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SPECIES IDENTIFICATION AND MOLECULAR ANALYSIS OF TERUBUK FISH FROM BENGKALIS STRAIT (RIAU, INDONESIA) USING *COI* MITOCHONDRIAL GENE AS A BARCODING MARKER

Tuty ARISURYANTI, Syafrizal ULUM, Lukman HAKIM

Laboratory of Genetics and Breeding, Faculty of Biology, Universitas Gadjah Mada, Jl. Tehnika Selatan, Sekip Utara, Yogyakarta 55281, Indonesia

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Terubuk fish (*Tenualosa* spp.) commonly found in Bengkalis Strait is an important fishery commodity with a high economic value. However, the exploitation of the fish especially during spawning and hatching time due to the demand of the fish for consumption affects the decrease in numbers of the fish in Bengkalis Strait every year. Therefore, research on species identification and molecular analysis of the terubuk fish have to be investigated due to no genetic information can be used to make regulations and policies related to fish conservation. This research aimed to identify and examine the genetic polymorphism of terubuk fish in Bengkalis Strait using the partial *COI* mitochondrial gene. The method applied in this research was a PCR with primer FishF2 and FishR2. Genetic identification of the terubuk fish was analysed using nucleotide BLAST and Identification Engine through BOLD and genetic variation was evaluated using the DnaSP program. Genetic distance was examined using Kimura 2 parameter (K2P) model. The phylogenetic tree was constructed using Bayesian Inference through the BEAST program. The result revealed that all samples of terubuk fish were identified as *Tenualosa macrura*. The terubuk samples investigated in this study have 4 haplotypes with 6 variable sites dan 1 parsimony site. The haplotype diversity and nucleotide diversity were 0.714 and 0.00353 respectively with a genetic distance of 0-0.9% (mean= 0.4%). This finding is first reported and the genetic information gained in this study is expected to be implemented for terubuk fish conservation, especially in Bengkalis Strait.

Key words: COI mitochondrial gene, genetic polymorphism, species identification, terubuk fish

Corresponding author: Tuty Arisuryanti, Laboratory of Genetics and Breeding, Faculty of Biology, Universitas Gadjah Mada, Jl. Tehnika Selatan Sekip Utara, Yogyakarta 55281, Indonesia. E-mail: tuty-arisuryanti@ugm.ac.id

INTRODUCTION

Mitochondrial DNA sequences provide an efficient tool for biodiversity research and contribute as the major source for identifying species and understanding genetic diversity and genetic relationships (DA SILVA and WILLOWS-MUNRO, 2016; BINGPENG *et al*., 2018; PAIXAO *et al*., 2018). Following the development of the polymerase chain reaction (PCR) procedure, many genetic techniques have been developed to measure and explore species identification and genetic diversity based directly or indirectly on DNA sequence differences among a group of animals including fish (HEY and PINHO, 2012; JOHNSON *et al*., 2018). *COI* mitochondrial gene is a standard gene frequently used for species-level identification and identifying the genetic diversity of a species (HEBERT *et al*., 2003; ČANDEK and KUNTNER, 2015), such as tiger shrimp (YUDHISTIRA and ARISURYANTI, 2019), arowana (NGILI *et al*., 2015), marine eels (PENINAL *et al*., 2017), Asian red catfish (SYAIFUDIN *et al*., 2017), mudskipper (RHA'IFA *et al*., 2021; AJI and ARISURYANTI *et al*., 2021), climbing perch and spotted barb fish (ARISURYANTI *et al*., 2019).

Bengkalis Strait is located in Riau Province which is a part of Malaka Strait and the borderline between Malaysia and Indonesia. The most popular fish which can be found in Bengkalis Strait is terubuk fish (*Tenualosa* spp.). The fish belongs to family Clupidae and has economic potency as food source in the community around the Strait (EFIZON *et al.*, 2012). However, the fish is now categorized as vulnerable due to exploitation during the spawning and hatching period. Recently, no genetic information related to the terubuk fish from Bengkalis Strait. Genetic information is very important as useful data for the government to implement rules and policies concerning terubuk fish conservation in its natural habitat. In addition, the morphology of terubuk fish species could not be detected easily and overlapped among species. Therefore, in this study, we identified what is the species of terubuk fish inhabited in Bengkalis Strait using a Barcoding *COI* gene marker and analyzed the *COI* gene polymorphism of terubuk fish collected from Bengkalis Strait. This finding is expected to gain genetic information which can be implemented for the conservation of the terubuk fish in its natural habitat.

MATERIALS AND METHODS

Sample collection

Ten samples of terubuk fish (code: TRB-01 - TRB-10) were collected from four sites of Bengkalis Strait (Figure 1). First site was located at Bukit Batu District (1˚22'26.846" S, 102˚9'58.223" E), second site was located at Sungai Apit District (1˚18'32.692" S, 102˚11'40.189" E), third site was located at Tasik Putri Puyu District (1˚23'7.033" S, 102˚12'27.194" E), and fourth site was located at Bengkalis District (1˚26'9.874" S, 102˚9'6.311" E). The terubuk fish were collected with help from fishermen and by the net. The terubuk fish were then documented (Figure 2). Muscle tissue from each fish was cut and put into sterile 1.5 ml tube filled with ethanol 99% for long period preservation. Next, the preserved muscle tissue and the whole terubuk fish were brought to the Laboratory of Genetics and Breeding, Faculty of Biology, Universitas Gadjah Mada (Yogyakarta, Indonesia). Each preserved muscle tissue was stored at -20ºC for further investigation and the whole fish was sent to *Musium Biologi*, Faculty of Biology, Universitas Gadjah Mada (Yogyakarta, Indonesia) as a voucher specimen.

