Characterization of the complete mitochondrial genome of Mauritian sardinella, *Sardinella jussieu* (Lacepède, 1803) collected in The Banten Bay, Indonesia

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Abstract

Fish in genus *Sardinella* are small pelagic species, which plays an important role in marine ecosystem as the first consumer. Those species are also commercially important, whose total catch reaches 278,600 tons in 2011 in Indonesia but their identification has been difficult for their morphological similarity. In this study, we reported *Sardinella jussieu* for the first time in Indonesian coastal area (Banten Bay, Indonesia 6°0'50.00"S - 106°10'21.00"E). We were able to confirm the species by both its morphological characteristics including the black spot at dorsal fin origin, the dusky pigmentation at caudal fin, 31 total scute numbers and DNA sequence identity in the GenBank database by the molecular analysis. Its total mitochondrial genome was determined by the combination of next generation sequencing and typical PCR strategy. The total mitochondrial genome of *Sardinella jussieu* (16,695 bp) encoded 13 proteins, 2 ribosomal RNAs, 22 transfer RNAs and the putative control region. All protein-coding gene started with ATG and typical stop codon ended with TAA or TAG except for ND4 in which AGA is used. Phylogenetic analyses of both COI region and full mitochondrial genome showed that *S. jussieu* is most closely related to *S. albella* and *S. gibossa*.

Background

Sardinella is a genus of fish in the family Clupeidae found in the Atlantic, Indian and the Pacific Ocean. The paddle-shaped supramaxilla bones are major characteristics, which help distinguish sardinella from other genera. Morphological characters distinguish *Sardinella* from all other *clupeoid* genera with the presence of two fleshy outgrowths on the hind margin of the gill opening [1]. According to FishBase (http://www.fishbase.org/), there are currently 22 recognized species in the genus of Sardinella. Sardinella is important not only in marine food webs as a base consumer supporting tuna, seabirds and marine mammals[2] but also in industry as the protein source with a low cost using as a bait for large fish or a feed in aquaculture.

Seven species in the genus Sardinella are currently known in Indonesian waters including *Sardinella fimbriata*, *Sardinella gibbosa, Sardinella lemuru, Sardinella albella, Sardinella atricauda, Sardinella branchysoma*, and *Sardinella melanura*, whose total catch in Indonesia reaches 278,600 tons in 2011 [3]. Morphological identification in Sardinella mainly by the characteristics in the gill racker, pelvic scute, scales, and otolith [4-6]. However, species identification in the genus of sardinella is often hard for its broad geographical ranges, overlapping distributions [2] and morphological similarities [7] especially in larval stages [8], which makes it difficult to manage the Sardinella resources in Indonesia.

In addition to the traditional morphological identification, the genetic information is now alternatively used for the species identification for its fast and exact results. The most widely used genetic markers are partial mitochondrial DNA sequences such as Cytochrome C Oxidase I (COI) or Cytochrome B (CytB) [9-11]. However, full mitochondrial genome sequences provide more information about its biogeographical or evolutional information than those fragmental sequences. Therefore, more than 5,000 mitochondrial genomes have been deposited in GenBank database (www.ncbi.nlm.nih.gov) from 33,500 species identified based

on morphological characteristics (www.fishbase.org).

In this study, we report the Mauritian sardinella, *Sardinella jussieu*, for the first time in Indonesian coastal waters, which was collected from the Banten Bay. *S. jussieu* was previously reported only in the Western Indian Ocean, Taiwan, Hong Kong, and Vietnam (www.fishbase.org). Morphological characteristics of *Sardinella jussieu* is distinguished within other sardinella species with the presence of black spot at dorsal fin origin and dusky pigmentation at caudal fin, total scute measurement is 31 and vertical striae on a scale not meeting at center and no perforation on hind part [1]. After confirmation of the species by the molecular COI markers, its total mitochondrial genome sequence was determined by the combination of the traditional PCR methods and next generation sequencing (NGS) techniques. The purpose of this study is to provide basic information of molecular on *Sardinella jussieau*. This information have importance role for further study on molecular systematic and genetic population of this fish.

