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Genetic diversity of indigenous catfish from Indonesia based on mitochondrial Cytochrome Oxidase Subunit II gene

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Abstract. *Budiariati V, Susmiati T, Waroh S, Putri RCA, Widayanti R. 2021. Genetic diversity of indigenous catfish from Indonesia based on mitochondrial Cytochrome Oxidase Subunit II gene. Biodiversitas 22: 593-600.* Catfish is one of the most demanding fish in Indonesia and served in a variety of traditional culinary. Due to their identical morphology and close relation between species in the order of Siluriformes, it is quite tricky to distinguish the species. This can be a threat to develop catfish production in Indonesia since there is a wide variety of catfish species in this mega biodiversity country. The study aimed to analyze the genetic diversity of Indonesian indigenous catfish especially those known as Baung fish by local people based on COII gene. The study also aimed to determine the phylogenetic relationship between the samples and compare them with the GenBank data. A total of 24 samples used in this study from 8 different rivers from 3 different islands and two samples were collected from coastal areas. The study results showed that there is genetic diversity of the Indonesian indigenous catfish based on COII gene. The sequences among 24 samples showed that from 691 nucleotides of COII gene, there were very subtle nucleotides differences of samples that originated from Bojonegoro, Magelang, and samples collected from Baru Beach, Yogyakarta. Based on COII amino acid sequences, 6 polymorphic amino acid sites were on-site number 64, 115, 123, 129, 144, and 165. The samples encoded LLB1 and LPB1 from Baru Beach, Yogyakarta, showed highest different amino acids in the six sites. Samples from the river of Central Java, Sumatra, and Kalimantan belonged to Bagridae family and consist of two different species *Hemibagrus* sp. and *Mystus* sp while samples from East Java belonged to Pangasiidae family. The Samples from coastal belonged to Ariidae family.

Keywords: Bagridae, cytochrome oxidase subunit II, Indonesian indigenous catfish, phylogenetic, Siluriformes

INTRODUCTION

Catfish is one of the most demanding fish in Indonesia and served in a variety of traditional Indonesian culinary delights. It also has high protein content and promising for being a good source of protein and other nutritional values (Kawiji et al. 2020; Mesomya et al. 2015). The fish are more adaptable and possess all the characteristics necessary for aquaculture including relatively high fecundity, ability for artificial spawning, adaptability to earthen ponds for culture, high tolerance to low dissolved oxygen, relatively high resistance against infectious diseases, and relatively high feed conversion efficiency (Jin et al. 2016). Catfish is one of the primary aquaculture species in the United States and its global importance is increasing in several countries in Asia including Indonesia (Liu 2008; Liu, 2011).

Catfish belong to the order of Siluriformes which is one of the largest orders of teleosts. Currently, 36 families and over 3,000 species are recognized, rendering catfishes among the most diverse vertebrate orders (approximately 1 in 10 actinopterygians or 1 in 20 vertebrates is a catfish) (Ferraris 2007; Kappas et al. 2016).

The fish's characteristics include whisker-like barbels, which are located on the nose, each side of the mouth, and on the chin, possess leading spines in their dorsal and pectoral fins (Arce et al. 2013; Armbruster, 2004). They are scaleless which is a characteristic that distinguishing them from most other teleost fish (Jin et al. 2016). However, due to their identical morphology and close relation between species in the order of Siluriformes, it is quite tricky to distinguish the species of the catfish. This can be a threat to develop catfish production in Indonesia since there is a wide variety of catfish species in this mega biodiversity country. Moreover, local people in Indonesia tend to use the same term to identify this type of fish. Analysis of genetic diversity is a critical measure in population studies, including for catfish, because by hinting on the evolutionary history of a population, it reveals the current and future health of the population since low levels of genetic diversity causes inbreeding depression in the short run and reduced evolutionary potential in the long run (Buj et al. 2014; Kirk and Freeland 2011).

Previous studies reported that indigenous catfish of Indonesia classified into *Hemibagrus nemurus*, which belong to Bagridae family, but there are some species that allocated in the Pangasiidae family based on cytochrome oxidase subunit III and species from Papua that belong to Ariidae family based on cytochrome B gene (Syaifudin et

al. 2017; Widayanti et al. 2019; Megarani et al. 2020). Genetic analysis itself recently highlighted related to the possibility for applications in fishery genetics (Kochzius 2009). The aims of this study was to analyze the genetic diversity of indigenous catfish from Indonesia based on mitochondrial cytochrome oxidase subunit II (COII) gene especially those known as Baung fish by the local people. The study also aimed to determine the phylogenetic relationship between the sample of indigenous catfish from Indonesia and compare them with the available sequence from the GenBank to develop DNA barcoding of catfish from different regions of Indonesia from the mitochondrial DNA sequences.

MATERIALS AND METHODS

Sample collections

A total of 24 samples from different regions of Indonesia known as Baung catfish for local people were collected and used as the samples of this study. The origin of the samples was from 8 different rivers from 3 different islands and two samples were collected from Baru Beach, Yogyakarta. The origin, number of samples as well as codes of the samples are presented in Table 1. Geographical map of sampling sites as shown in Figure 1. Each sample was taken individually from its habitat after being identified based on the morphologies. The samples used in this study were the tissues preserved in the RNA latter buffer (Qiagen) and then used for the total DNA isolation.