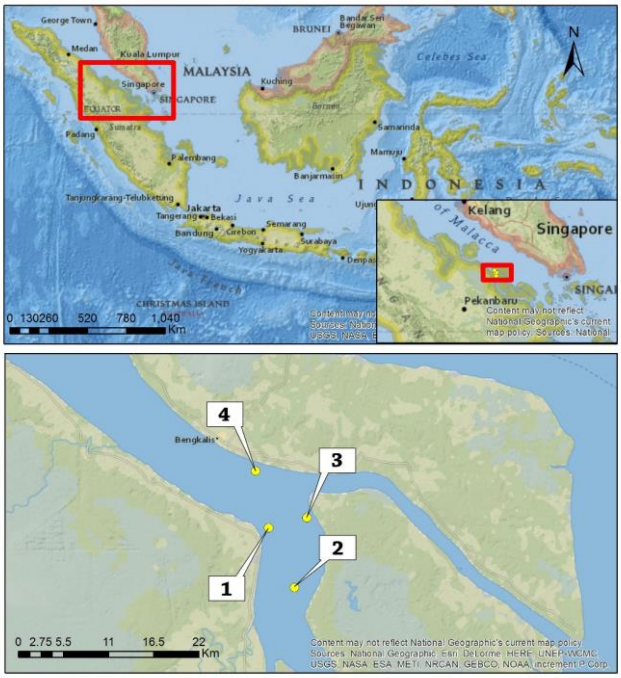

Figure 1. Map location of terubuk fish collection at Bengkalis Strait. The number corresponds to site collections

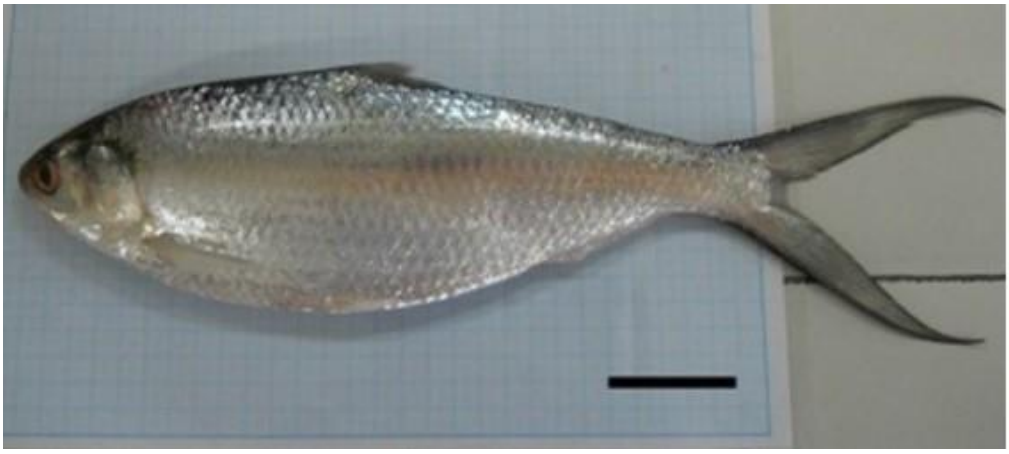

Figure 2. Terubuk fish collected from Bengkalis Strait (Riau, Indonesia). Bar scale = 5 cm.

DNA isolation, amplification and sequencing

Genomic DNA from each muscle fish (50-100 mg) was extracted using DNeasy blood and tissue kit (QIAGEN, Valencia, CA, USA) following the manufacturer's protocols. The total dilution volume of genomic DNA of each terubuk fish was 250 µL. Primers used for the amplification of the *COI* mitochondrial region were FishF2 (5'-TCG ACTAATCATAAAGATATCGGCAC-3') and FishR2 (5'-ACTTCAGGGTGACCGA AGAATCAGAA-3') (WARD *et al*., 2005). PCR reactions were carried out in 25 µL volume containing 12.5 µL MyTaq HS Red Mix PCR Kit (Bioline), 10-100 ng of genomic DNA, 2mM $MgCl_2$, 0.6 µM of each primer, and 5.5 µL ddH2O in a Thermal Cycler (Biorad). PCR conditions involved an initial denaturation at 95ºC for 2 min, then 35 cycles of 95ºC for 15 s, 50ºC for 30 s, 72ºC for 30 s and a final extension of 72ºC for 5 min. Amplified PCR products of terubuk fish were electrophoresed in 1% agarose gels with GelRed staining (Bioline) and then purified with ExoSAP-IT^{TM} (Applied Biosystem). Next, *COI* sequence reactions were visualized in both directions using the standard protocol for ABI Big Dye Terminator ver. 3.1. cycle sequencing kit (Applied Biosystem), 5-7 µl purified PCR product, and 0.8 µl of either primer per reaction. Sequence-reaction products were then loaded into an ABI 3500 Genetic Analyzer (Applied Biosystems). Amplicons were sequenced in both forward and reverse directions.