Methods

Sample collection and morphological measurement

Five individuals of *S. jussieu* were collected in the Banten Bay, Indonesia (6° 0'50.00"S 106°10'21.00"E) at January 2016 as the part of the regular fish survey (Fig. 1). Collected fish were directly stored in 96 % ethanol and kept at -20 ºC until the further analysis [12]. Morphological identification was made by their body shape, type of scale, fin feature, morphometric (i.e standard length, body width, and head length) and meristic characteristic (total number of scutes) [1, 13].

Genomic DNA Extraction and Next Generation Sequencing

Genomic DNA was extracted using an Accuprep® Genomic DNA Extraction Kit (Bioneer) according to manufacturer's instruction. A small portion of tail fin was dissected, which was further homogenized by the TissueLyser II (Qiagen). Purified genomic DNA was quantified with nanoDrop (Thermofisher Scientific D1000), aliquoted and stored at the -70ºC for further analysis.

Two universal primer sets targeting cytochrome c oxidase I (COI) region, Fish F1 and Fish R1 [10] and targeting cytochrome b (cyt-B) region, GLUDG-L and CB2-H [9] were used to obtain the partial sequences of each gene, respectively (Table 1). The quality of all the primers used in this experiment was analyzed by the OligoAnalyzer 3.1 (http://sg.idtdna.com/calc/analyzer) and commercially synthesized by Bioneer Co. (Korea). Each PCR mixture (20μL) contained 12,8 μL ultrapure water, 1 μL primer (0.5 μM, forward and reverse), 0.2 μL Ex Taq DNA polymerase (TaKaRa, Japan), 2 μL 10X Buffer, 2 μL dNTPs (1 μM, TaKaRa, Japan) and 100 ng genomic DNA as template. PCR was carried out under the following condition: initial denaturation step at 95ºC for 3 min, followed by 35 cycles of denaturation at 95ºC for 30 s, annealing at 50ºC for 30 s and extension at 72ºC for 45s (COI target sequence) or 30 s (Cyt-B target sequence). The process was completed with a final extension at 72ºC for 10 min. Two PCR products targeting partial sequences of COI and Cyt B were then purified with AccuPrep Gel purification kit (Bioneer, Korea) and ligated into a cloning vector (Promega, USA), sequenced in both directions.

In order to obtain two large PCR products (~ 8 kb), two pairs of sequence-specific primer sets (CYT-F and CO1-R and CO1-F and CYT B-R) were designed based on the obtained partial sequences of each region (Table 1). Each PCR reaction (30μL) contained 19,7 μL ultrapure water, 1 μL of each primer (0.5 μM,), 0.3 μL Ex Taq Hot Start Version DNA

polymerase (TAKARA, Japan), 3 µL 10X Buffer, 3 µL dNTPs (1 mM, Takara,Japan) and 100 ng genomic DNA as template. PCR was carried out with two-step PCR protocol for Long PCR under the following condition: initial denaturation step at 94°C for 3 min, followed by 30 cycles of denaturation at 98°C for 10s, annealing and extension at 68°C for 10min. The process was completed with a final extension at 72°C for 10 min. Two large PCR products were pooled together in equal concentration and fragmented to ~ 350 bp in length by Covaris M220 (Covaris Inc.). TruSeq® sample preparation kit V2 (Illumina, USA) was used for the construction of a library from fragmented sequence and quality and quantity of the constructed library was measured using 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA). Sequencing was performed by Illumina Miseq platform (2 x 300 bp pair ends) (Illumina, USA).

Assembly of mitochondrial genome by the Bioinformatic analysis

Raw reads from MiSeq sequencer, with under Qv 20 and more than ambiguous nucleotides were removed from raw read using CLC Genomic Workbench v 7.5 (CLC BIO Aarhus, Denmark). Mothür software was used to pairing forward and reverse sequence with more than 7 bp overlapped and without any mismatch. Paired sequence then assembled using Geneious R8 with minimum 20 bp of overlapping sequence and 100% overlap identity. Ambiguous sequences of the D-loop region was reconfirmed by the typical end-point PCR and with sequence specific primers (Sard_F and Sard_R) and DNA sequencing of its PCR products by Sanger sequencing method (Table 1).