The DNA for the genetic analysis was extracted from the tissue biopsy (30 mg). DNA Isolation Kit (Qiagen) was used for DNA extraction and purification. Extracted total DNA was detected by electrophoresis method and stored at -20˚C until further examination. Amplification of the targeted DNA fragments was done by polymerase chain reaction (PCR) method. The pair primer of COII gene was designed with Primer3 output program (http://wwwgenome.wi.mit.edu/cgi.bin/primr3.cgi/results_from-primer3) based on genetic sequence data of Hemibagrus nemurus (Access Number KJ573466.1) and Mystus vittatus (Access Number KX177968.1). The primer sequences for DNA amplification were Baung_COIIF 5' CCGCTCTGTCACTTTCTTTT 3' and Baung_COIIR 5' GCTCATTTGTGTCCTCCTTT 3' with the melting temperature of 53,1˚C and 53˚C. Amplification of the DNA was done in this condition: predenaturation 2 minutes at 94°C; denaturation 30 seconds at 94°C; annealing 45 seconds at 46°C; elongation 1 minutes 30 seconds at 72°C (35 cycles) and post-elongation 5 minutes at 72°C.

Table 1. Indigenous catfish samples from Indonesia

Origin	No. of samples	Sample codes
Martapura River, Banjarmasin, South Kalimantan	3	BJ1; BJ2; BJ3
Bengawan Solo River, Bojonegoro, East Java	3	BO1; BO2; BO3
Elo River, Magelang, Central Java	2	EM1; EM2
Kapuas River, Sintang, West Kalimantan	2	KS1; KS2
Kampar River, Pekanbaru, Riau	3	KR1; KR2; KR3
Mahakam River, Samarinda, East Kalimantan	3	MS1; MS2; MS3
Musi River, Palembang, South Sumatra	3	MP1; MP2; MP3
Progo River, Magelang, Central Java	3	PM1; PM2; PM3
Baru Beach, Yogyakarta	2	LLB1; LPB1

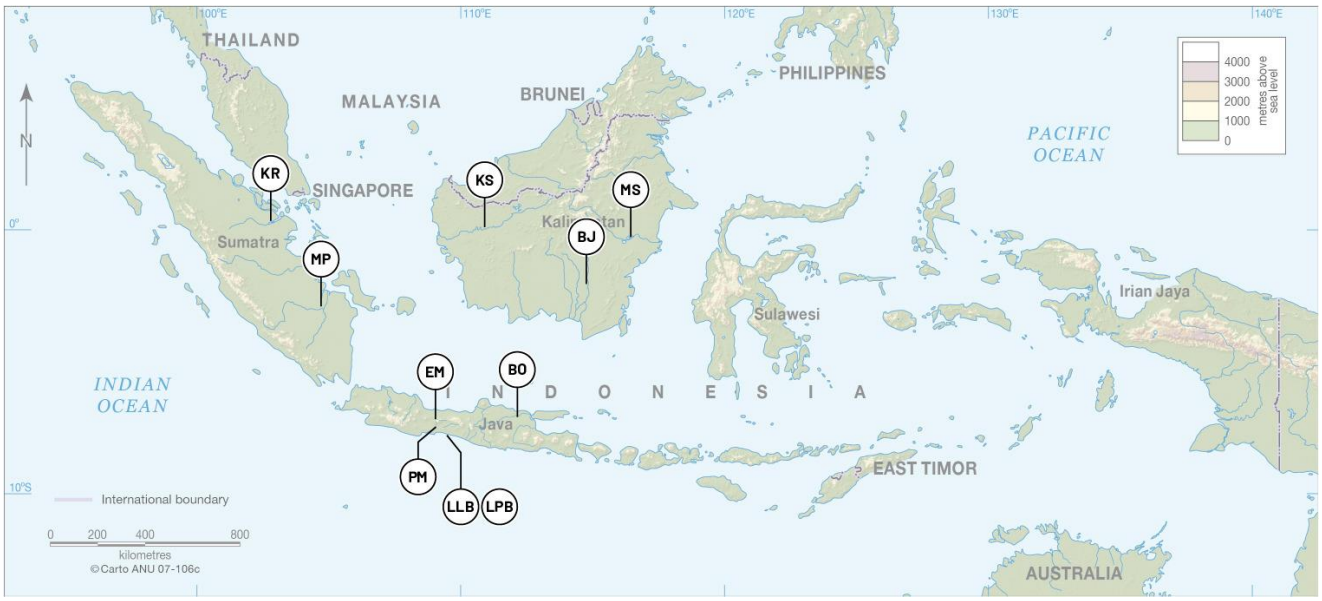

Figure 1. Geographical map of the sampling sites. BJ: Martapura River, Banjarmasin, South Kalimantan; BO: Bengawan Solo River, Bojonegoro, East Java; EM: Elo River, Magelang, Central Java; KS: Kapuas River, Sintang, West Kalimantan; KR: Kampar River, Pekanbaru, Riau, Sumatera; MS: Mahakam River, Samarinda, East Kalimantan; MP: Musi River, Palembang, South Sumatera; PM: Progo River Magelang, Central Java; LLB1, LPB1: Baru Beach, Yogyakarta, Indonesia