Sequence analysis

DNA sequences of the *COI* region were inspected and edited manually using SeqMan and EditSeq in Lasergene DNASTAR software package (DNASTAR Inc., Madion, USA). Chromatograms from forward and reverse primers were inspected for noisy and ambiguous base calling, and noisy tails were trimmed to perform consensus fragments. The consensus sequence of each terubuk fish sample was then translated to check the stop codon using vertebrae mitochondrial code. Next, the *COI* sequences were transferred into fasta format and aligned using opal in MESQUITE ver. 3.51 package (MADISON and MADISON, 2018) and ClustalW in MEGAX (KUMAR *et al*., 2018). The composition of mtDNA *COI* nucleotide was calculated with the MEGAX program. Genetic variability including the number of haplotypes, number of polymorphic sites, haplotype diversity and nucleotide diversity was evaluated using DnaSP ver. 6.0 program (ROZAS *et al*., 2017). Haplotype relationship was determined using a median-joining network constructed by NETWORK ver. 10.1 (https://www.fluxus-engineering.com). Kimura-2-parameter (K2P) model was used to estimate intra-population, intraspecific and interspecific genetic distance (HEBERT *et al*., 2003). Sequences of seven *Tenualosa macrura* (KX786670-KX786675 and KY570294), *T. reevesii* (KP112467), *T. toli* (KX786681), and T. *ilisha* (MF621554) taken from GenBank (www.ncbi.nlm.nih.gov) were used for comparative purposes whereas *COI* sequence of *Engraulis encrasicolus* (JF493424) was used as an outgroup. Phylogenetic relationships were also estimated using a Bayesian approach. The best-fit model of evolution was selected with jModelTest 2 (DARRIBA *et al*., 2012) under the Akaike information criterion (AIC) (POSSADA and BUCKLEY, 2004). BEAST program (SUCHARD *et al*., 2018) was used for Bayesian inference under the best-fit model. The analyses were run for 1,000,000 generations with a sampling frequency set to every 1,000 generations. If the standard deviation of split frequencies was below 0.01, the analysis was completed. The analysis used a relative burn-in of 25% for diagnostics. Consensus trees were visualised in FigTree 1.4.4. (RAMBAUT, 2018).

RESULTS

Sequence analysis

Seven of 10 *COI* region sequences of terubuk fish investigated in this study can be amplified clearly (Figure 3) whereas three *COI* sequences showed the unclear result. Therefore, the three *COI* sequences were not included in the further analyses. The *COI* fragment length was between 591 and 651 bp translated into 197-217 amino acids. No stop codons, gaps, and insertions/deletions were observed in any sequences. Next, the *COI* sequences from the terubuk fish examined in this study were compared to the *COI* sequence data from GenBank to confirm initial identification using Nucleotide BLAST and Identification Engine through BOLD. The analysis using both program revealed that the seven terubuk fish specimens have an identity 99.17-100% referred to *Tenualosa macrura* recorded in the GenBank and BOLD database with accession number KX786670.

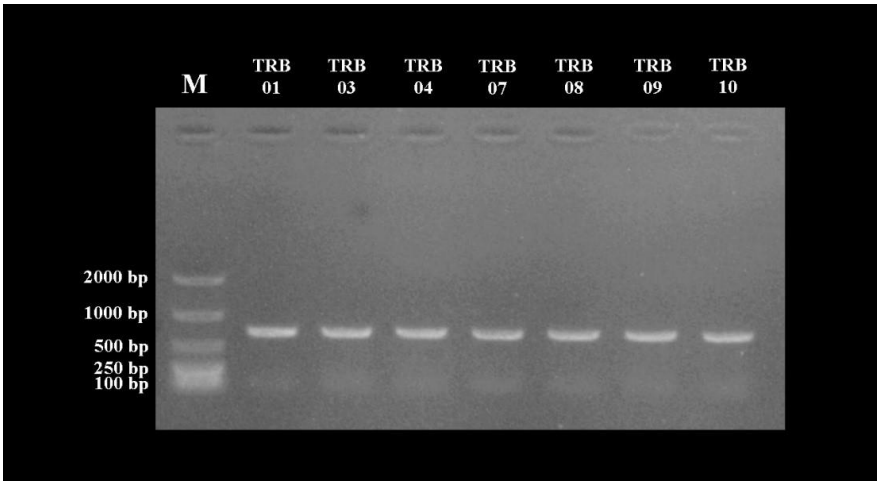

Figure 3. DNA profiles resulted from the PCR amplification of the *COI* mitochondrial gene of terubuk fish from Bengkalis Strait (Code TRB-01, TRB-03, TRB-04, TRB-07, TRB-08, TRB-09 and TRB-10). M is marker

Composition of mtDNA COI nucleotide

The number of nucleotides of all terubuk fish samples had no similar value. The *COI* nucleotide differences were between 0.2-0.4%. The average composition of *COI* nucleotide of the seven terubuk fish (*T. macrura*) were T=30.4%; C=28.7%; A=21.9%; and G=18.9%, while A+T=52.3% and C+G=47% respectively. If the *COI* sequences of seven terubuk fish from Bengkalis Strait combined with the terubuk fish (*T.macrura*) from Serawak recorded at the GenBank, the differences in the *COI* nucleotide were 0.1-0.3% (T), 0-0.4% (C), 0.1-0.3% (A), and 0.1-0.2% (G) (Table 1).

