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DNA Barcoding to Identify the Genetic Diversity of Gabus Sentani Fish (*Oxyeleotris heterodon*, Weber 1907) at Putali Gulf Sentani Lake

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Abstract. Gabus Sentani fish (*Oxyeleotris heterodon*, Weber 1907) is a native fish at Sentani Lake. The fish has an important economic value for the people who live in the banks of Lake Sentani. The high exploitation made the population decreasing and the status of this fish has not been evaluating by the IUCN. The decreasing population of gabus Sentani fish will make changes in genetic variation and will further lead to the occurrence of inbreeding. The purpose of this study was to determine the genetic variation in fish populations of Gabus Sentani fish in the Putali Gulf-Sentani Lake. The sample area is in the Gulf of Putali – Sentani Lake. It is because the gulf is one of the candidates for asylum fisheries habitat in the Sentani Lake. The analysis of genetic variation was conducted by using DNA Barcoding with Cytochrome Oxidase Gene Sub Unit 1 (COX 1). DNA amplification was obtained along 648 bp. Genetic distance ranges from 0,000 to 0,027. The phylogenetic tree formed forms three main groups. DNA Barcoding is effective for identifying the genetic diversity of gabus Sentani fish (*Oxyeleotris heterodon*, Weber 1907) located in Putali Gulf – Sentani Lake.

Keywords: Oxyeleotris heterodon; Cytochrome c Oxidase Subunit 1; DNA barcoding; Putali Gulf; Sentani Lake.

INTRODUCTION

Gabus Sentani fish (*Oxyeleotris heterodon*, Weber 1907) is an indigenous species found in Sentani Lake. The fish has an important role in an ecosystem. In Sentani Lake, gabus Sentani fish acts as a predator. The species found in the fish's hull include *Ophioeleotris aphoros* (fish), *Macrobrachium* (shrimp), nymphs, gastropods and detritus. Therefore, gabus Sentani fish is very important existence in Lake Sentani to maintain the ecosystem balance in the lake [1].

Gabus Sentani fish (*Oxyeleotris heterodon*, Weber 1907) has a high economic value [2]. People who live on the banks of Sentani Lake make the fish as an economic source. This activity is one of the causes of population decline in gabus Sentani fish (*Oxyeleotris heterodon*, Weber 1907). Communities that use these fish as a source of livelihood have not compensated for the decrease in population with cultivation. In addition to exploitation activities, the decline of fish populations in Sentani Lake is also caused by several other factors such as the introduction of predator and competitor species, climate change, and pollution [3]. Freshwater fish have limited distribution and low spreading ability, making them vulnerable to environmental changes [4]. Meanwhile, International Union for Conservation Nation (IUCN), has not made an evaluation effort on the status of gabus Sentani fish (*Oxyeleotris heterodon*, Weber 1907).

The use of Barcoding DNA proposed as a method for identifying species at the genetic level using short sequences (650 bp) [5]. DNA Barcoding is designed to provide information about species that are fast and accurate. One of the genes that can be used as a marker/marker is the genes derived from mitochondria. Gene Cytochrome Oxidase Sub Unit 1 (Gen COX 1) is one of the mitochondrial DNA genes used for markers [6]. Mitochondrial DNA can be used in studying the genetic structure in fish populations [7].

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Putali gulf is one part of Sentani Lake area. Putali gulf is located at S 02° 36' 59.1"E 140° 31' 32.1". Characteristics of Putali gulf is an area far from human settlements and is a litoral area there are many water plants, on the beach edge there is a sago tree. Water depth 4-12 meters. The color of the water is green [8]. Putali gulf is one of the four areas nominated to be one of the asylum areas in Sentani Lake. This study was conducted to identify the genetic diversity of gabus Sentani fish (*Oxyeleotris heterodon*, Weber 1907) that located in Putali Gulf – Sentani Lake.