Results and Discussion

Morphological and Molecular Identification of Sardinella jussieu

As the result of morphometric measurements, we determined that collected five fish were *S. jussieu*. Among the morphologically similar fish Sardinella species including *S. albella, S. atricauda*, *S. fimbriata, S. marquesensis, S. sindensis* and *S. gibbosa*, the scale and pigmentation patterns are useful characteristics to identify species [5, 13]. The average ratio of body depth (BD) to standard length (SL) of the collected samples was 27.5 % and total scute numbers were 31 (Table 2). Vertical striae on scales did not meet at center with no perforations on hind part of the scale and the pigmented dorsal and caudal fins were also identified (Fig. 2). Those morphological characteristics suggested that the collected samples were *S. jussieu*. The most closely related Sardinella species, *S. abella* and *S.gibbosa* are distinguished from *S. jussieu* in presence of scale perforations (Table 3). Molecular identification of five Sardinella samples confirmed the morphological identification. The COI sequence among five individuals (652 bp) similar and exhibited 100 % sequence identity to *Sardinella* sp. (GenBank accession number; KJ566769) collected from the coastal water in Thailand, and 99% to *S. jussieu* (GenBank accession no HQ231358) collected from Philippine [14]. Based on the morphological characteristics and DNA sequence identity, we concluded that five Sardinella samples collected in the Banten Bay, Indonesia were Mauritian sardinella, *Sardinella jussieu*.

Complete mitochondrial genome of the Sardinella jussieu

In order to have additional information of *S. Jussieu*, the complete mitochondrial genome sequence was determined by the NGS and bioinformatic sequence assembly. Its mitochondrial genome was 16,695 bp in length consisting of 13 protein-coding genes, 22 tRNA genes, two ribosomonal RNA genes, and the putative control region (Fig. 3). The base composition was 4,415 A (26%), 4,132 T (25%), 4,900 C (29%) and 3,248 G (19%), respectively. The purines and pyrimidines are A+T content (51%) slightly higher than G+C

content (49%). The highest A+T content was observed in the putative control region (66 %), which is similar to the other previous studies. The H strands encode 28 genes while the L strands encode 9 genes (Table 4). Among the protein-coding genes, three overlaps nucleotides up to 10 bp, ATP8-ATP6, ND4L-ND4, and ND5-ND6 were detected. The transfer RNA gene pair $tRNA^{-Ile}$-$tRNA^{-Gln}$ and t RNA^{-Thr}- $tRNA^{-Pro}$ overlaps one bp as well. A total 1,292 bp of noncoding nucleotides are apparent in the *S. jussieu* with 1,029 bp at putative control region and 263 remains spread over 11 intergenic nucleotides. 68,3% (11,397 bp) of total mitochondrial genome sequence encoded 13 proteins and the size of each gene ranged from 168 bp (ATP8) to 1,836 bp (ND5). Except for ND6, all protein-coding genes were encoded by H strand (Fig. 3). Although all 13 genes begins with typical start codon, ATG, there were several stop codons including typical ones such as TAA (CO1, COII, ATP8, ATP6, COIII, ND4L, ND5,CYTB) and TAG (ND2, ND3, ND6, ND1) and exceptional AGA in ND4 gene (Table 4). Overlapping nucleotides were identified in three pairs of protein-coding genes (10 nucleotides for ATP8 and ATP6, seven for ND4L and ND4, and four for ND5 and ND6).

The mitochondrial genome of *S. jussieu* contained 22 tRNA genes (Figure. 4), which showed the difference in their sizes from 68 bp (tRNA-Phe) to 71 (tRNA-Gln). 14 tRNA genes encode in H strand and 8 genes encoded in L strand (Fig. 3). The 12S rRNA gene (951bp) of *S. jussieu* was located between the tRNA-Phe and tRNA–Val, whereas 1,686 bp of 16S rRNA was between tRNA-Val and tRNA-Leu. Twenty-one tRNA structures were predicted to have typical three arms except for $tRNA_{ser}$, which showed two arms. That result was also identified in the other Sardinella species [15]. The putative control region of *S. jussieu* (1,029 bp) was longest among three other *Sardinella* species including *S. longicept* (958 bp) (GenBank accession number; NC033407), *S. albella* (986 bp) (GenBank accession number; NC016726), and *S. mederensis* (986 bp) (GenBank accession number; NC009587).

