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The Analysis of Genetic Variation in the Mitochondrial ND6 Gene and its Application for the Identification of Indonesian Catfish Species

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ABSTRACT

Background: Catfish is a freshwater and saltwater fish type with a wide distribution throughout the Indonesian archipelago. This study was conducted to analyze Indonesian catfish's genetic diversity and evolutionary relationships based on the mitochondrial ND6 gene.

Methods: Twenty-eight catfish samples were collected from nine rivers and one ocean in seven provinces of Indonesia. The MT-ND6 gene was amplified by using a set of primers (BaungND6F and BaungND6R). The MEGA X software was used to examine the MT-ND6 sequences for genetic variation and phylogenetic relation between Indonesian catfish populations.

Result: There were 167 nucleotide site variations and thirty-two amino acid variations in the MT-ND6 gene. The genetic difference among populations ranges from 0.9 to 25% and the average evolutionary divergence in the overall populations was 14%, with catfish BB and PM having the closest genetic distance, while the farthest is BF to MP and BF to MS. In conclusion, the phylogenetic tree indicated that there were four distinct catfish clades in this study: MP, MS, KR, PM, BB and KS had been grouped with *Hemibagrus nemurus* (Bagridae family), EM had been grouped with *Mystus vittatus* (Bagridae family), BSBJ had been grouped with *Pangasius pangasius* (Pangasiidae family) and PD and BF had been grouped with *Netuma thalassina* (Ariidae family).

Key words: Catfish, Genetic variation, Indonesian catfish, MT-ND6 gene, Phylogenetic study.

INTRODUCTION

Indonesia is a country that has abundant natural resources, which is called a mega biodiversity country (Pakpahan *et al.*, 2016; Ibrahim *et al.*, 2020). Water resources are an important part of Indonesia because they have abundant freshwater and saltwater fish (Allen and Adrim, 2003). Currently, nearly 40% of identified fish species are limited to freshwater habitats, which account for just 0.8% of the earth's surface (Dudgeon *et al.*, 2006). This fish is found in large rivers and flooded swamps. Indonesian catfish have high economic value and the population is classified as vulnerable to extinction (Aryani, 2015). The catfish group consists of about 3,000 species, 478 orders and 36 families of fish, including Bagridae, Siluridae, Pangasiidae, Akysidae, Schilbeidae, Sisoridae, Parakysidae, Ariidae, Plotosidae, Loricariidae, Chacidae, Clariidae, Kryptopterus and the others (Abell *et al.*, 2008; Vitt, 2021; Wong *et al.*, 2011; Widayanti *et al.*, 2021; Ng and Kottelat, 2013).

Indonesian catfish have different local names (common names) in several areas of Indonesia, such as Baon, Baong, Duri, Geso (Sumatra), Bawon (Betawi), Senggal, Singgah (Sunda), Tiken Bato (Kalimantan), Tagih, Tageh, Jendil and Baung fish (Java) (Widayanti *et al.*, 2021). In previous studies, catfish in Indonesia were grouped and classified into several genera and species, such as *Silurichthys* (*Silurihthys ligneoulus* from Kahayan River of Central Kalimantan) (Ng and Tan, 2011), *Pangasius* (*Pangasius rheophilus* from Berau and Kayan Rivers of East Kalimantan, *Pangasius pangasius* from Bengawan Solo River-Central Java) (Pouyaud and Teugels, 2000), *Mystus* (*Mystus singaringan* from Musi River-South Sumatra), *Barcoides* (*Barcoides macropterus* from Musi River-South Sumatra) and *Hemibagrus* (*Hemibagrus nemurus* from Musi and Penukal River-South Sumatra, *Hemibagrus wyckii* from Kampar Kanan River-Riau, *Hemibagrus olyroides* from Behau River-North Kalimantan, *Hemibagrus bongan* from Haloi River-West Kalimantan, *Hemibagrus lacustrinus* from Batang Ombilin River-West Sumatra, *Hemibagrus planiceps* from Cibogo River-West Java, *Hemibagrus caventus* from

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Trumon-Aceh and *Hemibagrus velox* from Dareh River-West Sumatra), *Netuma* (*Netuma thalassina* from Bomberay River-West Papua and Indian Ocean-Yogyakarta) (Aryani *et al.,* 2016; Syaifudin *et al*., 2017; Widayanti *et al*., 2021). However, there are still many types of Indonesian catfish in various areas that have not been identified and classified into which family, genus and/or species.

The relationship in a population or species is usually studied through a morphological approach (Qadri *et al*., 2017). The weakness of the morphological approach is the very high level of subjectivity (Buj *et al*., 2014; Ikabanga *et al*., 2017). One effort that needs to be made to determine the genetic status of Baung fish and to conserve genetic resources is to identify species at the molecular level through DNA barcoding (James, 2010; Dodson *et al*., 2015; Imtiaz *et al*., 2017). In this study, we address the question of the genetic diversity and the phylogenetic relationships within the Indonesian catfish.