Tabel 2. Amplicon product of mitochondrial DNA fragments (1242 bp) after being aligned with sequence data of *Hemibagrus nemurus* (NC_044863.1) from GenBank

Nucleotide sequence number (5'->3')	DNA fragments	Length of nucleotide sequences
7063-7080	tRNASer	18
7081-7084	intron	4
7085-7157	tRNA Asp	73
7158-7171	intron	14
7172-7862*	COII*	691*
7863-7936	tRNALys	74
7937	intron	1
7938-8105	ATP8	168
8096-8304	ATP6 partial	209

Note: * targeted gene COII

The amplicon products after being aligned with sequence data of COII gene of *Hemibagrus nemurus* (NC_044863.1) from GenBank is 1242 bp at the site number 7063 to 8304 which amplifies several DNA fragments as shown in Table 2.

Sequencing and phylogenetic analysis

The sequencing of the amplicon products was done by 1st Base Sequencing Int. (Singapore). The analysis of the sequences data was done using the MEGA program version X (Kumar et al. 2018). The sequences data were aligned with ClustalW followed by consensus editing and then compared to related sequence data from others catfishes recorded in the GenBank ((*Hemibagrus nemurus* (NC_044863.1); *Mystus_vittatus* (NC_032082.1); *Mystus cavasius* (NC_030187.1); *Hemibagrus wyckioides* (NC_024278.1); *Hemibagrus guttatus* (NC_023976.1); *Mystus rhegma* (NC_023223.1); Hemibagrus spilopterus (NC_023222.1); Liobagrus_marginatus (NC_022923.1); *Pseudobagrus ondon* (NC_022725.1); *Liobagrus anguillicauda* (NC_021602.1); *Exostoma labiatum* (NC_021601.1); *Pseudobagrus truncates* (NC_021395.1); Leiocassis crassilabris (NC_021394.1); *Pseudobagrus brevicaudatus* (NC_021393.1); *Pseudobagrus ussuriensis* (NC_020344.1); *Hemibagrus macropterus* (NC_019592.1); *Glyptothorax fokiensis fokiensis* (NC_018769.1); *Pelteobagrus eupogon* (NC_018768.1); *Pelteobagrus fulvidraco* (NC_015888.1); *Pangasius_larnaudii* (NC_015839.1); *Pareutropius debauwi* (NC_015837.1); *Malapterurus electricus* (NC_015833.1); *Heteropneustes fossilis* (NC_015827.1); *Diplomystes nahuelbutaensis* (NC_015823.1); *Bunocephalus coracoideus* (NC_015811.1); *Auchenoglanis occidentalis* (NC_015809.1); *Synodontis schoutedeni* (NC_015808.1); *Silurus asotus* (NC_015806.1); *Pimelodus_pictus* (NC_015797.1); *Clarias* sp. (NC_015749.1); *Centromochlus perugiae* (NC_015748.1); *Pterygoplichthys disjunctivus* (NC_015747.1); *Amphilius* sp. (NC_015746.1); *Amblydoras gonzalezi* (NC_015745.1); *Pseudobagrus brevicorpus* (NC_015625.1); *Pelteobagrus nitidus* (NC_014859.1); *Leiocassis longirostris* (NC_014586.1); *Cranoglanis bouderius* (NC_008280.1); *Corydoras rabauti* (NC_004698.1); *Pseudobagrus tokiensis* (NC_004697.1); *Ictalurus punctatus* (NC_003489.1); *Arius arius* (NC_036673.1); *Arius maculatus* (NC_045222.1); Outgroup: *Cyprinus carpio* (NC_001606.1)).

The COII targeted gene (691 bp) from the samples then analyzed to determine the genetic diversity using Kimura 2-parameter method, with 1000 replicates of the bootstrap method. The phylogenetic analysis was performed using Neighbor-joining to depict the relationship between the species and clusters between the individuals.

RESULTS AND DISCUSSION

Variation of nucleotides and amino acids sequences

Catfish have been the focus of varied research for many years due to high economic value related to their protein and other nutritional values (Kwasek et al. 2020). Catfishes are classified in the order Siluriformes which currently had 36 families and over 3,000 species are recognized (Ferraris 2007; Kappas et al. 2016). There is wide distribution of catfishes in Indonesia. It can be found in the rivers of Java, Sumatera, Kalimantan, Sulawesi, and Papua Island. Local people have used the morphological features to identify this species although determination based on the morphology itself may cause identification confound (Ng and Kottelat 2013).

Previous studies reported that geographical aspects in Indonesian lands influenced the speciation of catfish and resulted in genetic diversity of catfishes based on cytochrome-B and cytochrome oxidase subunit II gene (Widayanti et al. 2019; Megarani et al. 2020). Based on the previous reports, phylogenetic analysis using the DNA sequences is needed to reveal accurate species identification and support the conservation program. Finding the most appropriate gene sequences to distinguish the differences between species is also definitely needed to develop DNA barcodes as an effective species identification approach. Therefore, the aims of the study was to determine the molecular characteristic and phylogenetic relationship of the Indonesian catfish especially the fish known as Baung fish by local people using cytochrome oxidase subunit II gene.