MATERIALS AND METHODS

The study was conducted for ten months, starting from August 2016 until May 2017. Fish sampling was conducted by purposive sampling based on research, which proposed four locations of asylum candidates, but the researchers choose only one of the four locations, which is Putali Gulf [8]. Sampling is done by using gill net and caught five fish. Gabus Sentani fish caught immediately taken muscle that is on the posterior near the tail fin about 20 mg and stored in microtube labeled with a volume of 2 µL that has 96% ethanol.

DNA is extracted from the posterior muscle tissue around the caudal fin. DNA extraction was done by using Thermo Fisher kit. The purpose of this activity is to isolate the DNA from samples taken. The DNA extraction procedure was performed according to the Thermofisher book manual.

The analysis has done in Instrument Laboratory – Universitas Indonesia. DNA amplification was performed using a universal primer LCO 1490 (5'GGTCAACAAATCATA AAGATATTGG-3') and and HCO 2198 (5'TAAACTTCAGGGTGACCAAAA AATCA-3') [9]. Every 25µL PCR product contains NFW 1.5 µL, DNA isolated sample of 8 µL, LCO 1.5 µL, HCO 1.5 µL, and Mastermix 12,5 µL. Amplification of gabus Sentani fish was performed by using KAPA2G Robust HotStar Ready Mix kit. Primers consist of denaturation, annealing, and extension of DNA performed on PCR machines. The PCR conditions used were predenaturation in the PCR process consisting of predenaturation (94 °C, 2 minutes), denaturation (94 °C, 30 seconds), annealing (47 °C, 30 seconds), extension (72 °C, 45 second), elongation (72 °C, 10 minutes) and preservation (4 °C). The purpose of the step is to multiply DNA at specific desired locations by the length of fragments according to the specific primer used by the extraction DNA amplified by PCR using the Cytochrome Oxidase Sub Unit 1. The DNA amplification results are sequenced by the service provider Sequences of Macrogen to know the sequence of nucleotide bases.

Electrophoresis was performed on a 1 % agarose matrix already dyed one µL under 100 volts, 100 Ma for 30 min. The DNA profile is documented with the Geldoc tool. Sequencing aims to read the sequence of DNA bases in the amplified fragments. The sequence of readable DNA will be the barcode of the species. Sequencing is done by sending PCR products to a service provider company (Macrogen-Korea) for mitochondrial DNA sequences. Sequencing results can be viewed manually with the sequence navigator program (Applied Biosystem).

Sequencing chromatogram is evaluated using the Basic Local Alignment Search Tool (BLAST) software at National Center for Biotechnology Information (NCBI). The sequence of nucleotide bases of each species is compared with the multi-sequencing alignment using the Clustal W method on BioEdit and MEGA version 7. The phylogenetic tree is constructed by Neighbors-Joining (NJ) method based on Kimura 2 Parameter (K2P) matrix with boostrap 1.000 times.

RESULTS AND DISCUSSION

The DNA Barcoding study used muscle tissue taken from gabus Sentani fish (*Oxyeleotris heterodon*, Weber 1907) on Sentani Lake. The PCR yield after migrating to 1% agarose gel is shown in Fig. 1 according to the PCR product visualization results in Geldoc. Five muscle samples were successfully amplified perfectly along 648 bp. The results of the amplification in this study are in accordance with the character of Cytochrome Oxidase sub-unit one gene, which is short-term only about 648 bp [5, 10, 11]. The amplification results can be seen in Fig. 1.

The genetic distance between individuals of the gabus Sentani fish population (*Oxyeleotris heterodon*, Weber 1907) in Putali Gulf - Sentani Lake with the value of the matrix of mahalanobis is shown in Table 1. The genetic distance is the degree of gene difference in a population or species as measured by numerical quality [12].