MATERIALS AND METHODS

Sample collections

The samples were collected from 2017-2020, but this study was conducted from February to October 2021 in the Laboratory of Biochemistry and Molecular Biology, Faculty of Veterinary Medicine, Universitas Gadjah Mada. Twenty-eight catfish samples were collected from nine rivers (KR: Kampar River, Pekanbaru, Riau; MS: Musi River, Palembang, South Sumatra; PM: Progo River, Magelang, Central Java; EM: Elo River, Magelang, Central Java; BSBJ: Bengawan Solo River, Central Java; KS: Kapuas River, Sintang, West Kalimantan; BB: Martapura River, Banjarmasin, South Kalimantan; MS: Mahakam River, Samarinda, East Kalimantan; and BF: Bomberay River, Fakfak, West Papua) and one ocean (PD: Indian Ocean, Baru Beach, Yogyakarta) in seven provinces of Indonesia. The sampling location of the Indonesian catfish is presented in Fig 1. All individuals were taken individually from their habitat to be unrelated genetics to avoid bias in the analysis.

Genomic DNA isolation and MT-ND6 gene amplification

The catfish genome was isolated and the MT-ND6 gene was amplified using a set of primers BaungND6F: 5'-GAC CGV CAA TGA HTA GAA AT-3' and BaungND6R: 5'-TCA AGT CAT TAG TCC TGG TT-3'. The volume of PCR reagent was 50 µL, which included 25 µL of master mix, 1 µL of DNA template, 1 µL (10 pmol) of each primer and 22 µL of ddH2O. PCR program: An initial denaturing stage at 94°C 5 minutes was followed by 35 cycles at 94°C 30 s, 47°C 30 s and 72°C 90 s, with a final step at 72°C 6 min. All PCR amplicons were validated by 1.2% agarose gel electrophoresis with a 100 bp DNA marker. After being aligned with sequence data of ND6 complete gene of *Hemibagrus nemurus* (NC_044863.1) from the NCBI database, the amplicon result is 709 bp at the site number 13611-14319, which amplifies numerous DNA fragments, as shown in Table 1.

Table 1: Amplicon result of mitochondrial DNA fragments (709 bp) following alignment with *Hemibagrus nemurus* sequence data (NC 044863.1) from NCBI database.

Nucleotide sequence number (5′→3′)	DNA fragments	Length of nucleotide sequences
13611-13769	ND5 partial	159
13766-13769	Over lapping	-4
13766-14281*	Complement gene *ND6**	516*
14282-14319	tRNA-Glu partial	38

Note:* Targeted complement MT-ND6 gene.

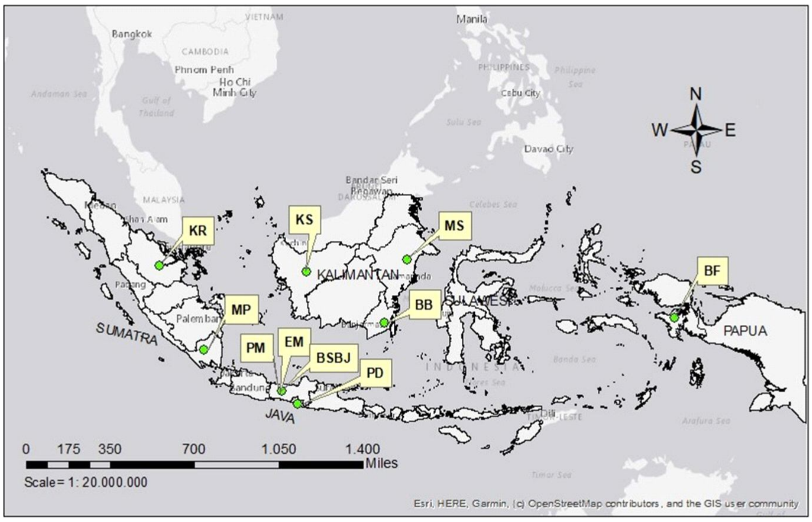


Fig 1: Sampling locations of Indonesian catfish.

Sequences and phylogenetic analysis

The amplicons of the mitochondrial ND6 (MT-ND6) gene were aligned using the ClustalW program (Thompson *et al.*, 1994) in MEGA X software (Kumar *et al.*, 2018) and edited based on sequencing chromatograms of forward and reverse sequences. The phylogenetic tree was examined using the neighbor-joining (NJ) approach and genetic diversity was estimated using the Kimura two-parameter method (Kimura, 1980) in software MEGA X version 10.1 (Kumar *et al.*, 2018). To construct a phylogenetic tree and identify the relation among catfish, the comparative species were downloaded from the NCBI database with accession numbers: *Pangasius pangasius* KC572135.1, *Pangasianodon gigas* AY762971.1, *Netuma thalassina* KU986659.1, *Mystus vittatus* KX177968.1, *Mystus cavasius* KU870465.1, *Hemibagrus wyckioides* KJ624624.1, *Arius maculatus* NC_045222.1, *Arius dispar* NC_048969.1, *Arius arius* KX211965.1, *Hemibagrus nemurus* NC_044863.1 and *Occidentarius platypogon* NC_037469.1.