The analysis of the sequences among 24 samples of the indigenous samples known as Baung fish by local people showed that from 691 nucleotides from COII *gene* there were very subtle nucleotides differences of samples which originated from Bojonegoro (Bengawan Solo River), Magelang (Elo River), and samples which were collected from Baru Beach Yogyakarta as shown in Table 3. The nucleotide differences resulted in the variation of amino acids between 24 samples. There were 6 polymorphic amino acid sites which were on site number 64, 115, 123, 129, 144, and 165. The samples encoded LLB1 and LPB1 from the Baru Beach showed highest different amino acids in that 6 six sites, followed by samples encoded BO1, BO2, BO3 which showed differences in 4 sites (amino acid site number 115, 123, 144, 165). The samples encoded EM1 and EM2 had 2 amino acid polymorphic site on site number 123 and 129. Meanwhile, the samples encoded KR1, KS1, and KS2 showed 1 difference of amino acid on site number 129 as shown in Table 4.

Table 4. Amino acid polymorphic site

Sample	Amino acid site					
	64	115	123	129	144	165
BJ1	V	N	V	A	V	L
BJ2	.	.	.	.	.	.
BJ3	.	.	.	.	.	.
BO1	.	D	I	.	I	V
BO2	.	D	I	.	I	V
BO3	.	D	I	.	I	V
EM1	.	.	I	N	.	.
EM2	.	.	I	N	.	.
KR1	.	.	.	T	.	.
KR2	.	.	.	.	.	.
KR3	.	.	.	.	.	.
KS1	.	.	.	T	.	.
KS2	.	.	.	T	.	.
LLB1	I	.	I	S	I	V
LPB1	I	.	I	S	I	V
MP1	.	.	.	.	.	.
MP2	.	.	.	.	.	.
MP3	.	.	.	.	.	.
MS1	.	.	.	.	.	.
MS2	.	.	.	.	.	.
MS3	.	.	.	.	.	.
PM1	.	.	.	.	.	.
PM2	.	.	.	.	.	.
PM3	.	.	.	.	.	.

Note: The identity of the samples shown in Table 1. Identification with the first sequence is denoted by a dot (.)

In this research, samples from Bengawan Solo River, Bojonegoro, East Java and from Elo River, Magelang, Central Java had high number of nucleotides differences as well as the amino acid variation. These results supported the confirmation that the samples were different species based on cytochrome-B and cytochrome oxidase subunit III gene (Widayanti et al. 2019; Megarani et al. 2020) whereas samples from the Progo River, Magelang, Central Java were less different and closer to the samples from other rivers from the different islands. These results strengthen the hypothesis that distinguishes the species based on morphological features is not fully accurate so that genetic-based identification is needed.

Comparing the samples from rivers or streams in Kalimantan, Sumatera, and Java revealed that the differences between the nucleotides and amino acid variations between samples were relatively not significantly different. The results indicate closely relation between indigenous catfishes from different rivers in the regions of Indonesia. In this study, we also add samples from Baru Beach Yogyakarta which had morphological similarity with samples taken from the various rivers and had been known by the local people as Baung fish, one of the local terminology of catfish in Indonesia. The samples from the coastal were included based on the morphology characteristic and it is possible to found the fish since catfish are highly diverse and distributed worldwide and commonly found in inland or coastal waters of all continents (Jin et al. 2016). Unfortunately, those samples originated from the beach were different from other sample-based on COII sequence which implies that the samples were different species.

The results of amino acid polymorphic sites were in line with the nucleotide differences. Based on COII amino acid sequences, 6 polymorphic amino acid sites were on site number 64, 115, 123, 129, 144, and 165. The most different sample which are the samples encoded LLB1 and LPB1 showed highest different amino acids in the six sites of amino acid polymorphic sites followed by samples BO1, BO2, BO3 (4 sites of amino acids differences), samples EM1 and EM2 (4 sites of amino acids differences) and samples KR1, KS1, and KS2 which had 1 differences of amino acids.

Genetic distances between samples of indigenous catfish from different regions of Indonesia

The differences of nucleotides that influenced the amino acid compositions also related to the genetic distance between species. The analysis of the genetic distance between the samples based on COII gene using Kimura 2-parameter method showed that samples encoded BJ1, BJ2, BJ3, MP1, MP2, MP3, KR2, MS1, MS2, MS3, PM1, PM2, PM3, KR3, KR1, KS1, KS2 were closer than those samples encoded BO1, BO2, BO3, EM1, EM2, LLB1, LPB1 which had higher score of genetic distance compared to other samples. This indicates genetic diversity between samples from different regions and also between individuals as shown in Table 5. The confirmation of the results was done by phylogenetic analysis to clarify the genetic diversity of the samples and determine the phylogenetic relationship with other species in the order of Siluriformes.

Phylogenetic relationships between Indonesian catfish and other catfish species

Order Siluriformes is one of the largest order of teleost which included not less than 4100 species, and representing approximately 12% of all teleosts and 6.3% of all vertebrates (Wilson and Reeder, 2005). It is reported that the order currently had 36 families and over 3,000 species are recognized (Ferraris 2007). The family of the order at least includes Amblycipitidae, Amphiliidae, Aspredinidae, Auchenipteridae, Bagridae, Callichtyidae, Clariidae, Cranoglanidididae, Diplomystidae, Doradidae, Heteropneustidae, Ictaluridae, Loricariidae, Malapteruridae, Mochokidae, Pangasiidae, Pimelodidae, Schilbeidae, Siluridae, and Sisoridae (Kappas et al. 2016). We used representatives of each family to compare the samples and other species from GenBank data and build the phylogenetic tree based on nucleotide and amino acid sequence. The comparative species are listed in the material and methods section.

