APLIKASI WILAYAH GEN CYTOCHROM-B DALAM STUDI POPULASI IKAN GABUS (*CHANNA STRIATA*) SECARA MOLEKULER

APPLICATION OF THE CYTOCHROME-B GENE REGION IN POPULATION STUDY OF STRIPED SNAKEHEAD (CHANNA STRIATA) BY MOLECULAR APPROACH

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ABSTRAK

Ikan gabus dengan perbedaan geografis yang berbeda dapat memiliki dampak keanekaragaman genetik. DNA barcoding merupakan suatu system yang dirancang untuk mengidentifikasi suatu spesies secara tepat dan akurat dengan menggunakan wilayah gen pendek dan terstandar. Penelitian ini bertujuan untuk mengetahui keragaman populasi ikan gabus pada empat wilayah di Jawa Timur. Penelitian ini menggunakan sekuen gen Cytochrome b (Cyt b) pada DNA mitokondria untuk menentukan variasi genetic populasi liar Channa striata. Hasil dari DNA barcode dengan Cytochrome c oxidase subunit I (COI) menunjukkan seluruh sampel secara valid sebagai spesies C. striata (99.84-99.85%), sementara itu pada sequence Cyt-b memiliki percent identity dengan sequence species yang sama berkisar 99.74 to100%. Pohon filogenetik yang direkonstruksi dengan menggunakan MEGAX menghasilkan adanya clade terpisah pada sequences dari Jawa Timur. Hal ini memperkuat adanya kesamaan populasi species C. striata di daerah aliran sungai (DAS) Brantas.

Kata Kunci: *Cyt-b* gen, keanekaragaman, populasi, ikan gabus.

ABSTRACT

Snakehead fish with different geographical differences can have an impact on genetic diversity. DNA barcoding is a system designed to identify a species precisely and accurately by using short and standardized gene regions. This study aims to determine the diversity of snakehead fish populations in four areas in East Java. This study used the Cytochrome b (Cyt b) gene sequence in mitochondrial DNA to determine genetic variation in the wild population of Channa striata. The results of DNA barcodes with Cytochrome c oxidase subunit I (COI) showed that all samples were validly C. striata species (99.84-99.85%), while Cyt-b sequences had percentage identity with the same sequence species ranging from 99.74 to 100%. The phylogenetic tree reconstructed using MEGAX resulted in a separate clade in the sequences from East Java. This strengthens the similarity of the population of C. striata species in the Brantas watershed.

Keywords: *Cyt-b* gene, diversity, population, *snakehead*.

1. INTRODUCTION

Channa striata have great potential in the field of aquaculture because it has benefits in the food industry and also in the pharmaceutical sector. In addition to its high protein content, *C. striata*haves a high albumen content. Unfortunately, *C. striata* snakehead fish farming activities in Indonesia have not been carried out seriously (Mustafa et al. 2012). Snakehead fish type *C. striata* have been widely exploited because the public knows its pharmaceutical potential (Jais et al. 1997). With this potential, snakehead fish is included in the group of fish with high economic value (Gustiano et al. 2019). Due to the high exploitation of snakehead fish by fishing in nature, it has an impact on the decline in the snakehead fish population (Courteney et al., 2004). Many studies on snakehead fish populations have been carried out by conducting massive surveys in public waters such as rivers (Pariyanto et al. 2021), swamps (Bijaksana 2012), and lakes (Burnawi and Pamungkas 2016, Wakiah et al. 2019). Approaches based on morphometric and morphological characteristics are still being carried out, however, molecular approaches are also being improved. This is done because the type of snakehead fish that is spread in public waters in Java has a fairly high diversity. In this study, population studies on snakehead fish *C. striata* from the Brantas watershed which include Lamongan, Gresik and Surabaya. Previous studies, research is still being done on the COI gene region (Kholil 2019, Lutfitasari 2020), and in this study on another region on Cytochrome b.