Total mitochondrial DNA sequence of *S. jussieu* showed 84-93% identity with those of currently known 3 other sardinella species among which *S. albella* is the most closely related to *S. Jussieu* (Fig. 5A). In order to know the better evolutional relationship of *S. jussieu*, its COI sequence was compared with those of the other 12 Sardinella species (Fig. 5B). As shown in the analysis by the full mitochondrial genomes, *S. ju*ssieu showed the most closely related to *S. albella* with 96 % sequence identity. In fact, DNA sequence identity of two species *S. albella* and *S. gibossa* was too high to be distinct each other in the COI region (Fig. 5B). This research confirm with cytochrome b and shown similar phylogenetic tree pattern as well. However, morphological keys to distinguise of two species were proposed the post pelvic ventral scutes and gill rackers number on a lower limb, both of *S. albella* and *S. gibbosa* frequently misidentified [16]. Comparison of complete mitochondrial sequences of both species for the better classification in further study. As the alterative, control region of mitochondrial genome will be the suitable sequence to distinguiste of the species, even sub-species (Ref). It is non coding region which have hight evolution rate envolves 2-5 time faster (Ref)

In this study, we identified that *S. jussieu* inhabits in Java island, Indonesia, as well as the two previously known Sardinella species, *S. albella* and *S. gibbosa*. Although *S. jussieu* is originally distributed in the western Indian Ocean from the western coast of southern India from Bombay South to Srilanka also Madagascar and Mauritius, the recent information it's also caught in Taiwan [17], Hongkong [18] and the Philippines [14]. The result strongly supported that *S. jussieu* is more widely distributed than we have thought and the large-scale survey should be made to know the spatiotemporal distribution of four sardinella species in Indonesia. We here reported the full-length mitochondrial genome sequence of *S. jussieu* collected from

Java island, which would provide the important information for the scientific management of Sardinella species in Indonesia. We expect more Sardinella species may exist in Java island and more information about the mitochondrial genome of the other unreported Sardinella species such as *S. gibbosa* would be a useful information for the molecular biological tools to discriminate different Sardinella species in Indonesia.

Conclusion

This study determined the complete mtDNA sequence of *S. jussieu* in Java Island, Indonesia for the first time. The mtDNA sequence is 16.695 bp in length and comprises the typical set of two rRNA, 22 tRNA genes, 13 protein coding genes, and putative control region. Mitochondrial genome structure of *S. jussieu* was identical to those in other Sardinella genus. Phylogenetic analysis using full mitochondrial genome exhibit that *S. jussieu* were most closely related to *S. albella.* However, comparison in the COI region showed that relationship between *S. albella* and *S. gibbosa* was ambigious and determination the complete mitochondrial DNA sequence of *S. gibbosa* is required for the better understanding of evolutional relationship between *S. jussieu* and those species. Those information would provide the basic information for the scientific management of Sardinella species in Indonesia

Declarations

List of Abbreviations

NGS: next generation sequencing

COI region: cytochrome c oxidase subunit 1 region

ND5 : NADH dehydrogenase subunit 4

ND5 : NADH dehydrogenase subunit 5

ND5 : NADH dehydrogenase subunit 6

Cyt-B : cytochrome B subunit

mtDNA : mitochondrial DNA

- Ethics approval and consent to participate

Not applicable

- Consent for publication

Not applicable

Availability of data and materials

The dataset(s) supporting the conclusions of this article is (are) included within the article.

Competing interest

The authors declare that they have no competing interests.

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Author contributions

S-PS carried out the whole process, participated in the whole experiment and drafted the manuscript. S-A added drafted in final phylogenetic analysis. H-WK participated in the design of the study and editing the manuscript. All author read and approved the final manuscript.

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Reference

1. Whitehead, P., *FAO species catalogue Vol. 7. clupeoid fishes of the world (suborder clupeoidei): An annotated and illustrated catalogue of the herrings, sardines, pilchards, sprats, shads, anchovies and wolf-herrings*. 1985, Food and Agriculture Organization of the United Nations.