The phylogenetic analysis was done to identify the taxon phenogram of the samples and the phylogenetic relationship of the Indonesian catfish samples and other related species. The analysis was done by constructing the phylogenetic tree using the NJ method. The phylogenetic tree between the samples and other species taken from GenBank shown in Figure 2. Figure 2 shows that the Indonesian catfish were separated into four clades or monophyletic groups, in conjunction with the other catfish species around the world. First, Indonesian catfish samples

PM1, PM2, PM3, KR1, BJ1, BJ2, BJ3, KR2, MP1, MP2, MP3, MS1, MS2, MS3 and KS2, KR1, KS1 were clustered with *Hemibagrus nemurus* (NC_044863.1) and *Hemibagrus wyckioides* (NC_024278.1). Samples EM1 and EM2 were clustered with *Mystus vittatus* (NC_032082.1) and *Mystus cavasius* (NC_030187.1). Furthermore, samples LLB1 and LPB1 were in the same groups with *Silurus asotus* (NC_015806.1); *Bunocephalus coracoideus* (NC_015811.1); *Centromochlus perugiae* (NC_015748.1). Samples BO1, BO2, BO3 were together with *Pangasius larnaudii* (NC_015839.1).

Table 3. Nucleotides differences of indigenous catfish from different regions of Indonesia

	BJ1	BJ2	BJ3	BO1	BO2	BO3	EM1	EM2	KR1	KR2	KR3	KS1	KS2	LLB1	LPB1	MP1	MP2	MP3	MS1	MS2	MS3	PM1	PM2	PM3
BJ1																								
BJ2	1.00																							
BJ3	1.00	0.00																						
BO1	90.00	90.00	90.00																					
BO2	90.00	90.00	90.00	0.00																				
BO3	90.00	90.00	90.00	0.00	0.00																			
EM1	73.00	72.00	72.00	102.00	102.00	102.00																		
EM2	73.00	72.00	72.00	102.00	102.00	102.00	0.00																	
KR1	10.00	10.00	10.00	95.00	95.00	95.00	74.00	74.00																
KR2	5.00	4.00	4.00	90.00	90.00	90.00	70.00	70.00	12.00															
KR3	4.00	3.00	3.00	93.00	93.00	93.00	73.00	73.00	11.00	5.00														
KS1	11.00	11.00	11.00	94.00	94.00	94.00	73.00	73.00	1.00	13.00	12.00													
KS2	9.00	9.00	9.00	94.00	94.00	94.00	73.00	73.00	1.00	11.00	10.00	2.00												
LLB1	88.00	88.00	88.00	90.00	90.00	90.00	88.00	88.00	85.00	87.00	89.00	84.00	84.00											
LPB1	88.00	88.00	88.00	90.00	90.00	90.00	88.00	88.00	85.00	87.00	89.00	84.00	84.00	0.00										
MP1	4.00	3.00	3.00	91.00	91.00	91.00	71.00	71.00	11.00	1.00	4.00	12.00	10.00	86.00	86.00									
MP2	4.00	3.00	3.00	91.00	91.00	91.00	71.00	71.00	11.00	1.00	4.00	12.00	10.00	86.00	86.00	0.00								
MP3	4.00	3.00	3.00	91.00	91.00	91.00	71.00	71.00	11.00	1.00	4.00	12.00	10.00	86.00	86.00	0.00	0.00							
MS1	6.00	5.00	5.00	91.00	91.00	91.00	71.00	71.00	11.00	7.00	6.00	12.00	10.00	89.00	89.00	6.00	6.00	6.00						
MS2	6.00	5.00	5.00	91.00	91.00	91.00	71.00	71.00	11.00	7.00	6.00	12.00	10.00	89.00	89.00	6.00	6.00	6.00	0.00					
MS3	6.00	5.00	5.00	91.00	91.00	91.00	71.00	71.00	11.00	7.00	6.00	12.00	10.00	89.00	89.00	6.00	6.00	6.00	0.00	0.00				
PM1	3.00	2.00	2.00	92.00	92.00	92.00	72.00	72.00	10.00	4.00	3.00	11.00	9.00	88.00	88.00	3.00	3.00	3.00	5.00	5.00	5.00			
PM2	3.00	2.00	2.00	92.00	92.00	92.00	72.00	72.00	10.00	4.00	3.00	11.00	9.00	88.00	88.00	3.00	3.00	3.00	5.00	5.00	5.00	0.00		
PM3	3.00	2.00	2.00	92.00	92.00	92.00	72.00	72.00	10.00	4.00	3.00	11.00	9.00	88.00	88.00	3.00	3.00	3.00	5.00	5.00	5.00	0.00	0.00	

Note: The identity of the samples shown in Table 1

Table 5. Genetic distances of indigenous catfish from different regions of Indonesia