Striped snakehead (*C. striata*) from the family Channidae which has a variety of kinds of habitats ranging from rivers, swamps, lakes, canals, and lakes, and can be found in rice fields. Channa consists of 34 species and is a fish native to the Asian region (Froese 2009). Out of the 34 species, in Indonesia, there are 10 species from the genus *Channa* (Gustiano et al. 2021). Beside the most dominant species *C. striata* which are spread in Sumatra, Kalimantan and Java, other species are *C. gachua* (Pinasti 2021), *C. pleurothalma* (Lubis et al. 2020), and *C. micropeltes* (Muhajirah et al. 2021). The distribution of *C. striata* in various regions has a different distribution including in swamps, rivers, rice fields, and lakes. This shows that *C. striata* are able to adapt and live in different conditions (Makmur 2017). Different habitats can show an influence on natural selection and changes in adaptive genetic composition (Fraser et al. 2014). Populations within one watershed will be more similar than populations from different watersheds, populations associated with short flows are expected to show less differentiation than populations separated by length or sections of the river (Kalinowski et al. 2008). In this study, specimens were collected in one area of the Brantas watershed in East Java. One of the gene regions used in this study is Cytochrome-c (Cyt-b) which has been widely used in various fish species (Bartáková et al. 2018, Chen et al. 2017, Garg and Mishra 2018), and the Cytochrome c oxidase subunit I (COI) as a region in the identification at the species level (Zhu et al. 2013).

The Cyt-b region gene is one of the genes in mitochondrial DNA that is often used in DNA analysis to identify species of fishery product raw materials and one of the molecular markers used in DNA barcoding research (Maulid et al. 2016, Sotelo et al. 2001). The use of the gene encoding Cyt-b has been used to identify counterfeit products that use tuna as raw material (Wulansari et al. 2015). The Cyt-b gene has functional implications in species that have unique metabolic needs including the need for a low-energy diet, large body size, adaptation to extreme needs and adaptation to high altitudes (Foote et al. 2011, McClellan et al. 2005). The existence of sequence variations in Cyt-b causes this gene to be widely used to compare species in the same genus or family, which based on the sequence of gene bases from Cyt-b, the area can provide phylogenetic information at the intraspecies level to the level between genera (Faizah 2008). In this study, snakehead fish from 4 regions were tested for kinship based on the Cyt-b DNA sequence as well as species confirmation based on DNA barcoding in the COI gene region.

2. MATERIAL AND METHODS

Sample Collection

Sampling included 4 locations, namely in Surabaya (7o18'25.0"S 112 o43'10.0"E), Gresik (7o03'26.0"S 112o34'26.0"E), Lamongan (7o04'33.0"S 112o16 '12.0"E), and Bojonegoro (7o09'00.0"S 111o52'12.0"E). Fish tissue was taken from the base of the tail (1 cm) and put into a sample bottle containing 70% ethanol (Andriyono and Suciyono 2020). Further experiments were carried out at the Toxoplasma Laboratory of the Tropical Diseases Institute (ITD) Universitas Airlangga Surabaya.

DNA Isolation and Amplification

DNA extraction using the gSYNCTM DNA Extraction kit (Maulid et al. 2016, Nuryanto et al. 2020). Amplification and visualization of DNA fragments was carried out by Polymerase Chain Reaction (PCR) using 2 pairs of universal primers. The first primer with a target gene for the Cyt-b region is GLUDG-L : 5' TGA CTT GAA RAA CCA YCG TTG and CB3-H 5' GGC AAA GAG AAA RTA TCA TTC 3' (Esa et al. 2012). The second primer, used for species identification using DNA barcoding regions in

the COI gene using a pair of Primers FishF1: 5' TCA ACC AAC CAC AAA GAC ATT OGC AC 3') and FishR1: 5' TAG ACT TCT GGG TGG CCA AAG AAT CA 3' (Panprommin et al. 2019). The PCR conditions include the predenaturation stage of 94ºC for 2 minutes, the denaturation process at 94ºC for 15 seconds, the annealing or attachment process at a temperature of 50ºC for 15 seconds and the extension process at 72ºC for 30 seconds and the post-extension process for 2 minutes at a temperature of 72ºC. The PCR product is then sent to the PT Genetics Science Indonesia company for the sequencing stage process (Nuryanto et al. 2020).

