Identification of Five Types Kampar River Fish with Genetic Approach

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Abstract. Fish identification in the mainland waters of Riau Province is generally carried out based on morphological characteristics. To identify fish species accurately in a short time, the DNA Barcoding method with a molecular approach is used. Five types of fish were taken from Kampar River and coded as MJ313, PJ36, SJ35, BJ33, and GJ41. The genes of these five fish were amplified, and PCR products were sequenced and analyzed using bioinformatics software. The editing of the sequencing results and the determination of the nucleotide composition were analyzed using Mega5 software. DNA sequences were aligned using the ClustalW software. The sequences obtained are then compared with GeneBank data using Basic Local Alignment Search Tools (BLAST). The results showed that the species with the code MJ313 and GJ41 were Channa striata, PJ36 were Osteochilus hasseltii, BJ33 were Mystus sp. Based on the sample examination, the species coded SJ35 cannot be determined because the marker band is low so that it is not visible. The results of the analysis on species coded SJ35 indicate that there is no COI report for species coded SJ35 on GeneBank.

1. Introduction
The potential of freshwater fish has been known to be very large, especially in terms of species and biodiversity. At least 1218 species from 84 families have been identified. 1172 species are native species from 79 families with 630 endemic species [1]. Besides storing potential, the diversity of fish species also poses challenges in the form of species identification and classification.

Identification of types and classification of freshwater fish has an important role in conducting studies related to management and conservation. Management and conservation efforts can only be carried out optimally if the biodiversity database is available [2]. The current limitations of identification and classification are one of the causes of the biodiversity crisis.

Identification of freshwater fish is a part of taxonomy process. Fish identification is based on certain morphological character and done by using taxonomy keys [3]. Many identification and classification of freshwater fish species is still carried out by taking into account the morphological characteristics. Identification in this way is not only time consuming, but also less accurate [4]. For example, these morphological method can not identify a fish which are still in developmental phase.

Species identification through the use of the DNA barcoding method is a solution developed to overcome the barriers to identification morphologically. The DNA barcoding promises better species identification by analyzing the mtDNA segment [5]. [6] stated that the identification method through DNA barcoding will provide reliable results and save time and costs. In addition, this method can provide a picture of the evolution of a species.

DNA barcoding provide the barcode sequence that can be matched to fish barcode library as a reference [7]. This sequences then provide the identity of fish species. On the other hand, the new findings which doesn’t have barcode library will lead to a new barcode sequence library [8]. Genetic
studies or DNA testing are carried out, because each type of living thing has its own characteristics and characteristics in its genes. This peculiarity is very important to know what kind or group the living creatures belong to.

In Riau Province, study from one of the oxbow lake Pinang Dalam, shown that there were 9 family, 21 genus and 28 species found in the lake [9]. Kampar river is known as a important habitat for many freshwater fish species. Study from [10], found that there were 14 family and 49 fish species in Kampar River. The research from [11], shown that at least there are 15 families and 23 genera found in Kampar River. The richness of fish biodiversity in Kampar River need to be examined by using DNA barcoding. DNA barcoding is needed to determine whether this type of fish species is new, or is old, but has undergone an adaptation process in its life evolution. The DNA of fish species in Kampar River also needed to be store in gene bank so we can compare the result of the study to other fish species from different location to understand the relationship with other fish species.

Like many other river, Kampar River is also facing the threat from non selective fishing, extensification of palm oil plantation and pollution such as heavy metals. The fish species in Kampar River could be an important economic resources in Riau Province. Therefore, the identification of fish species in Kampar River become very important to help management and conservation of fish species.

2. Method
Fish were obtained from fishermen in Kampar river, Riau Province. Genetic examination was carried out by taking a piece of fish muscle at the end of the fish tail with a size of 1 x 1 cm². These pieces are then chopped and put into a 1.5 ml tube filled with 99% alcohol. All samples then brought to laboratory for further analysis. The five samples were coded MJ313, PJ36, SJ35, BJ33, and GJ41.

The isolation and purification process was carried out by referring to the DNA isolation and purification kit protocol Dneasy Blood and Tissue Kit (Qiagen). The primer used to amplify the Cytochrome b gene is a universal primer which consists of:

L14841 (5’AAAGCTTCCATCCACATCTCAGCATGATGAAA3’) (Forward)
H15149 (5’AAACTGCAGCCCCTCAGAATGATATTTGTCCTCA3’) (Reverse)

PCR that has been obtained was migrated to agarose gel with a concentration of 1% in a solution of 1 X TBE (Tris-Borate-EDTA pH 8) and added 1 µl / ml of ethidium bromide. The electrophoresis process was carried out using a Fison Mode FEC 360 machine, the Large Horizontal Gen System. The gel that had solidified in the first well was added with 2 µl of loading dye and 2 µl of DNA ladder. In the second well and so on, it was inserted with a volume of 2 µl of loading dye plus 2 µl of the total DNA was migrated for 45 minutes with a voltage of 50 volts. Then the migrated DNA was visualized with the help of a UV transiluminator and photographed with a camera. A positive result is indicated by the presence of a DNA band that is formed.

At the stage of the data analysis, the sequencing results from the sample DNA were in the form of a nucleotide sequence which was then examined using the Bioedit program. Furthermore, the sequence data is aligned (multiple alignment) and compared with BLASTn (Basic Local Alignment Search Tool nucleotide) which is on GenBank on the site http://www.ncbi.nlm.nih.gov/BLAST. The results of the alignment of the nucleotides were then processed using the MEGA version 6.0 (Molecular Evolutionary Genetics Analysis) program which was used for genetic characterization analysis and phylogenetic tree construction.

3. Result and discussion
The result indicated that five fish samples from Kampar River can be amplified. All the sequence revealed no deletion, insertion or stop codons. A total of five COI barcodes were recovered from five fish samples. PCR products from five samples have fragment length as follows: SJ35 was 705 bp, MJ313 was 705 bp, PJ36 was 649 bp, BJ33 624 bp and GJ41 624 bp. All the amplified sequences contained mitochondrial COI sequences. There were no contaminant sequences such as from bacteria observed or pseudogenes. COI genes is selected
as the main material for DNA barcoding because the changes of amino acid sequences is more slowly that other gene in mitochondria [6].

The identification by using the particular part of mitochondrial DNA helps to give an obvious identity of animal species [12].

**Figure 1.** Neighbour-joining tree based on COI sequence data from samples SJ35, PJ36 and BJ33.

BLAST analysis showed that the species with the code MJ313 and GJ41 were Channa striata, PJ36 were Osteochilus hasseltii, BJ33 were Mystus sp. Based on the sample examination, the species coded SJ35 cannot be determined because the marker band is still low so that it is not visible. The results of the analysis on species coded SJ35 indicate that there is no COI report for species coded SJ35 on Gene Bank.

The research that are presented in this paper suggest the use of DNA barcoding or molecular approach to identify fish species rather than morphological method. DNA barcoding could also identify fish species which are still in ambiguously form such as larvae. This study could also be extended to the application of effective strategies for management and conservation of fish species in Kampar River.
4. Conclusion
The use of coded DNA can identify four out of five fish samples in the Kampar River, Riau Province. These species are Channa striata, Osteochilus hasseltii and Mystus sp. Species with code SJ35 indicate that there is no COI report registered with GeneBank.

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