelagic fish, such as *K. pelamis* because two clades (variants) were observed in Indonesian Archipelago Waters [36]. However, only one clade was founded in Indian Waters [14] and the Philippines Sulu-Celebes Sea [18]. This phylogenetic finding of *S. lemuru* with D-loop is developed perhaps as another hypervariable locus.

3.4 Indonesian throughflow (ITF)
Water mass transport due to ITF can occur in a narrow variety of pathways [37]. These results were observed with the INSTANT program during 2004-2006. These observed results with the INSTANT program during 2004-2006 has the intensity of speed 25-30% greater than the previous ITF water
mass transport in 2000. Interestingly, the ITF can connect all shallow and deep waters [38], then peaks in the SE monsoon. In addition, ITF spread potentially plankton larvae over great distances [38–40].

Based on observations of currents and salinity at a depth of 10 to 318 meters in the 2019 SE monsoon, the representative water mass driven by ITF current can be an agent of connectivity from *S. lemuru* in the Philippines to Bali Strait and salinity at a depth of 40 meters. See Figure 4 very clearly explains that the mass ITF water with relatively high salinity (34.5-35 PSU) flows from the east of the Philippines, into the Celebes Sea, then flows into the Makassar Strait. From the Makassar Strait, the ITF water masses branched out. Some to the Bali Strait and some to the Lombok Strait. The ITF water mass in the Bali Sea then flows towards the Bali Strait. The ITF water mass in the Lombok Strait hence flows into the Indian Ocean, but at around 10° south latitude and 114° east longitude turns north to the southern waters of East Java, along the east to the Banyuwangi Peninsula (Alas Purwo), then turn north into the Bali Strait.

The known circulation of the water mass (Figure 4) has a biological impact on the biota, namely: the distribution of each life phase (eggs-adults), migration orientation, fecundity, and swimming behavior at different depths supported by the osmoregulation system mechanism of biota which is specific in response to the profile of seawater density [41]. Therefore, assumed ITF water mass movements are the main environmental factors, then influence the genetic mixing of *S. lemuru* between separated waters by geographical distance.

![Figure 4](image-url)

*Figure 4.* Current circulation conditions and salinity at a depth of 40 m on August 2019 (SE Monsoon), which is suspected to be the agent of connectivity between Philippines [A] and Bali [B] waters.

4. Conclusion
The relatively higher variations of nucleotide bases occurred on *S. lemuru*, Canggu Bali. These higher variations happen because the stock is genetically mixed with *S. lemuru* of the Philippines. This event leads to a higher chance of maternal nucleotide base substitution. In addition, this study confirms the inter-waters relationship of *S. lemuru* stocks reinforced by the D-loop as a relatively polymorphic
marker, both in the Bali Strait and regionally. This study also provides information about the *S. lemuru* distribution, which is dispersed between waters due to translocation to the southern part (Indian Ocean) by ITF movements.

5. References

[1] Sulistyowati A 2019 Ribuan Lemuru di Pantai Batu Bolong merupakan Peristiwa Oseanografi Nusantara. https://www.kompas.id/baca/utama/2019/07/18/ribuan-lemuru-di-pantai-batu-bolong-merupakan-peristiwa-oseanografi. Accessed on 12 August 2021.

[2] Suyadnya I K A 2019 Wawancara nelayan jaring Canggu tentang perkembangan fenomena melimpahnya hasil tangkapan *Sardinella lemuru* di Pesisir Canggu, Bali

[3] Setyohadi D, Zakiyah A B and Wijaya A 2021 Upwelling Impact on *Sardinella lemuru* during the Indian Ocean Dipole in the Bali Strait, Indonesia *Fishes* **6** 8

[4] Lumban-Gaal J, Siswanto E, Mahapatra K, Natih N M N, Nurjaya I W, Hartanto M T, Maulana E, Adrianto L, Rachman H A, Osawa T, Rahman B M K and Permana A 2021 Impact of the strong downwelling (Upwelling) on small pelagic fish production during the 2016 (2019) negative (positive) indian ocean dipole events in the eastern indians ocean dipole events in the eastern indian ocean off java *Climate* **9** 1–11