Data Analysis

Sequencing results are presented in the form of chromatograms edited using Chromas 2.6 Software (Panprommin et al. 2019). The edited sequence results are stored in a fasta file, followed by BLASTN analysis on the GenBank database at NCBI (http://blast.ncbi.nlm.nih.gov/). The results of the BLASTN were then aligned to create a phylogenetic tree by editing using the alignment tools on the MEGAX Software (Kumar et al. 2018). The phylogenetic tree was reconstructed using the Neighbor Joining (NJ) method with 1000 replications. The genetic distance between individuals was calculated using the Kimura 2 Parameter (K2P) method.

3. RESULT

Species Identification

The results of the nucleotide sequences in this study were compared with the GeneBank nucleotide data in order to determine the similarity of the samples tested. The results of BLASTN (Table 1) on the four samples showed that the species *C. striata* had the access code MN057176.1. Of the four samples (Lamongan, Gresik, Surabaya, and Bojonegoro) were validly *C. striata* species based on COI DNA barcoding markers. When compared to the number of COI and Cyt-b sequences in the database, unfortunately only 234 DNA sequences in the Cyt-b region were deposited in the NCBI Genbank database. This shows that research on these gene markers is still low. The seven sequence numbers are access codes KR007701, KR007700, KR007699, MN057181, MN057176, MN057164, and MN057163. Meanwhile, in the COI sequence, the number of sequences registered with GenBank is more than the Cyt-b sequence, which is 396 sequences from various countries. The sequence is publicly accessible at the NCBI link (https://www.ncbi.nlm.nih.gov/).

Table 1. Nucleotide BLASTN results in GeneBank in the COI and Cyt-p partial region sequences.

No.	ID	Species name / Common name	COI		Cyt-b	
			% *Identity*	*GB Acc. No*	% *Identity*	*GB Acc. No*
1	1G	*Channa striata* / Striped snakehead	99,85%	MF496960	100%	MN057176
2	2G	*Channa striata* / Striped snakehead	99,84%	MF496960	99,75%	MN057176
3	3G	*Channa striata* / Striped snakehead	99,84%	KU692423	99,74%	MN057176
4	4G	*Channa striata* / Striped snakehead	99,84%	MF496960	100%	MN057176

Phylogenetic tree reconstruction

Phylogenetic tree reconstruction has been generated by MEGAX software begins with the alignment tools and then aligns it with ClustalW to see the diversity of nucleotide bases. The phylogenetic tree was analyzed using the Neighbor Joining Tree (NJ) method, with a 2-parameter Kimura evolution model and 1000x boostrap replication (Figure 1). The COI sequences were not reconstructed by phylogenetic reconstruction, the sequences were only used in the determination of specimens at the species level. From the four Cyt-b gene sequences of *C. striata* species and secondary data from the GeneBank database, it can be seen that all sequences in this study have the same kinship. For comparison, we added sequences for the same species with access codes for Genbank KU852458 (China), MN541366 (Vietnam), KX177965 (India), AB822531 (Laos) to determine the position of the sequence in this study. All sequences are closely related to sequences from China and Vietnam, but are quite far apart from sequences from India and Laos.