2. Willette, D., M. Santos, and M. Aragon, *First report of the Taiwan sardinella Sardinella hualiensis (Clupeiformes: Clupeidae) in the Philippines.* Journal of fish biology, 2011. **79**(7): p. 2087-2094.

3. MMAF, *Capture fisheries statistics of Indonesia, 2011*. 2012, Directorate General of Capture Fisheries: Jakarta. p. 190.

4. Homayuni, H., M. Marjani, and H. Mousavi-Sabet, *Descriptive key to the otoliths of three Sardinella species (Pisces, Clupeidae) from the northern Oman Sea.* Aquaculture, Aquarium, Conservation & Legislation-International Journal of the Bioflux Society (AACL Bioflux), 2013. **6**(3).

5. Bräger, Z. and T. Moritz, *A scale atlas for common Mediterranean teleost fishes.* Vertebrate Zoology, 2016. **66**.

6. Begg, G.A. and J.R. Waldman, *An holistic approach to fish stock identification.* Fisheries research, 1999. **43**(1): p. 35-44.

7. Sivakumaran, K., M. Manickasundaram, and V. Ramaiyan. *Problems of identification among species of Sardinella*. in *CMFRI Bulletin National Symposium on Research and Development in Marine Fisheries Sessions I & II 1987*. 1987. CMFRI; Kochi.

8. Ditty, J.G., E.D. Houde, and R.F. Shaw, *Egg and larval development of spanish sardine, Sardinella aurita (Family Clupeidae), with a synopsis of characters to identify clupeid larvae from the northern Gulf of Mexico.* Bulletin of Marine Science, 1994. **54**(2): p. 367-380.

9. Palumbi, S., et al., *The Simple Fool's Guide to PCR, Version 2.0, privately published document compiled by S. Palumbi. Dept.* Zoology, Univ. Hawaii, Honolulu, HI, 1991. **96822**.

10. Ward, R.D., et al., *DNA barcoding Australia's fish species.* Philosophical Transactions of the Royal Society of London B: Biological Sciences, 2005. **360**(1462): p. 1847-1857.

11. Vrijenhoek, R., *DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates.* Mol Mar Biol Biotechnol, 1994. **3**(5): p. 294-299.

12. Knebelsberger, T. and I. Stöger, *DNA extraction, preservation, and amplification.* DNA Barcodes: Methods and Protocols, 2012: p. 311-338.

13. Strauss, R.E. and C.E. Bond, *Taxonomic methods: morphology.* Methods for fish biology. American Fisheries Society, Bethesda, Maryland, 1990: p. 109-140.

14. Quilang, J.P., et al., *DNA barcoding of the Philippine endemic freshwater sardine Sardinella tawilis (Clupeiformes: Clupeidae) and its marine relatives.* The Philippine Agricultural Scientist, 2011. **94**(3).

15. Lavoué, S., et al., *Phylogenetic relationships among anchovies, sardines, herrings and their relatives (Clupeiformes), inferred from whole mitogenome sequences.* Molecular phylogenetics and evolution, 2007. **43**(3): p. 1096-1105.

16. Stern, N., B. Rinkevich, and M. Goren, *Integrative approach revises the frequently misidentified species of Sardinella (Clupeidae) of the Indo-West Pacific Ocean.* Journal of Fish Biology, 2016. **89**(5): p. 2282-2305.

17. Hu, W., et al., *Study on fish life history traits and variation in the Taiwan Strait and its adjacent waters.* Acta Oceanologica Sinica, 2015. **34**(2): p. 45.

18. Leung, A. *The epibenthic ichthyofauna of Tolo Harbour and Hong Kong's northeastern waters: a long term record of change*. in *Proceedings of the Eighth International Marine Biological Workshop: The Marine Flora and Fauna of Hong Kong and Southern China, Hong Kong University Press, Hong Kong*. 1997.