Table 1. Percentage of the composition of mtDNA COI nucleotide of terubuk fish collected from Bengkalis Strait and GenBank database

Sample	T(U)	C	A	G	A+T	C+G
TRB (Bengkalis Strait)	30.4	28.7	21.9	18.9	52.3	47.7
Tenualosa macrura (KX786670)	30.5	28.7	21.8	19.0	52.3	47.7
Tenualosa macrura (KX786675)	30.5	28.7	21.6	19.2	52.1	47.9
Tenualosa macrura (KX786674)	30.7	28.5	21.8	19.0	52.5	47.5
Tenualosa macrura (KX786673)	30.5	28.9	21.6	19.0	52.1	47.9
Tenualosa macrura (KX786671)	30.7	28.5	22.0	18.8	52.7	47.3
Tenualosa macrura (KY570294)	30.7	28.5	21.8	19.0	52.5	47.5
Tenualosa macrura (KX786672)	30.1	29.1	22.0	18.8	52.1	47.9

Genetic distance and intra-population variation

The mean K2P divergence of the terubuk fish from Bengkalis Strait was 0.4% (range 0-0.9%). In addition, four distinct haplotypes were detected with six variable sites and one parsimony site consisted of four transitions and two transversions. Four variable sites occurred in the third codon with no change in amino acid translation (synonymous) and two variable sites appeared in the first and third codon affected amino acid translation (nonsynonymous) (Table 2). Next, haplotype diversity and nucleotide diversity were 0.714 and 0.00353 respectively.

Table 2. Alignment of partial COI gene of seven terubuk fish samples investigated in this study (only variable sites are reported)

Codon site*		12	77	88	175	178
Nucleotide site**		333 456	222 233 901	222 666 234	555 222 345	555 333 234
TRB-01	(Hap-1)	CTG	CCA	GGG	GCC	CCA
TRB-03	(Hap-2)	...	...	...	..T	A.C
TRB-04	(Hap-2)	...	...	...	..T	A.C
TRB-07	(Hap-3)	...	..G	..A	..T	A.C
TRB-08	(Hap-4)	..A	...	...	..T	A..
TRB-09	(Hap-2)	...	...	...	..T	A.C
TRB-10	(Hap-2)	...	...	...	..T	A.C
Amino acid site***		12	77	88	175	178
TRB-01	(Hap-1)	L	P	G	A	P
TRB-03	(Hap-2)	.	.	.	.	T
TRB-04	(Hap-2)	.	.	.	.	T
TRB-07	(Hap-3)	.	.	.	.	T
TRB-08	(Hap-4)	.	.	.	.	T
TRB-09	(Hap-2)	.	.	.	.	T
TRB-10	(Hap-2)	.	.	.	.	T

*The number corresponds to the codon site

** The number corresponds to nucleotide base pair position

*** The number corresponds to the codon site with amino acid position

Dots (.) indicate identity in a particular position with the one of *T.macrura* (Code TRB-01)

Square showed nonsynonymous

Intraspecific analysis and haplotype networking

The 14 *COI* sequences of *T. macrura* (seven samples from Bengkalis Strait combined with seven samples from Sarawak which were taken from GenBank) were aligned and 522 bp consensus sequences were obtained which can be translated into 174 amino acid. Among the 14 sequences, 10 distinct haplotypes were detected with 13 variable sites and 3 parsimony sites. The 13 variable sites consisted of 8 transitions and 5 transversions (Tables 3 and 4). From Table 3, it can be seen that seven codons were synonymous and five codons were nonsynonymous. Haplotype diversity and nucleotide diversity were 0.89 and 0.0044 respectively. In addition, the haplotype network analysis with a Median Joining Network method revealed that the 10 haplotypes of 14 samples analysed were separated by 1-3 mutation points (Figure 4). From Figure 4, it can be seen that haplotype 1 and 4 were located in one group, as well as haplotypes 3, 5 and 7 were also located in another group. The grouping of those haplotypes does not reflect a geographic distribution.

Table 3. Alignment of partial COI gene of seven terubuk fish samples investigated in this study combined with seven T.macrura taken from GenBank database (only variable sites are reported)