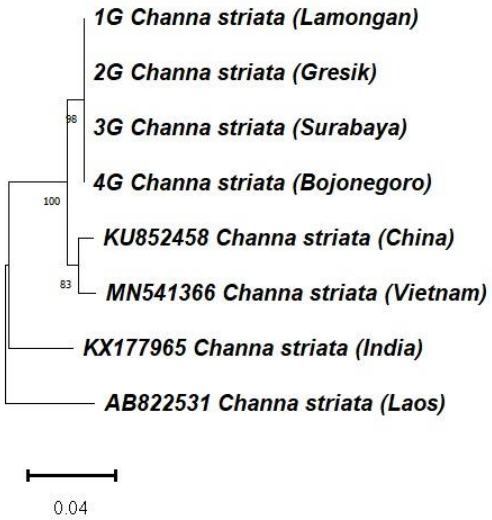

Figure 1. Phylogeographic Tree Based on Cytochrome-b was generated by MegaX through the neighbour-joining algorithm

Genetic distance

Genetic distance is the degree of difference in genes (nucleotide bases) within a species or population as measured by a numerical sum. Measurement of genetic distance was carried out using the MEGAX software (Kumar et al. 2018). The value of genetic distance can conclude a kinship relationship between populations of species from different areas by looking at the smaller the value of the genetic distance between species, the closer the relationship between the diversity of the population, as well as if the value of the genetic distance is greater, then the kinship between the population is further away. Various studies also explain that the genetic distance within the genus is lower than between genera, and the results of genetic distance analysis can affect the reconstruction of the phylogenetic tree (Khan et al. 2010).

The measurement of genetic distance from the four research samples was added by adding 4 species samples obtained from the Gene Bank database. The results of the calculation of genetic distance using the Cyt-b gene using MEGA X software, it is known that the intragenetic distance in the four samples of *C. striata* has no difference at all with a genetic distance of 0.0000. Meanwhile, the genetic distance from the Chinese sequence (KU852458) was 0.01924, and the farthest genetic distance from the Laos sequence (AB822531) was 0.07696 (Table 2).

Nucleotide Composition

The results of the analysis of the nucleotide composition of the four sample species were also compared with a number of sequences from the NCBI Database which showed that there were differences in the composition of the nucleotide bases possessed. The composition of the Unacil sequence from Indonesia shows a higher composition than Vietnam, Laos and India. This is in contrast to the proportion of cytocine which shows the lowest value compared to Cyt-b sequences from overseas samples. Meanwhile, the composition of adenine and guanine has relatively the same proportion in all analyzed sequences (Table 3).

Conservation status

Conservation status is one aspect that can be taken into account in the domestication of a species, based on the www.iucnredist.org site, it is known that the conservation status of Channa striata is LC (least concern). Meanwhile, CITES added the status of Striped snakehead to a fish that was not evaluated and grouped it into a harmless fish.

4. DISCUSSION

The existence of snakehead fish in Indonesia has been used as a food ingredient with a high protein content (Chasanah et al. 2015, Prastari et al. 2017), especially the albumin content which is quite good (Chasanah et al. 2015). There are ten species of snakehead fish in Indonesia, including *C. striata* (Gustiano et al. 2019, Gustiano et al. 2021). Due to the diversity of this type of fish, apart from paying attention to color patterns and other morphological characteristics, identification of fish species can also be done molecularly by knowing the differences in the DNA composition (Lakra et al. 2016, Zhu et al. 2013). This molecular identification approach is very helpful in a number of cases where samples are incomplete and damaged (Becker et al. 2015), or have undergone processing (Pollack et al. 2018). Species identification through genetic information of a population can be a turning point for sustainable cultivation programs through breeding programs (Gustiano et al. 2019). In this study, molecular identification was carried out to ensure that the species in this study were the same species, namely *C. striata*. The results of the BLASTN show that all samples from four areas in the Brantas watershed showed *C. striata* with a fairly high similarity, namely 99.84-99.85% (Table 1). Previous studies have stated that the distribution of *C. striata* in these four areas is also quite large (Amin et al. 2019, Hariati et al. 2019), although fish farming activities

have not been carried out on a massive scale, exploitation of natural catches is still being carried out.

