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Intraspecific Genetic Variation of Five Wild Indonesian Striped Snakehead (*Channa striata* (Bloch, 1793)) Populations Assessed Through *16S rRNA* Sequences

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ABSTRACT

The striped snakehead (Channa striata) belongs to the order Perciformes, suborder Channoidei, and the family Channidae. This fish is native to Sumatra, Java, and Kalimantan, but introduced to Sulawesi and Papua. Channa striata have potential as a food source and pharmacological agent. However, the study on the genetic variation of snakehead fish in Indonesia is currently limited to specific areas, thus the genetic data obtained is insufficient. Therefore, this study aimed to examine the intraspecific genetic variation of the striped snakehead from five distinct locations in Indonesia using the 16S rRNA mitochondrial gene as a genetic marker. The PCR method was conducted with two primers, 16Sar, and 16Sbr. The data obtained were then analyzed using DNASTAR, BLAST, Mesquite, MEGAX, BEAST, DnaSP, and NETWORK. The result revealed that all striped snakeheads displayed a high similarity (98.85-99.51%) to C. striata from GenBank. The 16S mtDNA sequences generated 5 haplotypes with 5 variable sites and 4 parsimony informative sites. The mean of haplotype diversity and nucleotide diversity were 0.706 ± 0.088 and 0.00325 ± 0.00052 respectively, with genetic divergence ranging from 0% to 0.73%. The phylogenetic tree revealed two distinct clades which were supported by a bootstrap value of 100% (based on Neighbor Joining, NJ and Maximum Likelihood, ML method) as well as a posterior probability value (Bayesian Inference, BI) of 1.00. These findings suggest an intraspecific genetic variation of wild striped snakehead populations in Indonesia, with two distinct groups consisting of the western part of Indonesia (Sumatera) and the central to the eastern part of Indonesia (Kalimantan, West Nusa Tenggara, and Papua. The findings of this study provide valuable insights into the conservation and cultivation of the striped snakehead through breeding programs in Indonesia.

Keywords: ***16S rRNA; Channa striata; genetic variation***

INTRODUCTION

Indonesia is an archipelagic country that has two hotspots, i.e. Sundaland and Wallacea. The presence of these two hotspots reveals the diversity of microbes, plants, and animals found in Indonesia, including freshwater fish (Lohman et al., 2011; Hubert et al., 2015). One of the freshwater fish that is widely consumed by the local people in Indonesia is the striped snakehead (*Channa striata* Bloch, 1793). This species is valued for its rich content of complex essential amino acids and abundant fatty acids, making it a significant source of animal protein. Moreover, striped snakehead exhibits promising potential as an anti-inflammatory, antibacterial, antinociceptive, and anticancer agent, and could serve as an alternative to serum albumin in humans (Gamaniel & Gwaza, 2017).

Channa striata belongs to the order Perciformes, the suborder Channoidei, and the family Channidae (Kottelat, 2013; Eschmeyer et al., 2018). This fish is found across Asia, particularly in West Asia (Pakistan, Bangladesh, and India), East Asia (China, South China, and South Korea), and Southeast Asia (Indonesia, Cambodia, Myanmar, Philippines, Vietnam, Thailand, and Malaysia). In Indonesia, *C. striata* is known to be a native freshwater fish of Sumatra, Kalimantan, and Java Island. However, in other regions including Sulawesi, the Maluku Islands, and Papua, this fish is considered as an introduced fish (Irmawati et al., 2017).

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The striped snakehead is known for having a cylindrical body shape, with a slightly flattened head located dorsolaterally in the anterior part of the head. This fish possesses a tubular anterior nose along with elongated dorsal fins and anal fins equipped with spines (Courtenay & Williams, 2004). The body coloration of the striped snakehead ranges from dark grayish to olive-brown on the dorsal side, while the ventral part is whitish with a distinctive V-line pattern (chevron-shaped) extending to the mid-lateral (Bhat et al., 2014; Tran et al., 2013).