Table 1. Primers used for the mitochondrial genome of *Sardinella jussieu*

Name	Sequence (5' to 3')	Product size (bp)
Fish F1	TCAACCAACCACAAAGACATTGGCAC	652
Fish R1	TAGACTTCTGGGTGGCCAAAGAATCA	
GLUDG- L	TGACTTGAARAAYCGTTG	451
GB2 - H	CCCTCAGAATGATATTTGTCCTCA	
CYTB-F	GCCTACGAAAAACCCACCCGCTCC	8,2 k
CO1-R	GTAAGGTCTACGGATGCCCCTGCG	
CYTB-R	AACGGAGGAGAAAGCGGTTGCGATG	8,7 k
CO1-F	CTTCCTGCTTCTCCTGGCCTCCTC	
SARD F	TTAAAGTCCTCCCTGAGGCCC	683
SARD R	TTAGGAGGGAGTCGTCAAATGC	

Table 2. General morphometric and meristic (total scute) of *S. jussieu*

Sample	measurement								
	Standart length/SL (mm)	Body depth/BD (mm)	Head Length/HL (mm)	Eye Diameter/ ED (mm)	BD/SL (%)	HL/SL (%)	ED/SL (%)	HL/ED (%)	total Scute
1	50	13.5	12	3.5	27.0	24.0	7.0	29.2	30
2	47	12.5	11	3	26.6	23.4	6.4	27.3	31
3	45	12.5	11	3	27.8	24.4	6.7	27.3	31
4	41	11.5	11	3	28.0	26.8	7.3	27.3	31
5	52	14.5	13	3.5	27.9	25.0	6.7	26.9	32
average	47	12.9	11.6	3.2	27.5	24.7	6.8	27.6	31

Table 3. Comparison of morphological characteristic of seven *Sardinella* species

Name	Scale		Fin	
	striae connected/overlapped	perforations	dark spot at dorsal fin origin	dark spot at caudal fin
S. fimbriata		√	√	√
S. gibbosa		√	√	√
S. albella		√	√	√
S. atricauda		√	√	√
S. brachysoma	√	√	√	√
S. melanura	√	√		√
S. jussieu			√	√

1

2 Table 4. Organization of the full length mitochondial genome of *Sadinella jussieu*

Feature	position Numbers			size (bp)	Strand	intergenic nucleotices	Codon		anticodon/position
							start	stop	
tRNA-Phe	1	-	68	68	H	-	-	-	GAA/31-33
12s rRNA	69	-	1019	951	H	0	-	-	-
tRNA-Val	1020	-	1091	72	H	0	-	-	TAC/1053-1055
16s rRNA	1092	-	2777	1686	H	0	-	-	-
tRNA-Leu	2779	-	2853	75	H	1	-	-	TAA/2814-2816
ND1	2854	-	3828	975	H	0	ATG	TAG	-
tRNA-Ile	3837	-	3908	72	H	8	-	-	GAT/3869-3871
tRNA-Gln	3908	-	3978	71	L	-1	-	-	TTG/3944-3946
tRNA-Met	3978	-	4046	69	H	-1	-	-	CAT/4008-4010
ND2	4020	-	5093	1074	H	-27	ATG	TAG	-
tRNA-Trp	5092	-	5163	72	H	-2	-	-	TCA/5125-5127
tRNA-Ala	5165	-	5233	69	L	1	-	-	TGC/5173-5175
tRNA-Asn	5236	-	5308	73	L	2	-	-	GTT/5273-5275
tRNA-Cys	5345	-	5410	66	L	36	-	-	GCA/5366-5368
tRNA-Tyr	5414	-	5484	71	L	3	-	-	GTA/5450-5452
COX1	5678	-	7036	1359	H	193	ATG	TAA	-
tRNA-Ser	7037	-	7107	71	L	0	-	-	TGA/7073-7075
tRNA-Asp	7112	-	7180	69	H	4	-	-	GTC/7142-7144
COII	7193	-	7897	705	H	12	ATG	TAA	-
tRNA-Lys	7884	-	7957	74	H	-14	-	-	TTT/7918-7920
ATP8	7959	-	8126	168	H	1	ATG	TAA	-
ATP6	8117	-	8800	684	H	-10	ATG	TAA	-
COIII	8800	-	9585	786	H	-1	ATG	TAA	-
tRNA-Gly	9585	-	9656	72	H	0	-	-	TCC/9618-9620
ND3	9600	-	10007	408	H	-57	ATG	TAG	-
tRNA-Arg	10006	-	10075	70	H	-2	-	-	TCG/1037-10039
ND4L	10076	-	10372	297	H	0	ATG	TAA	-
ND4	10366	-	11751	1386	H	-7	ATG	AGA	-
tRNA-His	11747	-	11815	69	H	-5	-	-	GTG/11747-11815
tRNA-Ser	11816	-	11883	68	H	0	-	-	GCT/11842-11844
tRNA-LEu	11884	-	11955	72	H	0	-	-	TAG/11916-11918
ND5	11956	-	13791	1836	H	0	ATG	TAA	-
ND6	13788	-	14309	522	L	-4	ATG	TAG	-
tRNA-Glu	14310	-	14378	69	L	0			TTC/14346-14348
CYTB	14385	-	15581	1197	H	6	ATG	TAA	-
tRNA-Thr	15526	-	15597	72	H	-56	-	-	TGT/15558-15560
tRNA-Pro	(15597	-	15666)	70	L	-1	-	-	TGG/15620-15622
control region	15667		16695	1029	H	0	-	-	-