[5] Sartimbul A, Nakata H, Rohadi E, Yusuf B and Kadarisman H P 2010 Variations in chlorophyll-a concentration and the impact on *Sardinella lemuru* catches in Bali Strait, Indonesia *Prog. Oceanogr.* **87** 168–74

[6] Arifan F and Wikanta D K 2011 Optimasi produksi ikan lemuru (Sardinella longiceps) tinggi asam lemak omega-3 dengan proses fermentasi oleh bakteri asam laktat *Pros. Semin. Nas. Sains dan Teknol. Fak. Wahid Hasyim Semarang* **2** 15–20

[7] Mahrus, Sumitro S B, Widodo N and Sartimbul A 2012 The Association between Genetic Variations and Omega-3 Production on *Sardinella lemuru* in Lombok Strait *IOSR J. Agric. Vet. Sci.* **1** 12–6

[8] Wujdi A 2013 Beberapa parameter populasi ikan lemuru (Sardinella lemuru) di Perairan Selat Bali *Widyariset* **16** 211–8

[9] Sartimbul A, Rohadi E, Ikhsani S N and 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** 744–52

[10] Pet J S, van Densen W L T, Machiels M A M, Sukkel M, Setyohadi D and Tumuljadi A 1997 Length-based analysis of population dynamics and stock identification in the sardine fisheries around East Java, Indonesia *Fish. Res.* **31** 107–20

[11] Luceño A J M, Torres M A J, Tabugo S R M and Demayo C G 2014 Describing the body shapes of three populations of *Sardinella lemuru* (Bleeker, 1853) from Mindanao Island, Philippines using relative warp analysis *Int. Res. J. Biol. Sci.* **3** 6–17

[12] Beaumont A R, Boudry P and Hoare K 2010 *Biotechnology and Genetics in Fisheries and Aquaculture* (Chichester; Ames, Iowa: Blackwell)

[13] Menezes M R, Kumar G and Kunal S P 2012 Population genetic structure of skipjack tuna *Katsuwonus pelamis* from the Indian coast using sequence analysis of the mitochondrial DNA D-loop region *J. Fish Biol.* **80** 2198–212

[14] Kumar G, Kocour M and Kunal S P 2014 Mitochondrial DNA variation and phylogenetic relationships among five tuna species based on sequencing of D-loop region *Mitochondrial DNA* **1**–5

[15] Kumar G, Kunal S P and Menezes M R 2012 Genetic stock structure of frigate tuna (Auxis thazard) along Indian Coast based on PCR-RFLP analyses of mtDNA d-loop region *Turkish J. Fish. Aquat. Sci.* **12** 893–903

[16] Hendiari I G A D, Sartimbul A, Arthana I W and Kartika G R A 2020 Keragaman genetik ikan lemuru (Sardinella lemuru) di wilayah Perairan Indonesia *Acta Aquat. Aquat. Sci. J.* **7** 28–36
[17] Bronstein O, Kroh A and Haring E 2018 Mind the gap! The mitochondrial control region and its power as a phylogenetic marker in echinoids *BMC Evol. Biol.* **18** 80

[18] Pedrosa-Gerasmio I R, Agmata A B and Santos M D 2015 Genetic diversity, population genetic structure, and demographic history of Auxis thazard (Perciformes), Sela crumenophthalmus (Perciformes), Rastrelliger kanagurta (Perciformes) and Sardinella lemuru (Clupeiformes) in Sulu-Celebes Sea inferred by mi *Fish. Res.* **162** 64–74

[19] Labrador K, Agmata A, Palermo J D, Ravago-Gotanco R and Pante M J 2021 Mitochondrial DNA reveals genetically structured haplogroups of Bali sardina (Sardinella lemuru) in Philippine waters *Reg. Stud. Mar. Sci.* **41** 1–14