Codon Site*		1	2	3	8	9	73	84	103	126	132	171	174
Nucleotide site position**					222	222	222	222	333	333	333	555	555
		123	456	789	234	567	111	555	000	777	999	111	222
		789	012	789	678	456	123	012					
TRB-01	(Hap-1)	AGT	CTT	CTG	CTG	AGC	CCA	GGG	TTG	CTC	ATC	GCC	CCA
TRB-03	(Hap-2)	...	...	...	...	...	...	...	...	...	...	..T	A.C
TRB-04	(Hap-2)	...	...	...	...	...	...	...	...	...	...	..T	A.C
TRB-07	(Hap-3)	...	...	...	...	...	..G	..A	...	...	...	..T	A.C
TRB-08	(Hap-4)	...	...	...	..A	...	...	...	...	...	...	..T	A..
TRB-09	(Hap-2)	...	...	...	...	...	...	...	...	...	...	..T	A.C
TRB-10	(Hap-2)	...	...	...	...	...	...	...	...	...	...	..T	A.C
KX786670	(Hap-2)	...	...	...	...	...	...	...	...	...	...	..T	A.C
KX786675	(Hap-5)	...	...	...	...	...	..G	...	...	...	...	..T	A.C
KX786674	(Hap-6)	...	...	...	...	...	...	...	...	...	..T	..T	A.C
KX786673	(Hap-7)	...	...	...	...	.C.	..G	...	...	...	...	..T	A.C
KX786671	(Hap-8)	...	...	...	...	...	...	...	..A	...	..T	..T	A.C
KY570294	(Hap-9)	...	...	...	...	...	...	...	...	..T	...	..T	A.C
KX786672	(Hap-10)	.C.	.A.	.C.	...	...	...	...	...	...	...	..T	A.C
Amino acid site *****		1	2	3	8	9	73	84	103	126	132	171	174
TRB-01	(Hap-1)	**S**	**L**	**L**	L	**S**	P	G	L	L	I	A	**P**
TRB-03	(Hap-2)	.	.	.	.	.	.	.	.	.	.	.	**T**
TRB-04	(Hap-2)	.	.	.	.	.	.	.	.	.	.	.	**T**
TRB-07	(Hap-3)	.	.	.	.	.	.	.	.	.	.	.	**T**
TRB-08	(Hap-4)	.	.	.	.	.	.	.	.	.	.	.	**T**
TRB-09	(Hap-2)	.	.	.	.	.	.	.	.	.	.	.	**T**
TRB-10	(Hap-2)	.	.	.	.	.	.	.	.	.	.	.	**T**
KX786670	(Hap-2)	.	.	.	.	.	.	.	.	.	.	.	**T**
KX786675	(Hap-5)	.	.	.	.	.	.	.	.	.	.	.	**T**
KX786674	(Hap-6)	.	.	.	.	.	.	.	.	.	.	.	**T**
KX786673	(Hap-7)	.	.	.	.	**T**	.	.	.	.	.	.	**T**
KX786671	(Hap-8)	.	.	.	.	.	.	.	.	.	.	.	**T**
KY570294	(Hap-9)	.	.	.	.	.	.	.	.	.	.	.	**T**
KX786672	(Hap-10)	**T**	**H**	**P**	.	.	.	.	.	.	.	.	**T**

*The number corresponds to the codon site

** The number corresponds to nucleotide base pair position

*** The number corresponds to the codon site with amino acid position

Dots (.) indicate identity in a particular position with the one of *T.macrura* (Code TRB-01)

Square showed nonsynonymous

Table 4. Grouping of the haplotype of T. macrura of COI mitochondrial gene fragment

Haplotype	Frequency	Individual code
Hap-1	1	TRB-01
Hap-2	5	TRB-03
		TRB-04
		TRB-09
		TRB-10
		KX786670
Hap-3	1	TRB-07
Hap-4	1	TRB08
Hap-5	1	KX786675
Hap-6	1	KX786674
Hap-7	1	KX786673
Hap-8	1	KX786671
Hap-9	1	KY570294
Hap-10	1	KX786672

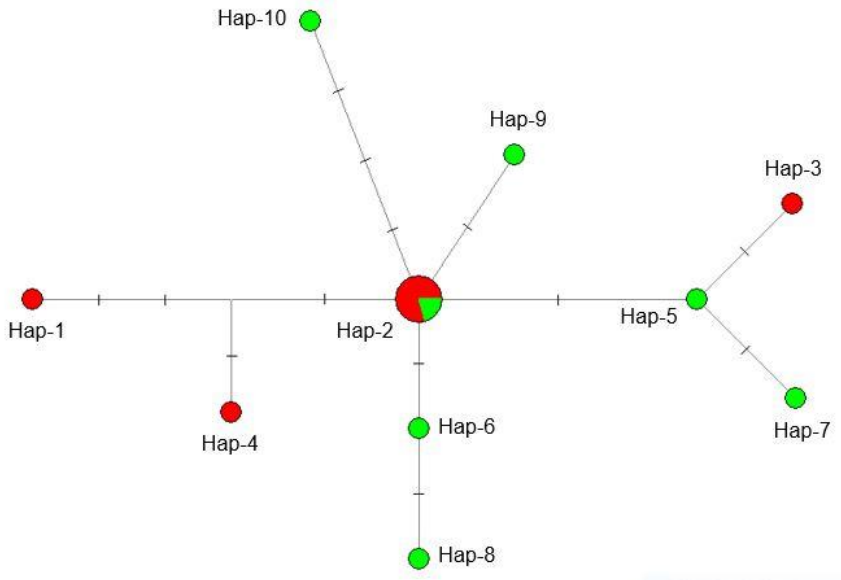

Figure 4. Haplotype networks according to *COI* mitochondrial genes of 14 *T. macrura*. Each circle represents one haplotype and the size represents the frequency (number of samples for each haplotype). The distance between the circles refers to the mutation difference between haplotypes. Samples of *T.macrura* from Bengkalis Strait are red and samples of *T.macrura* from Sarawak (Malaysia) are green

Phylogenetic relationship

The optimal model of nucleotide substitution for the *COI* matrix including the outgroup samples was the HKY model with gamma-distributed rate as inferred by the jModelTest 2 under the Akaike information criterion (AIC). The Bayesian analysis of *COI* mitochondrial data, together with additional sequences of *T. macrura*, *T. reevesii*, *T. toli*, and *T. illisha* taken from GenBank, revealed that the terubuk fish collected from Bengkalis Strait clade together with *T. macrura* from Sarawak (Malaysia) with the associated nodes supported by posterior probability of 1.00 (Figure 5).