3

4

Figure Legends

Figure 1. Sampling location on Banten Bay, Indonesia (red square)

Figure 2. Mauritian sardinella (*S. jussieu*) collected from Banten Bay, Indonesia (a). The fish scale of *S. jussieu* present no perforations and vertical striated with not meeting at center (b) according to Whitehead (1985)(c). Black scale bar = 1 cm.

Figure 3. Mitocondrial genomic organizarion of *Sardinella jussieu*

Figure 4. Putative secondary structure tRNA genes in mitochondrial genomic
Proposed structure of 22 tRNA genes encoded in the mitochondrial of *Sardinella jussieu*

Figure 5a. Phylogenetic tree of mitochondrial genome of four species belonging to Sardinella. The phylogenetic tree was constructed using molecular evolutionary genetic analysis ver.6.0 (MEGA 6, MEGA Inc. Englewood, NJ), program with the minimum evolution algorithm, the evolutionary distances were computed using Kimura 2-Parameter method.

Figure 5b. Phylogenetic tree of CO1 sequences of 18 species belonging to genus *Sardinella*. The phylogenetic tree was constructed using molecular evolutionary genetic analysis ver.6.0 (MEGA 6, MEGA Inc. Englewood, NJ), program with the minimum evolution algorithm, the evolutionary distances were computed using Kimura 2-Parameter method.

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Short Report

20:26 Characterization of the complete mitochondrial genome of Mauritian sardinella, *Sardinella jussieu* (Lacepede,1803), collected in the Banten Bay, Indonesia
Sinar Pagi Sektiana, Sapto Andriyono and Hyun-Woo Kim

20:27 Molecular characterization of three muscle alpha actin genes in mud loach (*Misgurnus mizolepis*; Cypriniformes)
Sang Yoon Lee, Jeong-Ae Lee and Yoon Kwon Nam

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Sinar

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FAAS-D-17-00042R1
Characterization of the complete mitochondrial genome of Mauritian sardinella, Sardinella jussieu (Lacepède, 1803) collected in Th
Sinar Pagi Kektiana; Sapto Andriyono; Hyun-Woo Kim
Fisheries and Aquatic Sciences

Dear Prof. Kim,

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Characterization of the complete mitochondrial genome of Mauritian sardinella, Sardinella jussieu (Lacepède, 1803
Sinar Pagi Kektiana; Sapto Andriyono; Hyun-Woo Kim
Fisheries and Aquatic Sciences

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We look forward to publishing your manuscript and I do hope you will consider Fisheries and Aquatic Sciences agai

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Sungchul C. Bai, Ph.D

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Characterization of the complete mitochondrial genome of Mauritian sardinella, Sardinella jussieu (Lacepède, 1803) collected in Th
Hyun-Woo Kim; Sinar Pagi Kektiana; Sapto Andriyono
Fisheries and Aquatic Sciences

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FAAS-D-17-00042
Characterization of the complete mitochondrial genome of Mauritian sardinella, Sardinella jussieu (Lacepède, 1803) collected in Th
Hyun-Woo Kim; Sinar Pagi Kektiana; Sapto Andriyono

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Authors: Hyun-Woo Kim; Sinar Pagi Kektiana; Sapto Andriyono
Corresponding author: Prof. Hyun-Woo Kim

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