RESULTS AND DISCUSSION

Genetic characterization of Indonesian catfish based on mtDNA gene

There were 167 variable sites in the MT-ND6 whole gene across all Indonesian catfish samples, nucleotide substituted, but no deletions or insertions were detected. Based on comparisons with the catfish MP1 sequence, the sequence alignment was performed using the ClustalW algorithm in the Mega X software. A dot in Fig 2 and Fig 3 indicates the homology of each sample to catfish MP1. A few unique mutation sites have been identified, which can be used as genetic markers for the population: BSBJ sites 4, 13, 33; EM sites 12, 123, 124; MS sites 21; PM sites 251, 300; BF sites 336, 396, 472, 504; PD sites 45, 77, 243, 246, 402, 440 (Fig 2 and Fig 3).

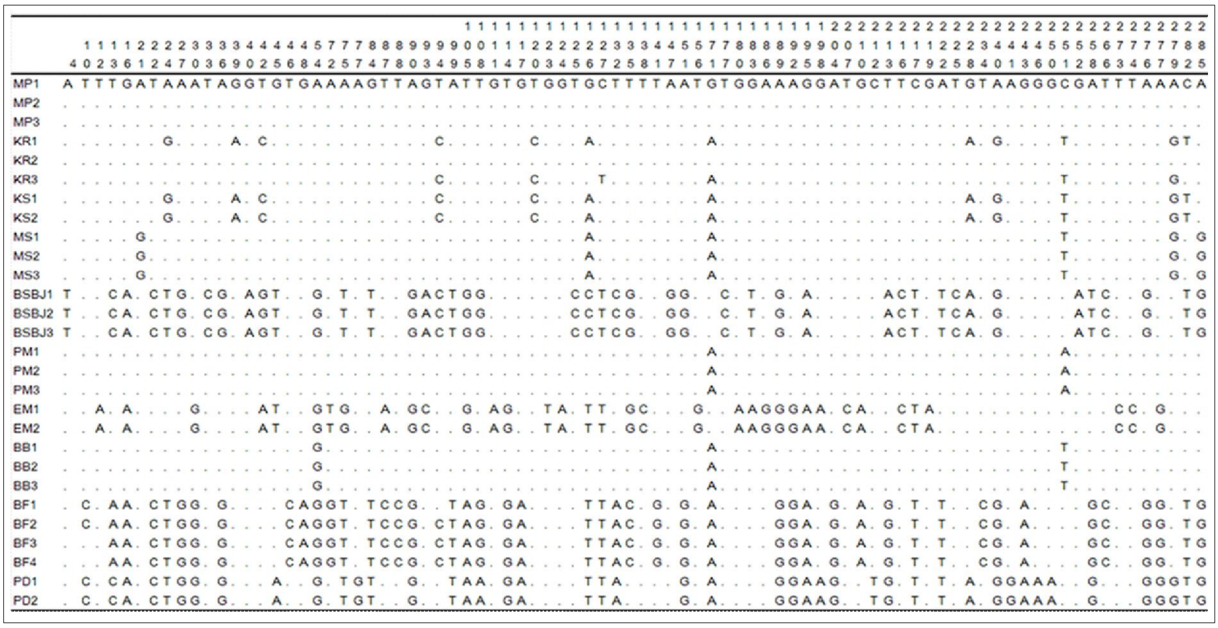
A total of 173 amino acids for each Indonesian catfish were analyzed with complete sequences of the MT-ND6 gene. All samples had 32 different amino acid variants, two different groups of amino acid variations in the MT-ND6 gene, namely catfish MP1-BB3 and EM1-PD2 (Fig 4). Based on the 32 amino acid variations, there were no amino acids in catfish KR1 and KR3, which differed from those in catfish KS1, KS2, MS1, MS2 MS3, BB1, BB2 and BB3.

Bootstrapping (1000 repetitions) was used to estimate genetic distances and the Kimura two-parameter model was used to conduct analyses. The genetic difference among Indonesian catfish populations ranges from 0.9 to 25%; the closest is between catfish BB and PM, while the furthest is between catfish BF-MP and BF-MS. The value of base substitutions at each site in the overall population was determined to be 0.14 (14%) after averaging all sequence pairings among groups (Table 2). The catfish from Kalimantan (MS, KS and BB), Sumatra (MP and KR) and Java (PM) were all classified as *Hemibagrus spp*., with a genetic distance of 0.9 - 4.5%.

The mtDNA is a common target for species identification and phylogenetic studies, which help to understand the evolutionary history of humans and animals without recombination and has a high mutation rate (Postillone and Perez, 2017; Baird *et al.*, 2017; Song *et al.*, 2016). Due to the lack of well-defined traits and high variability in measures usually employed to identify species in other catfish groups, determining species of catfish primarily on physical characteristics is relatively difficult. As a result, molecular identification offers more precise information on species diversification and evolutionary relationships (Buj *et al.*, 2014; Ikabanga *et al.*, 2017). Ng and Kottelat (2013) reported that three new species were described based on morphological identification: *H. divaricatus* from western Peninsular Malaysia, *H. lacustrinus* from Danau Singkarak and its related drainages in western Sumatra and *H. semotus* from northeastern Borneo. The mtDNA Cyt B gene was used to assess the congruence of morphologically and genetically based taxonomies among the Southeast Asian catfish genus Hemibagrus Bleeker. The most significant exception is the definition of a morphologically cryptic group from North Borneo. Within the genus Hemibagrus, the *H. nemurus* species represent a separate and widely distributed genetic structure. *H. nemurus* from West Java appears to be a subspecies of *H. capitulum* (Dodson and Lecomte, 2015). The many varieties of catfish that occur in Indonesia are occasionally misidentified by Indonesians in several areas. This is due to the very high similarity of catfish. In this study,

Table 2: Calculations of evolutionary divergence between groupings based on sequence pairings.