Extensive studies on the genetic diversity of *C. striata* using mitochondrial DNA have been widely conducted in Asian countries such as India (Lakra et al., 2010; Baisvar et al., 2018), the Philippines (Aquino et al., 2011), Thailand (Adamson et al., 2012), Vietnam (Nguyen & Duong, 2016), and Malaysia (Jamaluddin et al., 2011; Song et al., 2013). Furthermore, the research using *COI* gene (Aquino et al., 2011; Jamaluddin et al., 2011; Song et al., 2013; Tan et al., 2015; Nguyen and Duong, 2016;), *Cyt b* (Adamson et al., 2012; Baisvar et al., 2018; Rahayu et al., 2021), *D-loop* (Baisvar et al., 2019), and a combination of *COI* & *16S* rRNA (Lakra et al., 2010) were also conducted. Based on those studies, they mainly focused on using *COI* gene as a genetic marker for this species.

The use of alternative genetic markers offers additional perspectives and contributes to a more comprehensive understanding of the evolutionary history and genetic relationships of species. The partial mitochondrial *16S* ribosomal RNA (*16S* rRNA) has been shown to be a potential genetic marker for identifying and categorizing mammals, birds, shrimp (Kitano et al. 2007; Yang et al. 2014), and fishes (Chakraborty & Iwaktuki, 2006; Quraishia et al., 2015; Jahan et al., 2017; Saad, 2019; Arisuryanti et al., 2020). Due to its higher conservation compared to *COI* gene (Mohanty et al., 2015), an alteration of a few nucleotides of *16S* rRNA might demonstrate a significant degree of genetic variation inside or among populations (Cawthorn et al., 2012; Yang et al., 2014). Therefore, the approach using the *16S* gene provides complementary information to previous studies that primarily relied on the *COI* gene.

Despite the potential of the *16S* rRNA gene as a genetic marker, genetic studies on *Channa striata* using the *16S* rRNA gene have been limited to specific locations, thus the genetic data obtained is insufficient. This study aimed to analyze the intraspecific genetic variation of the striped snakehead from five different locations in Indonesia using the *16S* rRNA gene as a genetic marker. This genetic information obtained will greatly aid the conservation efforts for *C. striata* and its habitat. In addition, the data can be used for the development of striped snakehead fish cultivation through breeding programs.

MATERIALS AND METHODS

Sampling Collection and Storage

A total of 18 striped snakehead specimens were collected from 5 different locations (Figure 1), namely Batang Hari River (Jambi), Ogan River (South Sumatra), Arut River (Central Kalimantan), Lake Lebo Taliwang (West Nusa Tenggara) and Lake Sentani (Papua) (Table 1). After collecting the samples, all

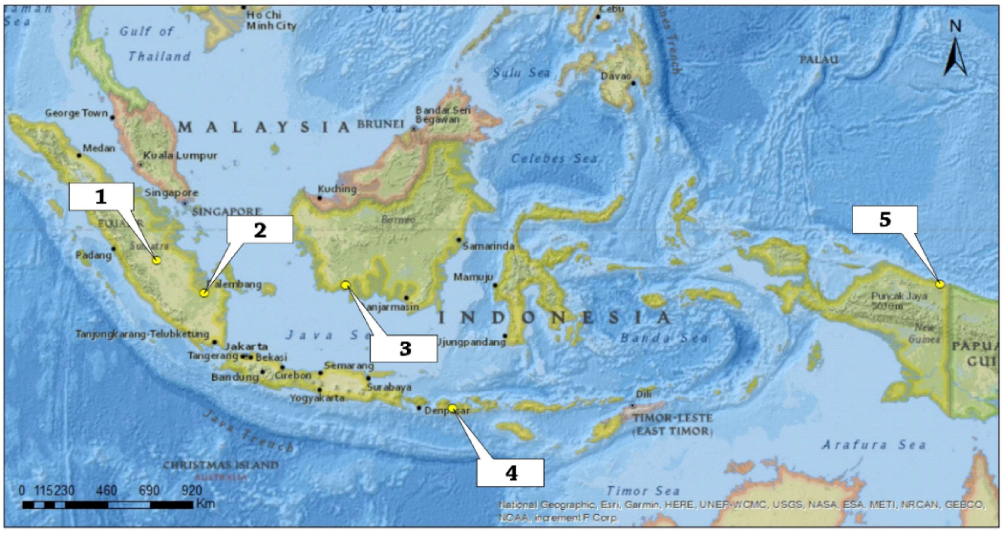

Figure 1. Map sampling sites of *C. striata* investigated in this study. 1= Batang Hari River, Jambi (GBB); 2= Ogan River, South Sumatra (GBO); 3= Arut River, Central Kalimantan (GBA); 4= Lake Lebo, West Nusa Tenggara (GBL); 5= Lake Sentani, Papua (GBS).

striped snakehead specimens were cleaned and stored in 99% ethanol. The preserved fish samples were then sent to the Laboratory of Genetics and Breeding, Faculty of Biology UGM, and stored at -20°C for further analysis.