	BJ1	BJ2	BJ3	BO1	BO2	BO3	EM1	EM2	KR1	KR2	KR3	KS1	KS2	LLB1	LPB1	MP1	MP2	MP3	MS1	MS2	MS3	PM1	PM2	PM3
BJ1																								
BJ2	0.00																							
BJ3	0.00	0.00																						
BO1	0.14	0.15	0.15																					
BO2	0.14	0.15	0.15	0.00																				
BO3	0.14	0.15	0.15	0.00	0.00																			
EM1	0.12	0.11	0.11	0.17	0.17	0.17																		
EM2	0.12	0.11	0.11	0.17	0.17	0.17	0.00																	
KR1	0.01	0.01	0.01	0.15	0.15	0.15	0.12	0.12																
KR2	0.01	0.01	0.01	0.15	0.15	0.15	0.11	0.11	0.02															
KR3	0.01	0.00	0.00	0.15	0.15	0.15	0.12	0.12	0.02	0.01														
KS1	0.02	0.02	0.02	0.15	0.15	0.15	0.12	0.12	0.00	0.02	0.02													
KS2	0.01	0.01	0.01	0.15	0.15	0.15	0.12	0.12	0.00	0.02	0.01	0.00												
LLB1	0.14	0.14	0.14	0.15	0.15	0.15	0.14	0.14	0.14	0.14	0.14	0.13	0.13											
LPB1	0.14	0.14	0.14	0.15	0.15	0.15	0.14	0.14	0.14	0.14	0.14	0.13	0.13	0.00										
MP1	0.01	0.00	0.00	0.15	0.15	0.15	0.11	0.11	0.02	0.00	0.01	0.02	0.01	0.14	0.14									
MP2	0.01	0.00	0.00	0.15	0.15	0.15	0.11	0.11	0.02	0.00	0.01	0.02	0.01	0.14	0.14	0.00								
MP3	0.01	0.00	0.00	0.15	0.15	0.15	0.11	0.11	0.02	0.00	0.01	0.02	0.01	0.14	0.14	0.00	0.00							
MS1	0.01	0.01	0.01	0.15	0.15	0.15	0.11	0.11	0.02	0.01	0.01	0.02	0.01	0.14	0.14	0.01	0.01	0.01						
MS2	0.01	0.01	0.01	0.15	0.15	0.15	0.11	0.11	0.02	0.01	0.01	0.02	0.01	0.14	0.14	0.01	0.01	0.01	0.00					
MS3	0.01	0.01	0.01	0.15	0.15	0.15	0.11	0.11	0.02	0.01	0.01	0.02	0.01	0.14	0.14	0.01	0.01	0.01	0.00	0.00				
PM1	0.00	0.00	0.00	0.15	0.15	0.15	0.11	0.11	0.01	0.01	0.00	0.02	0.01	0.14	0.14	0.00	0.00	0.00	0.01	0.01	0.01			
PM2	0.00	0.00	0.00	0.15	0.15	0.15	0.11	0.11	0.01	0.01	0.00	0.02	0.01	0.14	0.14	0.00	0.00	0.00	0.01	0.01	0.01	0.00		
PM3	0.00	0.00	0.00	0.15	0.15	0.15	0.11	0.11	0.01	0.01	0.00	0.02	0.01	0.14	0.14	0.00	0.00	0.00	0.01	0.01	0.01	0.00	0.00	