	MP	KR	KS	MS	BSBJ	PM	EM	BB	BF	PD
MP										
KR	0.023									
KS	0.045	0.028								
MS	0.020	0.025	0.032							
BSBJ	0.203	0.208	0.206	0.203						
PM	0.012	0.023	0.038	0.014	0.203					
EM	0.172	0.183	0.190	0.177	0.223	0.174				
BB	0.012	0.023	0.035	0.015	0.203	0.009	0.169			
BF	0.250	0.249	0.249	0.250	0.184	0.250	0.231	0.247		
PD	0.229	0.226	0.220	0.217	0.166	0.223	0.227	0.220	0.115	



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         1 1 1 1 2 2 2 2 3 3 3 3 4 4 4 4 4 5 7 7 7 8 8 8 9 9 9 9 0 0 1 1 1 2 2 2 2 2 2 3 3 3 4 4 5 5 7 7 8 8 8 8 9 9 9 0 0 1 1 1 1 1 1 2 2 2 3 4 4 4 4 5 5 5 5 6 7 7 7 7 7 8 8
       4 0 2 3 6 1 2 4 7 0 3 6 9 0 2 5 6 8 4 2 5 7 4 7 8 0 3 4 9 5 8 1 4 7 0 3 4 5 6 7 2 5 8 1 7 1 6 1 7 0 3 6 9 2 5 8 4 7 0 2 3 6 7 9 2 5 8 4 0 1 3 6 0 1 2 8 6 3 4 6 7 9 2 5
MP1    A T T T G A T A A A T A G G T G T G A A A A G T T A G T A T T G T G T G G T G C T T T T A A T G T G G A A A G G A T G C T T C G A T G T A A G G G C G A T T T A A A C A
MP2    . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
MP3    . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
KR1    . . . . . . . G . . . . A . C . . . . . . . . . . . . C . . . . . . C . . . A . . . . . . . . A . . . . . . . . . . . . . . . . . . . A . G . . . . T . . . . . . . G T .
KR2    . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
KR3    . . . . . . . . . . . . . . . . . . . . . . . . . . . C . . . . . . C . . . . T . . . . . . . A . . . . . . . . . . . . . . . . . . . . . . . . . T . . . . . . . G . .
KS1    . . . . . . . G . . . . A . C . . . . . . . . . . . . C . . . . . . C . . . A . . . . . . . . A . . . . . . . . . . . . . . . . . . . A . G . . . . T . . . . . . . G T .
KS2    . . . . . . . G . . . . A . C . . . . . . . . . . . . C . . . . . . C . . . A . . . . . . . . A . . . . . . . . . . . . . . . . . . . A . G . . . . T . . . . . . . G T .
MS1    . . . . . G . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . A . . . . . . . . A . . . . . . . . . . . . . . . . . . . . . . . . . T . . . . . . . G . G
MS2    . . . . . G . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . A . . . . . . . . A . . . . . . . . . . . . . . . . . . . . . . . . . T . . . . . . . G . G
MS3    . . . . . G . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . A . . . . . . . . A . . . . . . . . . . . . . . . . . . . . . . . . . T . . . . . . . G . G
BSBJ1  T . . C A . C T G . C G . A G T . . G . T . T . . G A C T G G . . . . . . C C T C G . . G G . . C . T . G . A . . . . . A C T . T C A . G . . . . . A T C . . G . . T G
BSBJ2  T . . C A . C T G . C G . A G T . . G . T . T . . G A C T G G . . . . . . C C T C G . . G G . . C . T . G . A . . . . . A C T . T C A . G . . . . . A T C . . G . . T G