The K2P distance among all *COI* haplotypes within *T. macrura* was quite variable ranging from 0.0 to 1.2% (mean=0.46%). The lowest genetic distance (0.0%) was found among TRB-03, TRB-04, TRB-09, TRB-10, and KX786670. This data revealed the close genetic relationship among the four terubuk fish investigated in this study and one terubuk fish from Serawak. This occurrence matched with the haplotype network analysed by a Median Joining Network which put all the five samples of terubuk fish in one haplotype (Hap-2) (Figure 4). The highest genetic distance (1.2%) was found between TRB-01 and KX786672.

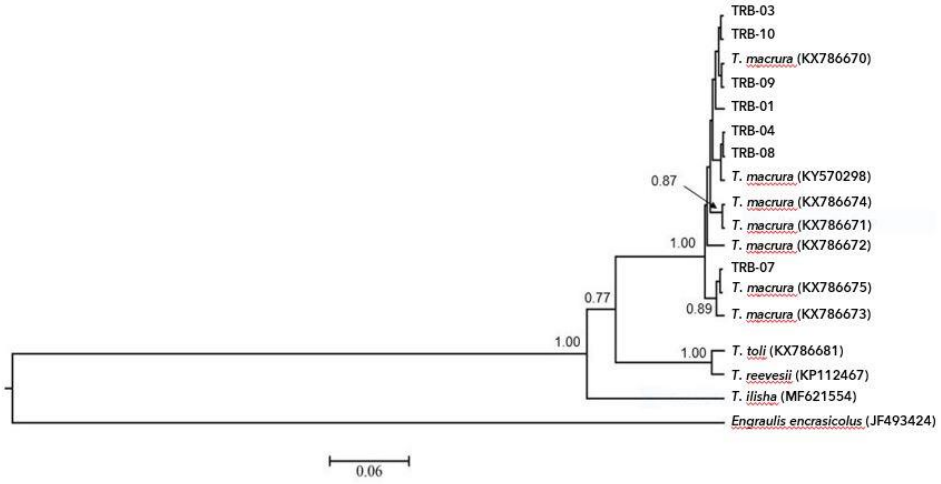

Figure 5. Bayesian tree inferred from mitochondrial *COI* sequences. The tree was produced from 10^6 generations using the HKY+G model. The number of each node represents posterior probabilities and the scale corresponds to substitutions/site

DISCUSSION

Using DNA barcoding we have found the identity of the seven terubuk fish is more than 99% with *Tenualosa macrura* (KX786670) indicating that all terubuk fish collected from Bengkalis Strait have been identified as *Tenualosa macrura*. According to YANG *et al.* (2014), if the identity is between 98-100% with species recorded at the GenBank it means the specimens examined can be identified as that species with high similarity. This authentication of the species

is also supported by the monophyletic lineage of the shad fish investigated in this study with *T. macrura* from Sarawak with a low genetic distance (0.46%). According to ZEMLAK *et al.* (2009), the threshold of 3.5% indicates the existence of a close genetic relationship and still in the same species. Therefore, the correct identification can help the implementation of rules and policies for the conservation of *T. macrura* in its natural habitat.

Median Joining Network also revealed that three terubuk fish (TRB-01, TRB-07 and TRB-08) were separated from the main clade and distinguished the three haplotypes as distinctive individuals. This divergence might indicate that the three individuals may come from other populations due to migration behaviour. The species included in the genus *Tenualosa* commonly have long-distance migration (AZIZ *et al*., 2015; ARJUNAIDI *et al*., 2016). Therefore, there is a possibility of admixture among *T. macrura* from a different population.

The seven *T.macrura* samples investigated in this study exhibited low genetic variation which was indicated by 3-5 bp (1.13%) nucleotide differences with one amino acid divergence even though the haplotype diversity was high. The low genetic variation correlates with the previous study conducted by AZIZ *et al*. (2015) who studied the genetic diversity of *T. toli* in Daru and Mukah (Sarawak, Malaysia) using cytochrome b mitochondrial gene (*Cyt-b*) and by ARJUNAIDI *et al*. (2016) who investigated the genetic diversity of *T. ilisha* from Perak River (Malaysia) using *COI* and *16S* mitochondrial genes. These previous studies reported that the number of the terubuk fish caught had decreased drastically and might cause a decrease in genetic variation in the population. The less diversity of *T. macrura* from Bangkalis Strait might be due to overexploitation of the fish during spawning and hatching season affecting a decrease in population (EFIZON *et al*., 2012). The exploitation activities eventually cause the genetic drift effects which decrease in variability of the nucleotide sequence of *T. macrura COI* gene. As a consequence small genetic diversity will not be able to produce enough stocks for the future population due to inbreeding depression. For a long period, this will cause the loss of one of the most important fishery resources in Bengkalis Strait, severing the socio-economics of the local citizen. EFIZON *et al.* (2012) reported that the number of *T.macrura* has decreased since the 1980s due to overexploitation. Their investigation found that the death rate of fishing (F) during the period of research was higher than the natural mortality rate (M), so the total mortality rate (Z) during the period of research was determined by the rate of death fishing (F). The mortality rate of the fish during the research period was due to the sampling or fishing factor by fishermen at the time of the spawning process. It means the F value is higher than the M value. This finding revealed the evidence that the decrease in *T.macrura* is due to overfishing.