DNA Extraction, Amplification, and Sequencing

The genomic DNA was extracted from 50-100 mg of muscle tissue from each preserved specimen using the DNeasy Blood and Tissue Kit (QIAGEN, Valencia, USA) following the manufacturer's protocols. Polymerase chain reaction (PCR) was used to amplify the partial mitochondrial *16S* rRNA gene using two primers, 16Sar (5'- CGCCTGTTTATCAAAAACAT-3') and 16Sbr (5'-CCGGTCTGAACTCAGATCACGT-3'). The PCR was performed in a 25 µl volume comprising 10-100 ng of genomic DNA, 12.5 µl MyTaq HS Red Mix PCR (Bioline), 2mM $MgCl_2$, 0.6 µM of each primer, and 5.5 µl ddH2O.

The amplification conditions consisted of an initial pre-denaturation at 95°C for 2 minutes, followed by 35 cycles of denaturation at 95°C for 15 seconds, annealing at 50°C for 30 seconds, and extension at 72°C for 30 seconds. A final extension of 5 minutes at 72°C was also carried out (Arisuryanti et al. 2020).

The electrophoresis of PCR products was performed on a 1% agarose gel stained with FloroSafe (Bioline) and buffered with Tris-acetate EDTA (TAE) at 50 volts for 20 minutes. The visualization was conducted under UV light. All amplification samples were delivered to First Base (Malaysia) for purification and sequencing in both forward and reverse directions using the Big Dye Terminator (Applied Biosystems) and the ABI 3730xl Genetic Analyzer via PT. Genetika Science (Jakarta) (Applied Biosystems).

Data Analysis

SeqMan and EdiSeq (Lasergene, DNASTAR) were used to visually evaluate the chromatograms for reading errors. Each contiguous sequence of the gene segment was examined for unexpected stop codons and gaps. The correct sequences were then converted into FASTA format. Each sample sequence was analyzed using BLAST (blast.ncbi.nlm.nih.gov) to verify species identity from the GenBank data. The sequences were then aligned using Opal on Mesquite v.3.51 program (Maddison & Maddison, 2018) and ClustalW on the MEGAX program (Kumar et al., 2018).

The MEGAX program was used to determine the nucleotide composition. The genetic distance was calculated using the MEGAX program with the Kimura-2 Parameter (K2P) model, and the results were summarized in a Neighbor-Joining (NJ) tree. The phylogenetic tree was reconstructed using the Neighbor-Joining and Maximum Likelihood methods with 1,000 bootstraps in the MEGAX program (Kumar et al., 2018) and Bayesian Inference in the BEAST program (Suchard et al., 2018). The Akaike Information Criterion (AIC) provided in jModelTest 2.1.10 (Darriba et al., 2012) was used to determine the best fit evolutionary model. The best sequence substitution model for this study was GTR with Gamma (GTR + G). The posterior probabilities distribution was estimated using the Markov chain Monte Carlo (MCMC) method, which ran for 10^6 generations with a sampling frequency of every 1,000 generations. The analysis used a relative burn-in of 25% for diagnostics. FigTree 1.4.4 was used to show the consensus trees (Rambaut 2019).

Genetic variations (polymorphic sites, number of haplotypes, haplotype diversity, nucleotide diversity) within the striped snakehead samples were calculated using DnaSP ver 6.0 (Rozas et al., 2017). The haplotype network of striped snakehead fish was investigated using the Median Joining Network in the NETWORK ver.10.1 program (https://www.fluxus-engineering.com).