BSBJ3  T . . C A . C T G . C G . A G T . . G . T . T . . G A C T G G . . . . . . C C T C G . . G G . . C . T . G . A . . . . . A C T . T C A . G . . . . . A T C . . G . . T G
PM1    . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . A . . . . . . . . . . . . . . . . . . . . . . . . . A . . . . . . . . . .
PM2    . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . A . . . . . . . . . . . . . . . . . . . . . . . . . A . . . . . . . . . .
PM3    . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . A . . . . . . . . . . . . . . . . . . . . . . . . . A . . . . . . . . . .
EM1    . . A . A . . . . G . . . . A T . . G T G . . A . G C . . G . A G . . T A . T T . G C . . . G . . A A G G G A A . C A . . C T A . . . . . . . . . . . . . . C C . G . . .
EM2    . . A . A . . . . G . . . . A T . . G T G . . A . G C . . G . A G . . T A . T T . G C . . . G . . A A G G G A A . C A . . C T A . . . . . . . . . . . . . . C C . G . . .
BB1    . . . . . . . . . . . . . . . . . . G . . . . . . . . . . . . . . . . . . . . . . . . . . . . A . . . . . . . . . . . . . . . . . . . . . . . . . T . . . . . . . . . .
BB2    . . . . . . . . . . . . . . . . . . G . . . . . . . . . . . . . . . . . . . . . . . . . . . . A . . . . . . . . . . . . . . . . . . . . . . . . . T . . . . . . . . . .
BB3    . . . . . . . . . . . . . . . . . . G . . . . . . . . . . . . . . . . . . . . . . . . . . . . A . . . . . . . . . . . . . . . . . . . . . . . . . T . . . . . . . . . .
BF1    . C . A A . C T G G . G . . . . C A G G T . T C C G . . T A G . G A . . . . T T A C . G . G . A . . . . G G A . G . A . G . T . T . . C G . A . . . . G C . . G G . T G
BF2    . C . A A . C T G G . G . . . . C A G G T . T C C G . C T A G . G A . . . . T T A C . G . G . A . . . . G G A . G . A . G . T . T . . C G . A . . . . G C . . G G . T G
BF3    . . . A A . C T G G . G . . . . C A G G T . T C C G . C T A G . G A . . . . T T A C . G . G . A . . . . G G A . G . A . G . T . T . . C G . A . . . . G C . . G G . T G
BF4    . . . A A . C T G G . G . . . . C A G G T . T C C G . C T A G . G A . . . . T T A C . G . G . A . . . . G G A . G . A . G . T . T . . C G . A . . . . G C . . G G . T G
PD1    . C . C A . C T G G . G . . . A . . G . T G T . . G . . T A A . G A . . . . T T A . . . . G . A . . . . G G A A G . . T G . T . T . A . G G A A A . . G . . . G G G T G
PD2    . C . C A . C T G G . G . . . A . . G . T G T . . G . . T A A . G A . . . . T T A . . . . G . A . . . . G G A A G . . T G . T . T . A . G G A A A . . G . . . G G G T G
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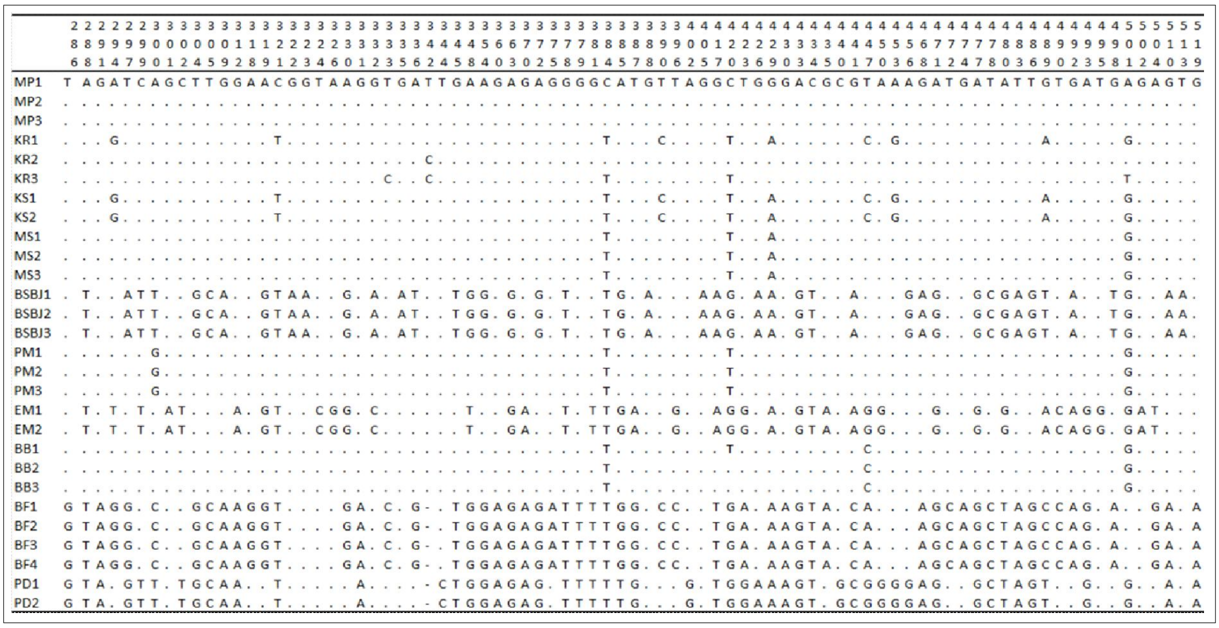
Fig 2: The variable sites from position 4 to 285 in the Indonesian catfish ND6 gene.