The result of the research on the identification and genetic polymorphism of terubuk fish from Bengkalis Strait inferred from the *COI* mitochondrial gene was the first to be reported. Therefore, this finding is to be expected to contribute to help in the management and restoration of stockfish species. In addition, the genetic information gained in this study can be used to arrange and develop the *COI* library of *T.macrura* in Indonesia.

CONCLUSION

This study successfully identified terubuk fish from Bengkalis Strait which has decreased drastically each year due to overexploitation. The terubuk fish investigated in this study was identified as *Tenualosa macrura*. The genetic analysis indicated the low genetic

variation on the level of intra-population and intraspecific population and showed a close genetic relationship with *T.macrura* from Sarawak.

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REFERENCES

AJI, K.W., T., ARISURYANTI (2021): Molecular identification of mudskipper fish (*Periophthalmus* spp.) from Baros Beach, Bantul, Yogyakarta. Journal of Tropical Biodiversity and Biotechnology, *6(3)*: 66391.

ARISURYANTI, T., R.L., HASAN, K.L., AYU, N., RATMAN, L., HAKIM (2019): Genetic identification of freshwater fish species through DNA Barcoding from Lake Lebo Taliwang, West Nusa Tenggara. Journal of Tropical Biodiversity and Biotechnology, *4(3)*: 107-112.

ARJUNAIDI, N.N., M.F., ZAKARIA, A.H.A., AZIS, M.S., SHAHREZA, T.N.A., JAAFAR, Y.G., SEAH, N.A., ASMA (2016): Authentication of *Tenualosa* species in Perak River, Malaysia: application of morphological measurement and molecular analysis of partial *CO1* and *16S* genes to resolve species ambiguity. AACL Bioflux, *9*: 1355-1363.

AZIZ, A.H.A., Z., TARMIZI, M.N., ALI, A.N., ARIFFIN, D.D.M., ABDULLAH, L.W., LIAN, Y.G., SEAH, T.N.A.M., JAAFAR, A.M.A., BOLONG, A.A.A., KASIM, M.S., SHAHREZA (2015): Mitochondrial DNA diversity of terubok (*Tenualosa toli*) from Daro and Mukah, Sarawak inferred by partial *Cytochrome b* (*Cyt-B*). J. Fish. Aquat. Sci., *10*: 92-101.

BINGPENG, X., L., HESHAN, Z., ZHILAN, W., CHUNGUANG, W., YANGUO, W., JIANJUN (2018): DNA barcoding for identification of fish species in the Taiwan Strait. PLoS ONE, *13(6),* e0198109.

ČANDEK, K., M., KUNTNER (2015): DNA barcoding gap: reliable species identification over morphological and geographical scales. Mol. Ecol. Res., *15(2)*: 268-277.

DARRIBA, D., G.L., TABOADA, R., DOALLO, D., POSADA (2012): jModelTest 2: more models, new heuristics and parallel computing. Nat. Methods, *9(8)*: 772.

DA SILVA, J.M., S., WILLOWS-MUNRO (2016): A review of over a decade of DNA barcoding in South Africa: a faunal perspective. Afr. Zool., *51(1)*: 1-12.

EFIZON D., O.S., DJUNAEDI, Y., DHAHIYAT, B., KOSWARA (2012): Kelimpahan populasi dan tingkat eksploitasi ikan terubuk (*Tenualosa macrura*) di Perairan Bengkalis, Riau. Berkala Perikanan Terubuk, *40*:52 – 65. (In Indonesian Language)

HEBERT, P.D., A., CYWINSKA, S.L., BALL, J.R., DEWAARD (2003): Biological identifications through DNA barcodes. Proc. R. Soc. Lond. [Biol] *270*: 313-321.

HEY, J., C., PINHO (2012): Population genetics and objectivity in species diagnosis. Evolution *66*: 1413-1429.

JOHNSON, B.M., B.M., KEMP, G.H., THORGAARD (2018). Increased mitochondrial DNA diversity in ancient Columbia River basin chinook salmon *Oncorhynchus tshawytscha*. PLoS ONE *13*: e0190059.

KUMAR, S., G., STECHER, M. LI, C., KNYAZ, K., TAMURA (2018): MEGA X: Molecular evolutionary genetics analysis across computing platforms. Mol. Biol. Evol., *35*: 1547-1549.

MADDISON W.P., D.R., MADDISON (2018): Mesquite: A modular system for evolutionary analysis. Version 3.51 (http://www.mesquiteproject.org.)

NGILI Y., D., R., LANTANG, E., UBYAAN, I.Y., IMELDA, PALIT (2015): Mitochondrial DNA genetic diversity of arowana fish local variants on the D-loop hypervariable region: Study in the southern region of Papua-Indonesia. Der Pharma Chemica, *7*: 223-228.

PAIXAO, R.V., J., RIBOLLI, E., ZANIBONI-FILHO (2018): Genetic variation of the endangered neotropical catfish *Steindachneridion scriptum* (Siluriformes: Pimelodidae). Front. Genet., *9*, article 48.

PENINAL S., J., SUBRAMANIAN, A., ELAVARASI, M., KALAISELVAM (2017): Genetic identification of marine eels through DNA barcoding from Parangipettai coastal waters. Genom. Data, *11*: 81-84.