Intensive catching of snakehead fish allows the population to decrease in nature. Fish populations in four areas of the Brantas watershed were analyzed based on gene characteristics in the Cyt-b region. The BLASTN results obtained from the Cyt-b 570 bp sequence in this study were similar to the species *C. striata* (MN057176) with a percent identity value of 99.74-100% (Table 1). This shows that the comparison results given are similar and reliable, with the e value getting closer to one, the e value cannot be trusted (Narita et al. 2014). The Cyt-b sequence also completes the confirmation of the species in this study as *C. striata* species. This Cyt-b sequence has been widely used in studies of diversity and population genetics in various types of fish (Aziz et al. 2015, Kartavtsev 2011, Sun et al. 2019). Population studies carried out in this study showed that *C. striata* from four sites had similarities in their nucleotide base sequences. This is shown in the reconstruction of the resulting phylogenetic tree.

Grouping species based on phylogenetic trees (Figure 1), can provide position information in population studies. The position of a species will be seen in a line or in a separate clade. The reconstruction of the phylogenetic tree also depends on the resulting boothstap value. The boostrap value shows the high accuracy of the branching formed. The greater the boostrap value, the higher the topological accuracy of the phylogenetic tree reconstruction (Kumar et al. 2000). In this phylogenetic reconstruction, the resulting bootstrap values are 83, 98 and 100 respectively at the branching points of the phylogenetic tree. The phylogenetic tree analysis aims to determine the relationship between species, and to study population studies of species which are generally depicted in a branching line like a tree (Irawan 2013). Phylogenetic tree analysis using DNA molecular data can describe the evolution between species (Dharmayanti 2011).

The reconstruction of the phylogenetic tree based on the Cyt-b DNA sequence formed one clade consisting of Indonesian samples (Surabaya, Gresik, Lamongan, and Bojonegoro), while the other clade consisted of sequences from outside Indonesia including those from China KU85248, and Vietnam MN541366. Likewise, the sequence from India KX177965 and the Laos sequence AB822531 were separated from the sample from Indonesia. This confirms that the Cyt-b sequence can be used in studies of species populations from a number of areas. The existence of genetic flow (gene flow) and the activity of introduction by humans is one of the causes of this diversity (Laudien et al. 2003). Although the distribution of *C. striata* in Asia is quite wide, but with various adaptations in each geographic area, it results in genotypic adaptations with different nucleotide sequences. A number of barriers to the spread of freshwater fish species are the presence of sea and land separation, as well as rivers with various currents. Some species eventually breed and adapt in lakes, rivers and swamps with various regional characteristics (Adamson et al. 2012). Based on the results of the genetic distance calculation, the four samples from Indonesia have a genetic distance value of 0.0000 (Table 2). It is clear that the four samples have a very close relationship with the low value of the resulting genetic distance. This can also indicate that the populations of the four samples have the same geographical kinship and have the same watershed, namely the Berantas River and the Bengawan Solo River. Populations associated with short streams are expected to show less differentiation than populations separated by lengths or sections of stream (Kalinowski et al. 2008). The genetic distance difference which is quite far is shown by samples from India and Laos with genetic distance values of 0.06400 and 0.07696 respectively, this shows that the kinship relationship of *C. striata* from Indonesia with India and Laos has a very distant relationship. Based on the results obtained in this study, the Cyt b gene was successfully used to study intrapopulation variation.