Note: The identity of the samples shown in Table 1

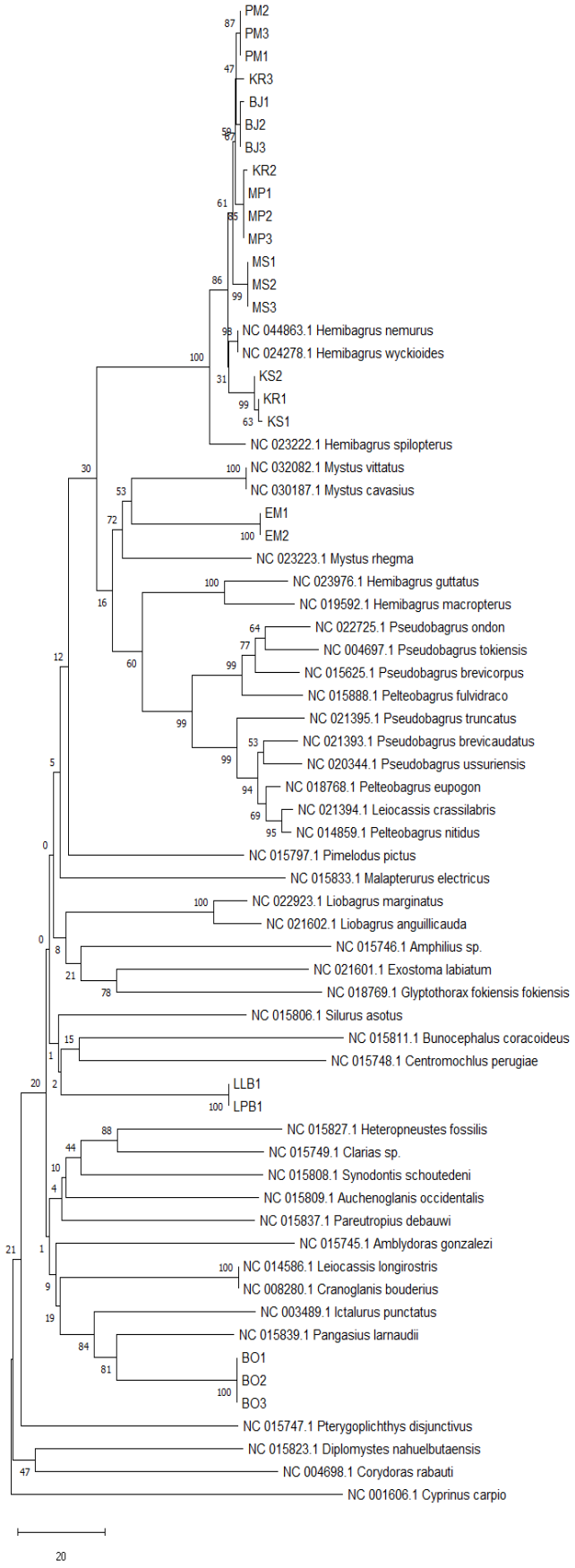

Figure 2. Phylogram of Indonesian catfish based on the Cytochrome Oxidase Subunit II nucleotide sequence

The analysis of the phylogenetic tree based on amino acid sequence shown in Figure 3. Based on amino acid sequences, the Indonesian catfish samples were classified into four monophyletic groups. Samples MS2, MS3, MS1, KR3, KR2, PM3, PM2, PM1, MP1, MP3, MP3, BJ3, BJ2 and samples KR1, KS1, KS2 were together with *Hemibagrus nemurus* (NC_044863.1) and *Hemibagrus wyckioides* (NC_024278.1). Samples EM1 and EM2 were clustered with *Mystus_vittatus* (NC_032082.1), *Mystus cavasius* (NC_030187.1), and *Mystus rhegma* (NC_023223.1). Samples LLB1 and LPB1 were in the same clade with *Auchenoglanis occidentalis* (NC_015809.1), *Leiocassis longirostris* (NC_014586.1), and *Cranoglanis bouderius* (NC_008280.1). Meanwhile, samples BO1, BO2, BO3 were grouped with *Pangasius_larnaudii* (NC_015839.1).

Based on the phylogram, samples PM1, PM2, PM3, KR1, BJ1, BJ2, BJ3, KR2, MP1, MP2, MP3, MS1, MS2, MS3 and KS2, KR1, KS1 were in the same clade with *Hemibagrus* sp. The species *Hemibagrus nemurus* has been reported as the species that originated from Southeast Asian (Dodson et al. 1995; Dodson 1999). It makes sense that the species from the river of varied regions of Indonesia were *Hemibagrus* sp that belong to Bagridae family. Samples from the same Province PM1, PM2, PM3 from Progo River, Magelang, Central Java and samples EM1 and EM2 from Elo River, Magelang, Central Java clustered in two different clades. Sample EM1 and EM2 were in the same group with *Mystus vittatus* and *Mystus cavasius* while samples from Progo River identified as *Hemibagrus* sp. The two different groups however still in same family of Bagridae. Interestingly, the samples from different islands encoded KS1, KS2 from Kapuas River, Sintang, West Kalimantan and KR1 from Kampar River, Pekanbaru, Riau, Sumatera showed close relationship and clustered in the same branch of phylogeny. These findings showed that geographical aspect influenced the genetic diversity but it is possible that species from different islands may have close genetic relation and the samples taken from the same regions can also be a different species but classified in the same family. These results are in line with previous study which reported that genetic diversity in fishes can be influenced by habitat type and environmental factors including their geographical differences (Nicol et al. 2017; Martinez et al. 2018). Further research regarding the effect of geographic location differences on the genetic diversity of catfish is needed.

The samples encoded BO1, BO2, BO3 from Bengawan Solo River, Bojonegoro, East Java were clustered in the same group with *Pangasius larnaudii*. which belong to Pangasiidae family. The phylogenetic analysis of samples LB1 and LPB1 was not clearly defined using the comparative species then we did separate phylogenetic analysis for those samples with the addition of sea catfishes species from the Ariidae family (Denadai et al. 2012). The results showed that samples LLB1 and LPB1 were clustered in the same groups with *Arius arius* (NC_036673.1) and *Arius maculatus* (NC_045222.1) as shown in Figure 4. This described that species from river and coastal from Indonesia belong to different families.