POSADA, D., T.R., BUCKLEY (2004): Model selection and model averaging in phylogenetics: advantages of Akaike information criterion and Bayesian approaches over likelihood ratio tests. Syst. Biol., *53(5)*: 793-808.

RHA'IFA, F.A., D.J. AUDREA, L. HAKIM, T. ARISURYANTI (2021): DNA barcode of barred mudskipper (*Periophthalmus argentilineatus* Valenciennes, 1837) from Tekolok Estuary (West Nusa Tenggara, Indonesia) and their phylogenetic relationship with other Indonesian barred mudskippers. Journal of Tropical Biodiversity and Biotechnology, *6(2)*: jtbb59702.

RAMBAUT, A. (2018): FigTree v 1.4.4. (http://tree.bio.ed.ac.uk/software/figtree/).

ROZAS, J., A., RERRER-MATTA, J.C., SANCHEZ-DELBARRIO, S., GUIRAO-RICO, P., LIBRADO, S.E., RAMOS-ONSINS, A., SANCHEZ-GRACIA (2017). DnaSP 6: DNA sequence polymorphism analysis of large data sets. Mol. Biol. Evol., *13(12)*: 3299-3302.

SUCHARD, M.A., P., LEMEY, G., BAELE, D.L., AYRES, A.J., DRUMMOND, A., RAMBAUT (2018): Bayesian phylogenetic and phylodynamic data integration using BEAST 1.10. Virus Evol., *4(1)*: vey016.

SYAIFUDIN M., D., JUBAEDAH, M., MUSLIM, A., DARYANI (2017): DNA authentication of Asian Red Catfish *Hemibragus nemurus* from Musi and Penukal River, South Sumatra Indonesia. Genet. Aquat. Org., *1*: 43-48.

WARD, R.D., T.S., ZEMLAK, B.H., INNES, P.R., LAST, P.D.N., HEBERT (2005): DNA barcoding Australia's fish species. Philos. T. R. Soc. B, *360(1462)*: 1847-1857

YANG, L., Z., TAN, D., WANG, L., XUE, M.X., GUAN, T., HUANG, R., LI (2014): Species identification through Mitochondrial rRNA genetic analysis. Sci. Rep., *4*: 4089.

YUDHISTIRA, A., T., ARISURYANTI (2019): Preliminary findings of cryptic diversity of the giant tiger shrimp (*Penaeus monodon* Fabricius, 1798) in Indonesia inferred from COI mitochondrial DNA. Genetika, 51: 261-270.

ZEMLAK, T.S., R.D., WARD, A.D., CONNELL, B.H., HOLMES, P.D.N., HEBERT (2009): DNA barcoding reveals overlooked marine fishes. Mol. Ecol. Resour., 9(Suppl. 1): 237-242.

IDENTIFIKACIJA VRSTE I MOLEKULARNA ANALIZA RIBE TERUBUK IZ BENGKALISKOG TESNACA (RIAU, INDONEZIJA) KORIŠĆENJEM *COI* MITOHONDRIJALNOG GENA KAO BARKODING MARKERA

Tuty ARISURYANTI, Syafrizal ULUM, Lukman HAKIM

Laboratorija za genetiku i oplemenjivanje, Biološki fakultet, Universitet Gadjah Mada, Jl. Tehnika Selatan, Sekip Utara, Džogdžakarta, Indonezija

Izvod

Riba Terubuk (*Tenualosa* spp.) koja se obično nalazi u moreuzu Bengkalis je važna ribarska roba sa visokom ekonomskom vrednošću. Međutim, eksploatacija ribe, posebno tokom mresta, zbog potražnje ribe za konzumaciju utiče na smanjenje broja ribe u moreuzu Bengkalis svake godine. Zbog toga se istraživanja o identifikaciji vrsta i molekularnoj analizi ribe terubuk moraju istražiti jer se genetske informacije ne mogu koristiti za donošenje propisa i politika u vezi sa očuvanjem ribe. Ovo istraživanje je imalo za cilj da identifikuje i ispita genetski polimorfizam ribe terubuk u moreuzu Bengkalis koristeći delimični *COI* mitohondrijski gen. Metoda primenjena u ovom istraživanju je PCR sa prajmerom FishF2 i FishR2. Genetička identifikacija ribe terubuk je analizirana korišćenjem nukleotidnog BLAST-a i identification Engine-a kroz BOLD, a genetska varijacija je procenjena korišćenjem DnaSP programa. Genetička udaljenost je ispitana korišćenjem modela Kimura 2 parametara (K2P) i sumirana metodom spajanja suseda. Filogenetsko stablo je konstruisano pomoću programa BEAST. Rezultat je otkrio da su svi uzorci ribe terubuk identifikovani kao *Tenualosa macrura*. Uzorci terubuka koji su istraženi u ovoj studiji imaju 4 haplotipa sa 6 varijabilnih mesta. Diverzitet haplotipa i raznovrsnost nukleotida iznosili su 0,714 i 0,00353 respektivno sa genetskom distancom od 0-0,9% (srednja vrednost = 0,4%). Ovaj nalaz je prvi put objavljen, a očekuje se da će genetske informacije dobijene u ovoj studiji biti primenjene za očuvanje senovitih riba, posebno u moreuzu Bengkalis.

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