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       2 2 2 2 2 2 3 3 3 3 3 3 3 3 3 3 3 3 3 3 3 3 3 3 3 3 3 3 3 3 3 3 3 3 3 3 3 3 3 3 3 3 3 3 3 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 5 5 5 5 5 5
       8 8 9 9 9 9 0 0 0 0 0 0 1 1 1 2 2 2 2 2 3 3 3 3 3 3 4 4 4 4 5 6 6 7 7 7 7 7 8 8 8 8 8 9 9 0 0 1 2 2 2 2 3 3 3 3 4 4 4 5 5 5 6 7 7 7 7 7 8 8 8 8 9 9 9 9 9 0 0 0 1 1 1
       6 8 1 4 7 9 0 1 2 4 5 9 2 8 9 1 2 3 4 6 0 1 2 3 5 6 2 4 5 8 4 0 3 0 2 5 8 9 1 4 5 7 8 0 6 2 5 7 0 3 6 9 0 3 4 5 0 1 7 0 3 6 8 1 2 4 7 8 0 3 6 9 0 2 3 5 8 1 2 4 0 3 9
MP1    T A G A T C A G C T T G G A A C G G T A A G G T G A T T G A A G A G A G G G G C A T G T T A G G C T G G G A C G C G T A A A G A T G A T A T T G T G A T G A G A G T G
MP2    . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
MP3    . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
KR1    . . . G . . . . . . . . . . . T . . . . . . . . . . . . . . . . . . . . . . . T . . . C . . . . T . . A . . . . . . C . G . . . . . . . . . . A . . . . . G . . . . .
KR2    . . . . . . . . . . . . . . . . . . . . . . . . . . C . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
KR3    . . . . . . . . . . . . . . . . . . . . . . . C . . C . . . . . . . . . . . . T . . . . . . . . T . . . . . . . . . . . . . . . . . . . . . . . . . . . . T . . . . .
KS1    . . . G . . . . . . . . . . . T . . . . . . . . . . . . . . . . . . . . . . . T . . . C . . . . T . . A . . . . . . C . G . . . . . . . . . . A . . . . . G . . . . .
KS2    . . . G . . . . . . . . . . . T . . . . . . . . . . . . . . . . . . . . . . . T . . . C . . . . T . . A . . . . . . C . G . . . . . . . . . . A . . . . . G . . . . .
MS1    . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . T . . . . . . . . T . . A . . . . . . . . . . . . . . . . . . . . . . . . . G . . . . .
MS2    . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . T . . . . . . . . T . . A . . . . . . . . . . . . . . . . . . . . . . . . . G . . . . .
MS3    . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . T . . . . . . . . T . . A . . . . . . . . . . . . . . . . . . . . . . . . . G . . . . .
BSBJ1  . T . . A T T . . G C A . . G T A A . . G . A . A T . . T G G . G . G . T . . T G . A . . . A A G . A A . G T . . A . . . G A G . . G C G A G T . A . . T G . . A A .
BSBJ2  . T . . A T T . . G C A . . G T A A . . G . A . A T . . T G G . G . G . T . . T G . A . . . A A G . A A . G T . . A . . . G A G . . G C G A G T . A . . T G . . A A .
BSBJ3  . T . . A T T . . G C A . . G T A A . . G . A . A T . . T G G . G . G . T . . T G . A . . . A A G . A A . G T . . A . . . G A G . . G C G A G T . A . . T G . . A A .
PM1    . . . . . . G . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . T . . . . . . . . T . . . . . . . . . . . . . . . . . . . . . . . . . . . . G . . . . .
PM2    . . . . . . G . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . T . . . . . . . . T . . . . . . . . . . . . . . . . . . . . . . . . . . . . G . . . . .
PM3    . . . . . . G . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . T . . . . . . . . T . . . . . . . . . . . . . . . . . . . . . . . . . . . . G . . . . .
EM1    . T . T . T . A T . . . A . G T . . C G G . C . . . . . . T . . G A . . T . T T G A . . G . . A G G . A . G T A . A G G . . . G . . G . G . . A C A G G . G A T . . .
EM2    . T . T . T . A T . . . A . G T . . C G G . C . . . . . . T . . G A . . T . T T G A . . G . . A G G . A . G T A . A G G . . . G . . G . G . . A C A G G . G A T . . .
BB1    . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . T . . . . . . . . T . . . . . . . . . C . . . . . . . . . . . . . . . . . . G . . . . .
BB2    . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . T . . . . . . . . . . . . . . . . . . C . . . . . . . . . . . . . . . . . . G . . . . .
BB3    . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . T . . . . . . . . . . . . . . . . . . C . . . . . . . . . . . . . . . . . . G . . . . .
BF1    G T A G G . C . . G C A A G G T . . . . G A . C . G - . T G G A G A G A T T T T G G . C C . . T G A . A A G T A . C A . . . A G C A G C T A G C C A G . A . . G A . A
BF2    G T A G G . C . . G C A A G G T . . . . G A . C . G - . T G G A G A G A T T T T G G . C C . . T G A . A A G T A . C A . . . A G C A G C T A G C C A G . A . . G A . A
BF3    G T A G G . C . . G C A A G G T . . . . G A . C . G - . T G G A G A G A T T T T G G . C C . . T G A . A A G T A . C A . . . A G C A G C T A G C C A G . A . . G A . A
BF4    G T A G G . C . . G C A A G G T . . . . G A . C . G - . T G G A G A G A T T T T G G . C C . . T G A . A A G T A . C A . . . A G C A G C T A G C C A G . A . . G A . A
PD1    G T A . G T T . T G C A A . . T . . . . . A . . . . - C T G G A G A G . T T T T T G . . . G . T G G A A A G T . G C G G G G A G . . G C T A G T . . G . . G . . A . A
PD2    G T A . G T T . T G C A A . . T . . . . . A . . . . - C T G G A G A G . T T T T T G . . . G . T G G A A A G T . G C G G G G A G . . G C T A G T . . G . . G . . A . A
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Fig 3: The variable sites from position 286 to 519 in the Indonesian catfish ND6 gene.

no morphological comparison was carried out. The current study of Indonesian catfish based on the MT-ND6 gene sequences revealed four groupings.