Table 1. Sampling location, sample code, geographic reference, and sample size of *C. striata*

Location	Sample Code	Latitude (S)	Longitude (E)	Sample Size (N)
Lake Lebo Taliwang, West Nusa Tenggara	GBL	08º34'0"	116º13'0"	1
Arut River, Central Kalimantan	GBA	2°40'10.6"	111°38'08.3"	5
Lake Sentani, Papua	GBS	2°36′36.7″	140°31′11.6″	5
Batang Hari River, Jambi	GBB	1°29'22.0"	102°26'51.0"	3
Ogan River, South Sumatra	GBO	3°13'21.0"	104°46'30.6"	4

RESULTS AND DISCUSSION

Results

Species Verification and Nucleotide Composition

The results of the Nucleotide BLAST analysis (https://blast.ncbi.nlm.nih.gov/) revealed that all striped snakehead samples have 98.85-99.51% similarity to *C. striata* samples with accession number KU986899 from the GenBank database. The average compositions of four nucleotides for all samples were 30.64% (A), 21.21% (T), 22.55% (G), and 25.59% (C) (Table 2). The range of T, C, A, and G nucleotide composition between samples was 30.52-30.92 % (A), 21.08-21.33 % (T), 22.29-22.69 % (G), and 25.50-25.70 % (G), respectively.

Genetic Variation

The 489 bp of mtDNA*16S* sequences revealed 5 variable sites and 4 parsimony informative sites. The variable sites were found on the base pair position of 154, 171, 182, 496, and 498 (Table 3). A total of five haplotypes were discovered (Table 4). Three haplotypes were shared and two were unique haplotypes. The most common haplotype was haplotype 1 (H1), which was found in 9 different samples from the central and eastern parts of Indonesia (GBL, GBS, and GBA populations). Two populations from Sumatera were found to have population-specific haplotypes, which were haplotype 4 (H4) from the Batang Hari River population (GBB) and haplotype 5 (H5) from the Ogan River population (GBO). The haplotype network formed a linear line network with each haplotype differing by one nucleotide, except for haplotype 1 and haplotype 5, which differ by two nucleotides (Figure 2). The mean of haplotype diversity (Hd) was 0.706 ± 0.088, whereas the nucleotide diversity (ð) was 0.00325 ± 0.00052. Haplotype diversity ranged from 0.00 (Arut and Ogan River) to 0.700 (Lake Sentani) and nucleotide diversity from 0.00 (Arut and Ogan River) to 0.00169 (Lake Sentani) (Table 5).

Genetic Distance and Phylogenetic Tree

The lowest percentage of genetic divergence was 0% between the Arut River (GBA) population and the

Table 2. Percentage composition of mtDNA *16S* nucleotide of five Indonesian wild *C. striata* populations

Sample Code	A	T(U)	G	C	A+T	G+C
GBL	30.52	21.29	22.69	25.50	51.81	48.19
GBS	30.52	21.33	22.61	25.54	51.85	48.15
GBA	30.52	21.29	22.69	25.50	51.81	48.19
GBB	30.92	21.08	22.29	25.70	52.01	47.99
GBO	30.72	21.08	22.49	25.70	51.81	48.19

Table 3. Polymorphic positions in haplotypes in *16S* region (489 bp) of Indonesian wild *C. striata.* The number above corresponds to the nucleotide base pair position.

Sample Code	Haplotype	Polymorphic Site				
		154	171	182	496	498
GBL-01	H1	G	T	G	G	T
GBS-01	H1	.	.	.	.	.
GBS-02	H1	.	.	.	.	.
GBS-03	H2	.	.	.	T	C
GBS-04	H1	.	.	.	.	.
GBS-05	H3	.	.	.	T	.
GBA-01	H1	.	.	.	.	.
GBA-02	H1	.	.	.	.	.
GBA-03	H1	.	.	.	.	.
GBA-04	H1	.	.	.	.	.
GBA-05	H1	.	.	.	.	.
GBB-01	H4	A	C	A	.	.
GBB-02	H4	A	C	A	.	.
GBB-03	H4	A	C	A	.	.
GBO-01	H5	.	C	A	.	.
GBO-02	H5	.	C	A	.	.
GBO-03	H5	.	C	A	.	.
GBO-04	H5	.	C	A	.	.

Table 4. Grouping of the haplotype of Indonesian wild *C. striata* based on the *16S* mitochondrial gene fragment

Haplotype	Sample number	Sample Code	Location
H1	9	GBL-01	Lake Lebo Taliwang, West Nusa Tenggara
		GBS-01 GBS-02 GBS-04	Lake Sentani, Papua
		GBA-01 GBA-02 GBA-03 GBA-04 GBA-05	Arut River, Central Kalimantan
H2	1	GBS-03	Lake Sentani, Papua
H3	1	GBS-05	Lake Sentani, Papua
H4	3	GBB-01 GBB-02 GBB-03	Batang Hari River, Jambi
H5	4	GBO-01 GBO-02 GBO-03 GBO-04	Ogan River, South Sumatra