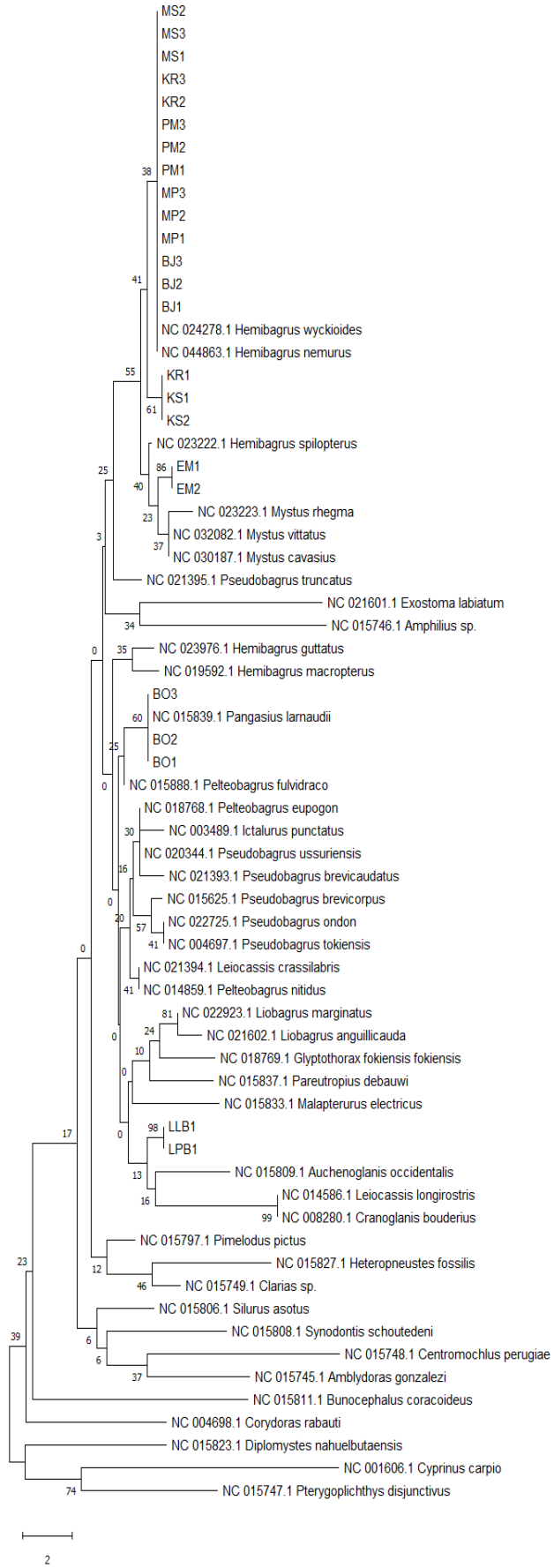

Figure 3. Phylogram of Indonesian catfish based on the Cytochrome Oxidase Subunit II amino acid sequences

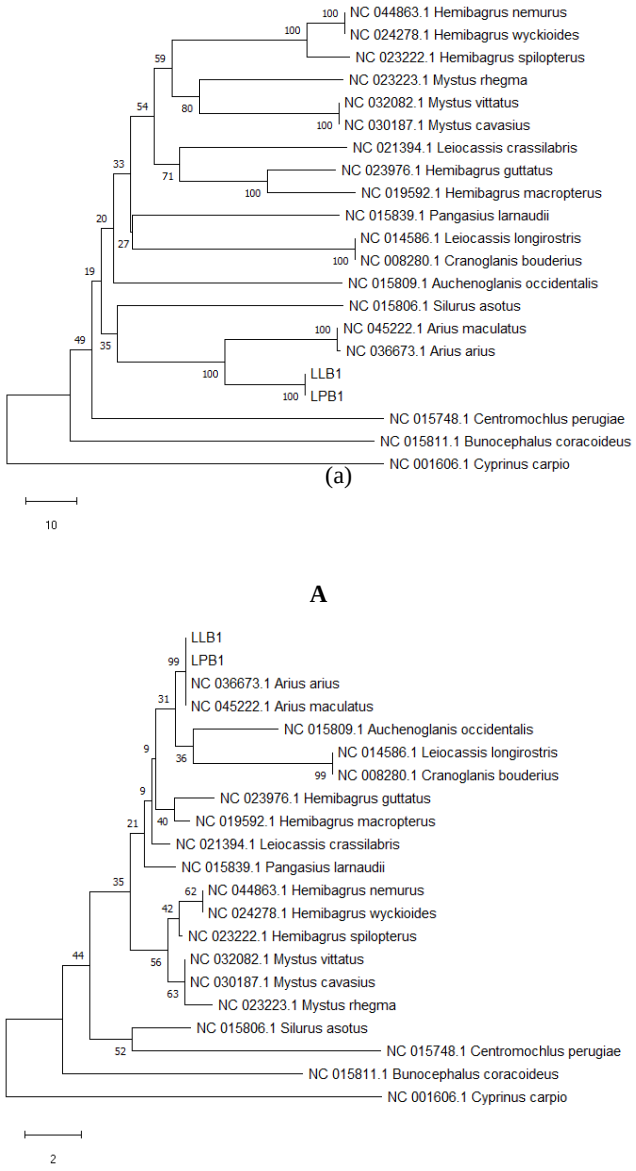

Figure 4. Phylogram of samples LLB1 LPB1 on the Cytochrome Oxidase Subunit II nucleotide (A) and amino acid sequences (B)

This study concludes that there is genetic diversity of the indigenous catfish from Indonesia based on mitochondrial cytochrome oxidase subunit II gene. Samples from the Central Java, Sumatra, and Kalimantan river belonged to Bagridae family and consist of two different species *Hemibagrus* sp. and *Mystus* sp while samples from East Java belonged to Pangasiidae family, and sample is taken from coastal belonged to Ariidae family. Besides, we can also conclude that the COII *gene* especially its amino acid polymorphic site can be used to distinguish catfishes (order Siluriformes) in Indonesia.

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