[20] Willette D A and Santos M D 2012 Correcting widespread misidentifications of the highly abundant and commercially important sardine species Sardinella lemuru, Bleeker, 1853 in the Philippines *Appl. Ichthyol.* **29** 881–5

[21] Kumar G, Kunal S P, Menezes M R and Meena R M 2012 Three genetic stocks of frigate tuna Auxis thazard thazard (Lacepede, 1800) along the Indian coast revealed from sequence analyses of mitochondrial DNA D-loop region *Mar. Biol. Res.* **8** 992–1002

[22] Rohlf F J 2015 *Getting Started Guide NTSYSpc: Numerical Taxonomy and Multivariate Analysis System* v.2.2 (New York: Applied Biostatics Inc)

[23] Astarini I A, Ningsih E Y, Simanungkalit D, Ardiana S A, Danie Al Malik M, Yusmalinda N L A, Sembiring A, Pertawi N P D, Cahyani N K D and Collins A 2021 Genetic variation of longtail tuna thunnus tonggol landed in four fish markets in Indonesia based on mitochondrial dna *Biodiversitas* **22** 1644–51

[24] Walsh P S, Metzger D A and Higuchi R 1991 Chelex 100 as a medium for simple extraction of DNA for PCR-based typing from forensic material. *Biotechniques* **10** 506–13

[25] Allen G, Erdmann M and Purtiwi P 2017 Descriptions of four new species of damselfishes (Pomacentridae) in the Pomacentrus philippinus complex from the tropical western Pacific Ocean. *J. Ocean Sci. Found.* **25** 47–76

[26] Lee W J, Conroy J, Howell W H and Kocher T D 1995 Structure and evolution of teleost mitochondrial control regions *J. Mol. Evol.* **41** 54–66

[27] Rozen S and Skaletsky H J 1996 1997 1998 *Primer3*, Whitehead Institute for Biomedical Research http://www.genome.wi.mit.edu/genome_software/other/primer3.html

[28] Madden T 2013 The BLAST sequence analysis tool *BLAST Seq. Anal. Tool* 1–17

[29] Kumar S, Stecher G, Li M, Knyaz C and Tamura K 2018 MEGAX: Molecular Evolutionary Genetic Analysis across computing platform *Mol. Biol. Evol.* **35** 1547–9

[30] Saitou N and Nei M 1987 The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol. Biol. Evol.* **4** 406–25

[31] Felsenstein J 1985 Confidence limits on phylogenies: An approach using the bootstrap *Evolution (N. Y.)* **39** 783–91

[32] Schlitzer R 2020 *Ocean Data View*, Alfred Wegener Institute for Polar and Marine Research Bremerhaven, germany http://odv.awi.de

[33] Wujdi A, Suwarso and Wudianto 2013 Biologi reproduksi dan musim pemijahan ikan lemuru (Sardinella lemuru Bleeker 1853) di Perairan Selat Bali *BAWAL Widya Ris. Perikan. Tangkap* **5** 49–57

[34] Kartika G R A, Sartimbul A and Widodo W 2017 Varian genetik Sardinella lemuru di Perairan Selat Bali *J. Kelaut. Indones. J. Mar. Sci. Technol.* **10** 21–8

[35] Nei M and Kumar S 2000 *Molecular Evolution and Phylogenetics* (New York: Oxford University Press)

[36] Jackson A M, Ambriayanto, Erdmann M V, Toha A H A, Stevens L A and Barber P H 2014 Phylogeography of commercial tuna and mackerel in the Indonesian Archipelago *Bull. Mar. Sci.* **90** 471–92

[37] Susanto R D, Wei Z, Adi T R, Zheng Q, Fang G, Fan B, Supangat A, Agustiadi T, Li S, Trenggono M and Setiawan A 2016 Oceanography Surrounding Krakatau Volcano in the Sunda Strait, Indonesia *Oceanography* **29** 264–72
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