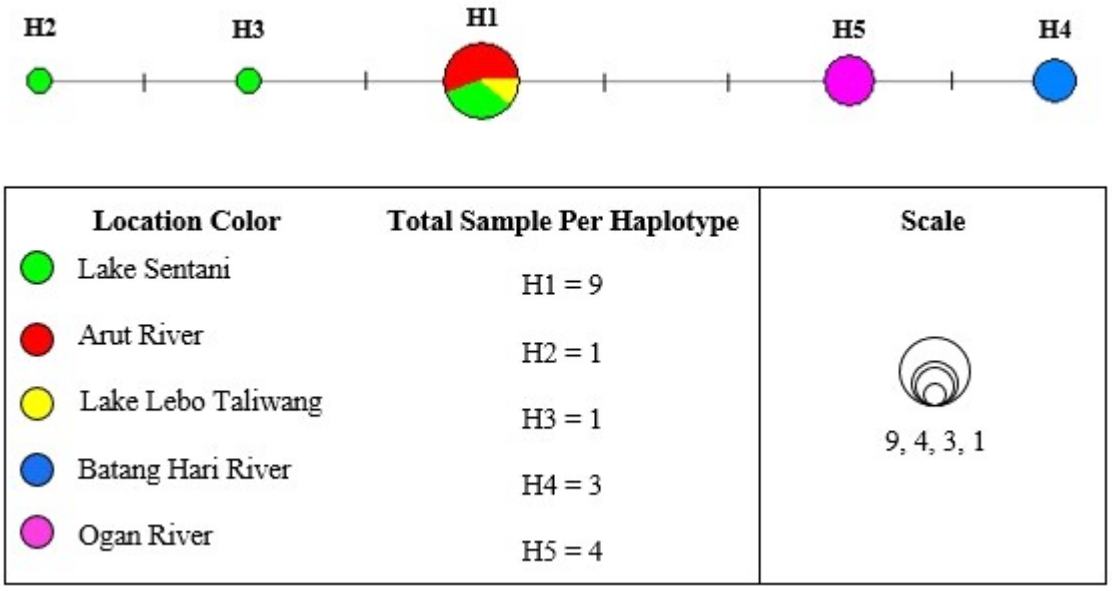

Figure 2. Haplotype network showing the relationship among *16S* mitochondrial haplotypes of Indonesian wild *C. striata*

Table 5. Haplotype and nucleotide diversity in the *16S* region of four Indonesian wild *C. striata* populations

Population	Haplotype diversity	Nucleotide diversity
Lake Sentani (GBS)	0.700±0,218	0.00169±0.00059
Arut River (GBA)	0.00 ± 0.00	0.00 ± 0.00
Batang Hari River (GBB)	0.667±0,314	0.00108±0.00051
Ogan River (GBO)	0.00 ± 0.00	0.00 ± 0.00

Lake Lebo Taliwang (GBL) population, while the highest was 0,73% between the Batang Hari River (GBB) population and the Lake Sentani population (Table 6). A total of 21 sequences were used in the phylogenetic analysis, including 3 samples from GenBank as follows: *Channa striata* (KT358478), *Channa gachua* (HM117234), and *Channa punctata* (HM117217). The phylogenetic tree topology of the NJ, ML, and BI trees was found to be identical (Figure 3).

Table 6. Mean percentage nucleotide sequence divergence of a 498 bp fragment of the *16S* mitochondrial gene among five populations of *C. striata* in this study

	GBL	GBS	GBA	GBB	GBO
GBL					
GBS	0.12				
GBA	0.00	0.12			
GBB	0.61	0.73	0.61		
GBO	0.40	0.52	0.40	0.20	

Discussion

Genetic diversity is an important aspect of the evolutionary process and it represents the ability of species or populations to adapt to environmental conditions. Haplotype and nucleotide diversity are essential measures of genetic variation. The higher the number, the greater the genetic variation and diversity in the population (Falush et al., 2003; Spielman et al., 2004; Liu, 2017).