In addition to genetic distance, this study also analyzed the composition of A, T, G and C nucleotides (Table 3) by equating the lengths of the tested sequences. The results of this analysis showed that Channa striata based on the Cytb gene from Surabaya, Gresik, Lamongan, Bojonegoro, had identical sequence percentages, namely Thymine (T) 31.8%, Citocine (C) 29.9%, Adenine (A) 24 .8%, Guanine (G) 13.6%. Meanwhile, in other sequences KU852458 (China), MN541366 (Vietnam), India (KX177965), and Laos (AB822531) have slightly different proportions. In this study, it was shown that the nucleotide composition of the four samples from Surabaya, Gresik, Lamongan, and Bojonegoro had the same nucleotide composition, indicating that the Channa striata species in the four samples had a very close relationship and could be said to be the same species. The composition of A+T is higher than that of G+C in the Cyt-b region (Johns and Avise 1998)

Based on its conservation status, according to the IUCN Red List in 2019, *C. striata* species is included in the Least Concern (LC) category or has a low risk (Table 4). This *C. striata* species according to CITES data has not been evaluated (Not Evaluated), which indicates that it is still safe for trade. *C. striata* is quite abundant in the wild, which causes not much cultivation activities are carried out. However,

monitoring and controlling activities for catching *C. striata* in the wild using non-environmentally friendly fishing gear need serious attention.

CONCLUSION

The results of the analysis of the Cyt-b gene sequence of snakehead fish (*C. striata*) from the four geographic areas have the same genetic population because the samples were taken from the same watershed. The genetic distance value between species from the four regions has a genetic distance value of 0.000. The genetic distance is quite far between *C. striata* from Indonesia and *C. striata* from China and Vietnam of 0.01924 and 0.01220, and the farthest genetic distance from India is 0.06400 and Laos is 0.07696. Further research and sampling from different geographical and geographical locations are needed to complete the genetic information (*Cyt-b* gene) for *C. striata* species in Indonesia and at the same time determine the number of possible haplotypes. The wide distribution of *C. striata* in Southeast Asia, therefore management and conservation need to be considered. Breeding activities in *C. striata* aquaculture can be done by taking broodstock sources from a number of areas to increase the heterogeneity of the genes they have.

Tabel 2. Summary of genetic distance of streped sneakehead Channa striata was generated from MegaX.

No.	Samples ID / Species name /origin	1G Lamongan	2G Gresik	3G Surabaya	4G Bojonegoro	KU852458 China	MN541366 Vietnam	AB822531 Laos	KX177965 India
1	1G *Channa striata* Lamongan								
2	2G *Channa striata* Gresik	0,00000							
3	3G *Channa striata* Surabaya	0,00000	0,00000						
4	4G *Channa striata* Bojonegoro	0,00000	0,00000	0,00000					
5	KU852458 *Channa striata* (China)	0,01924	0,01924	0,01924	0,01924				
6	MN541366 *Channa striata* (Vietnam)	0,02120	0,02120	0,02120	0,02120	0,01536			
7	AB822531 *Channa striata* (Laos)	0,07696	0,07696	0,07696	0,07696	0,08372	0,07930		
8	KX177965 *Channa striata* (India)	0,06400	0,06400	0,06400	0,06400	0,07056	0,06840	0,07260	

Table 3. The nucleotide composition in the Cyt b sequence by equating the length of the aligned sequence is 529bp.

No.	Nama Spesies	Basa Nukleotida				Total
		T(U) %	C %	A %	G %	
1	1G *Channa striata* (Lamongan)	31.8	29.9	24.8	13.6	529
2	2G *Channa striata* (Gresik)	31.8	29.9	24.8	13.6	529
3	3S *Channa striata* (Surabaya)	31.8	29.9	24.8	13.6	529
4	4G *Channa striata* (Bojonegoro)	31.8	29.9	24.8	13.6	529
5	KU852458 *Channa striata* (China)	31.2	30.2	24.8	13.8	529
6	MN541366 *Channa striata* (Vietnam)	30.2	31.8	24.4	13.6	529
7	AB822531 *Channa striata* (Laos)	30.2	31.8	24.4	13.6	529
8	KX177965 *Channa striata* (India)	30,8	30,8	24,7	13,7	529

Ket : T : Timin, U : Urasil, A : Adenin, G : Guanin, C : Citosin

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