The genetic distance between individuals from the population of gabus Sentani fish (*Oxyeleotris heterodon*, Weber 1907) in Putali Gulf - Sentani Lake ranged from 0,000 to 0.027. The genetic distance is expressed with a number that ranges from 0--1 or 0--100%. If the genetic distance is equal to 0, then there can be no difference (identical), whereas if the genetic distance is equal to 1, then it is completely different. The greater the genetic distance, the greater the genetic relationship between these populations [12].

FIGURE 1. Visualization of PCR Products using Agarose Gel Electrophoresis (AGE 1%).

TABLE 1. The genetic distance of gabus Sentani fish population in Putali Gulf – Sentani Lake

	Y1	Y2	Y3	Y4
Y1				
Y2	0,002			
Y3	0,027	0,025		
Y4	0,000	0,002	0,027	
Y6	0,000	0,002	0,027	0,000

The value contained in Table 1, all values below 1, means there is no difference between individuals. The lowest genetic value is found in individuals Y1, Y4, and Y6. The three individuals have a low genetic value; allegedly the three individuals are hereditary. The low genetic value of inbreeding will decrease the fitness of individual to natural changes and increased lethal alleles [13].

Seen also from Table 1, Y3 has a fairly distant kinship relationship with individuals Y1, Y4, and Y6. It is presumably possible that Y3 comes from a different population with other individuals coming from Putali Gulf - Sentani Lake. Sentani Lake is a type of landslide lake that is the stagnant waters formed by the shifting of land that forms the basin so that the steep type of lake formed and surrounded by steep hills and grooved like bay [2]. From the topography of Sentani Lake, it is suspected that gabus Sentani fish from other populations can move from one bay to another on Sentani Lake.

Trees of phylogeny are formed to show the closeness of relationships between individuals with each other. The result of the reconstruction of phylogeny tree on Sentani fish from Putali Gulf – Sentani Lake by Neighbors-Joining method (NJ) using MEGA version 7.0 software. The boostrap value used is 1000 times. The boostrap value becomes the deciding factor of a belief in the phylogeny tree formed. The greater the boostrap value, the higher the confidence value of the phylogeny tree formed [14]. The phylogenetic tree is presented in Fig. 2.

The formed phylogeny tree shows the existence of three groups formed, namely first group (Y1, Y4, Y6), second group (Y2), and third group (Y3). The phylogenetic tree formed shows the genetic diversity of gabus Sentani fish in Putali Gulf – Sentani Lake. All the samples that form the three groups in the identified phylogenetic tree have different nucleotide sequences with the appearance of different branch lengths in each group.

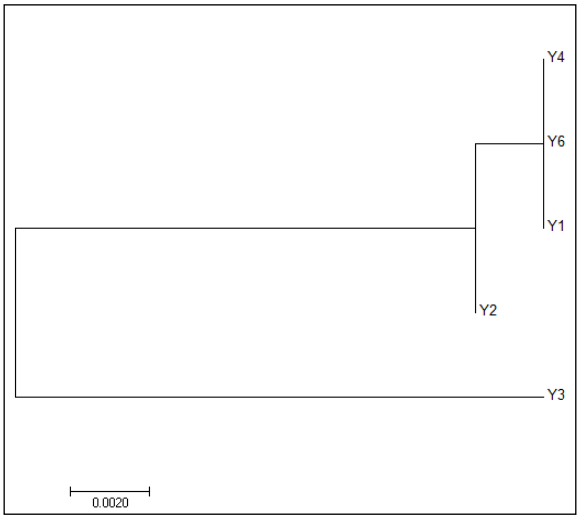

FIGURE 2. Phylogeny Tree of Gabus Sentani Fish at Putali Gulf – Sentani Lake

CONCLUSIONS

DNA Barcoding with the Cytochrome Oxidase Sub Unit 1 gene has been successfully identified the genetic diversity of gabus Sentani fish in Putali Gulf – Sentani Lake. The phylogenetic tree formed produces three main groups of gabus Sentani fish population. In addition to identifying genetic diversity, DNA barcoding is also effective for looking at individual kinship relationships in a population.

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