Phylogenetic relationship of Indonesian catfish based on MT-ND6 gene sequences

The sample taxa were identified and examined using the neighbor-joining algorithm to generate a phylogenetic tree (Saitou and Nei, 1987). The tree is drawn to scale and the length of the branches is the same as the unit used to infer the evolutionary distance of the phylogenetic tree. The Kimura 2-parameter algorithm calculated the evolutionary distances (Kimura, 1980). The total number of sites in the final dataset was 519 bp. A phylogenetic tree was created using 28 unique Indonesian catfish and several catfish from other countries (NCBI database) (Fig 5). The formation of groups in the phylogenetic tree occurs based on nucleotide variations in the MT-ND6 gene sequences. The phylogenetic tree divides Indonesian catfish into four groups with catfish from other countries. Catfish MP, MS, KR, PM, BB and KS were grouped with *H. Nemurus*; EM with *Mystus vittatus*; BSBJ with *Pangasius pangasius* and PD and BF with *Netuma thalassina*.

Megarani *et al.* (2020) reported the genetic characteristics and phylogenetic structure of nine populations of Indonesian indigenous catfish using Cytochrome B (Cyt B) sequences. Indonesian catfishes were separated into five clades; *Hemibagrus nemurus* and *Hemibagrus wyckioides*, *Sperata seenghala* and *Hemibagrus spilopterus*, *Pseudolais pleurotaenia*, *Mystus cavasius* and *Potamosilurus latirostris*. In the current study, Indonesian catfishes were split into four groups together with catfish from other countries. Catfish MP, MS, KR, PM, BB and KS were grouped with *H. Nemurus* (Bagridae family), EM was grouped with *Mystus vittatus* (Bagridae family), BSBJ were grouped with *Pangasius pangasius* (Pangasiidae family) and PD and BF were grouped with *Netuma thalassina* (Ariidae family). Differences in the use of mtDNA genes for genetic characterization and phylogenetic studies will provide in-depth information on the determination of species and subspecies. The existence of Indonesian catfish in freshwater, which is grouped with the Ariidae family in this study (BF samples from Bomberay River-West Papua), is still not known about its history and process and it needs to be further studied.

The intricacy of the region's biogeographical history and a lack of well-defined morphological traits make taxonomy and phylogenetic reconstruction of catfishes distributed across Southeast Asia difficult (Dodson *et al.*, 2015). According to the genetic diversity and phylogenetic analyses of catfish from Indonesia using mitochondrial COX-III and 12S rRNA gene, Indonesian catfish are classified into four groups; *Hemibagrus nemurus/Hemibagrus spilopterus, Pangasius pangasius/Pangasianodon gigas, Mystus vittatus/Mystus rhegma and Netuma thalassina* (Widayanti *et al.*, 2019; Widayanti *et al.*, 2021). In this present research, the outcomes of this study were nearly the same, indicating that utilizing the MT-ND6 and 12S rRNA target genes, the genetic variety of Indonesian catfish could be divided into four groups. The genetic difference among Indonesian catfish populations based on MT-ND6 ranges from 0.9-25% and the average of evolutionary divergence in the overall populations was determined to be 14%, with catfish BB and PM having the closest genetic distance, while the farthest is BF to MP and BF to MS.

																1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
					1	2	4	5	7	8	8	8	9	9	0	0	0	0	0	0	1	1	1	2	2	2	3	4	4	4	6	6
	2	4	5	6	4	6	2	1	1	1	4	9	3	6	0	1	2	7	8	9	1	2	5	4	7	9	0	4	5	7	5	8
MP1	M	F	F	D	V	Y	V	I	A	T	A	V	M	L	A	A	L	S	G	Y	G	W	V	V	G	I	G	V	T	A	S	A
MP2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
MP3	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
KR1	.	.	.	.	.	.	.	.	.	.	V	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
KR2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
KR3	.	.	.	.	.	.	.	.	.	.	V	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
KS1	.	.	.	.	.	.	.	.	.	.	V	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
KS2	.	.	.	.	.	.	.	.	.	.	V	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
MS1	.	.	.	.	.	.	.	.	.	.	V	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
MS2	.	.	.	.	.	.	.	.	.	.	V	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
MS3	.	.	.	.	.	.	.	.	.	.	V	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
PM1	.	.	.	.	.	.	.	.	.	.	E	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
PM2	.	.	.	.	.	.	.	.	.	.	E	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
PM3	.	.	.	.	.	.	.	.	.	.	E	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
BB1	.	.	.	.	.	.	.	.	.	.	V	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
BB2	.	.	.	.	.	.	.	.	.	.	V	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
BB3	.	.	.	.	.	.	.	.	.	.	V	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
EM1	.	L	.	N	.	.	I	.	.	.	.	.	V	F	V	M	.	G	.	C	A	.	.	M	.	V	.	.	V	.	G	T
EM2	.	L	.	N	.	.	I	.	.	.	.	.	V	F	V	M	.	G	.	C	A	.	.	M	.	V	.	.	V	.	G	T
BSBJ1	L	.	L	N	M	.	A	V	.	.	.	A	.	F	V	.	A	G	N	.	D	Y	.	.	.	V	S	.	V	.	.	.
BSBJ2	L	.	L	N	M	.	A	V	.	.	.	A	.	F	V	.	A	G	N	.	D	Y	.	.	.	V	S	.	V	.	.	.
BSBJ3	L	.	L	N	M	.	A	V	.	.	.	A	.	F	V	.	A	G	N	.	D	Y	.	.	.	V	S	.	V	.	.	.
BF1	.	L	I	N	.	.	.	V	.	.	.	A	V	V	.	.	A	G	.	.	S	.	.	M	C	V	.	I	V	.	G	.
BF2	.	L	I	N	.	.	.	V	.	.	.	A	V	V	.	.	A	G	.	.	S	.	.	M	C	V	.	I	V	.	G	.
BF3	.	.	I	N	.	.	.	V	.	.	.	A	V	V	.	.	A	G	.	.	S	.	.	M	C	V	.	I	V	.	G	.
BF4	.	.	I	N	.	.	.	V	.	.	.	A	V	V	.	.	A	G	.	.	S	.	.	M	C	V	.	I	V	.	G	.
PD1	.	L	L	N	.	C	.	V	V	A	T	.	V	V	V	V	A	.	.	.	S	.	A	M	C	L	.	I	V	G	G	.
PD2	.	L	L	N	.	C	.	V	V	A	T	.	V	V	V	V	A	.	.	.	S	.	A	M	C	L	.	I	V	G	G	.

Fig 4: Amino acid variations in the MT-ND6 gene of Indonesian catfish.

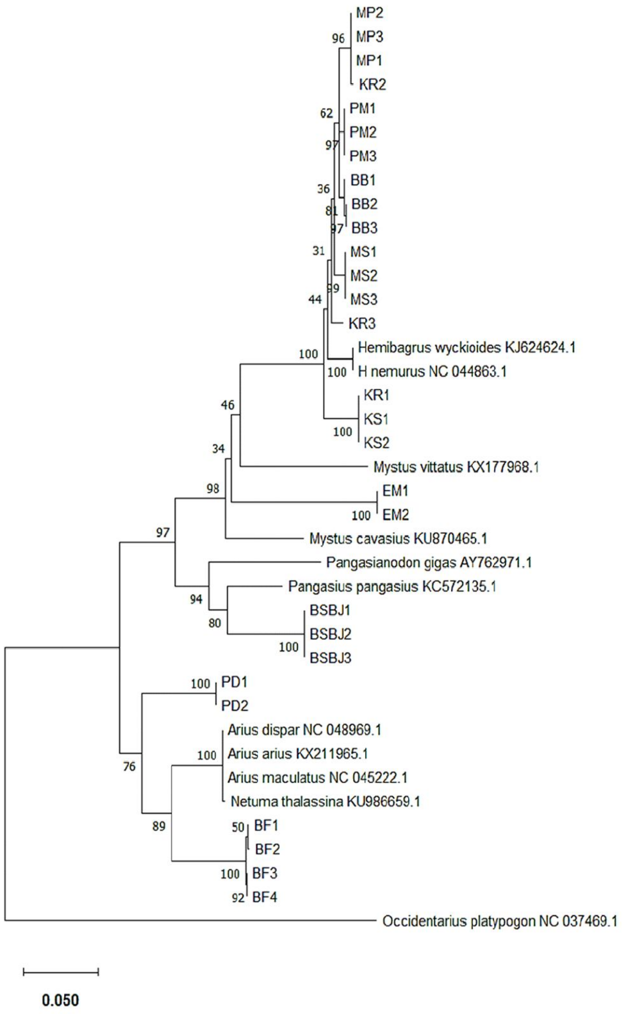

Fig 5: Phylogenetic tree of the Indonesian catfish based on the MT-ND6 sequences.

CONCLUSION

In conclusion, based on the MT-ND6 gene, BB and PM catfish have the closest genetic distance, while the farthest is BF to MP catfish and BF to MS catfish. The phylogenetic tree confirmed that the samples in this study were divided into four clades: MP, MS, KR, PM, BB and KS had been grouped with *Hemibagru nemurus* (Bagridae family), EM had been grouped with *Mystus vittatus* (Bagridae family)), BSBJ had been grouped with *Pangasius pangasius* (Pangasiidae family)) and PD and BF had been grouped with *Netuma thalassina* (Ariidae family).

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Conflict of interest: None.

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