This study revealed overall high haplotype diversity (Hd = 0.706 ± 0.088) and low nucleotide diversity (ð = 0.00325 ± 0.00052) of wild *C. striata* from 5 different populations. Two populations from Batang Hari River (GBB) and Lake Sentani (GBS) displayed the presence of high haplotype diversity (Hd >0.5) and low nucleotide diversity (ð < 0.005). High haplotype diversity but low nucleotide diversity suggested that the striped snakehead populations in this study had recently diverged from one another (Grant & Bowen, 1998). Furthermore, according to Bowen et al. (2001), this condition might be the consequence of rapid growth in population and the accumulation of mutations after populations suffered a bottleneck. In contrast, two populations from Arut River (GBA) and Ogan River (GBO) showed no genetic diversity (Hd and ð= 0), possibly as a result of several factors, such as small effective population size, species distribution, inbreeding, isolation, migration, habitat fragmentation, and human activities (Newman & Pilson, 1997; Luikart et al., 1998; Wang et al., 2006; Alam et al., 2022).

The A>C>G>T nucleotide composition pattern was consistent with the study conducted by Lakra et al. (2010) using *16S* rRNA mtDNA in eight species belonging to Genus *Channa*. Moreover, the average composition of A+T (51.86%) was greater than G+C (48,14%), which according to Nei & Kumar (2000) is still within the range of vertebrates.

The 489 bp of mtDNA*16S* sequences generated 5 variable sites. This result was to be expected considering that the *16S* gene is one of the most conserved mitochondrial genes, having a low mutation rate even among diverse taxa (Yang, 2014). The majority of nucleotide variation was caused by transition, with a transition-to-transversion ratio of 4:1. This ratio was consistent with the previous studies

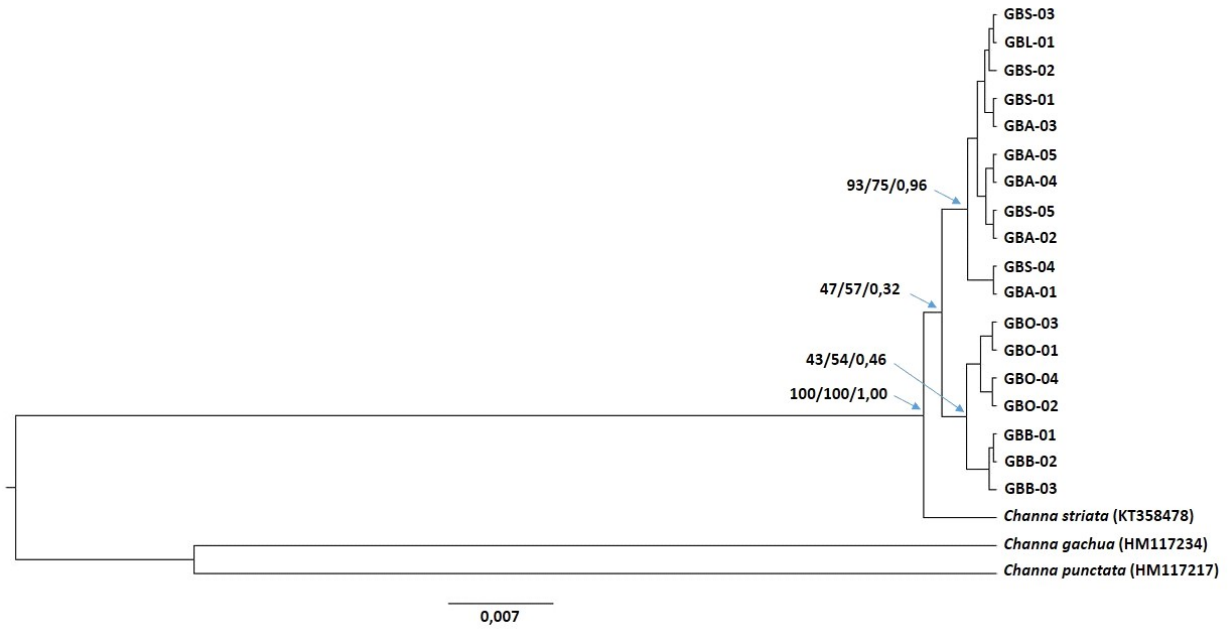

Figure 3. Phylogenetic tree of *C. striata* inferred from *16S* mitochondrial gene sequences. The number of each node represents bootstraps for NJ and ML and posterior probabilities for Bayesian Inference. The scale corresponds to substitution/site.

on mtDNA in fish, which found that generally, the transition exceeded the number of transversions (Vinson et al., 2004; Ward et al., 2005).

The phylogenetic tree revealed two distinct clades of *C. striata* in this study, which were supported by a bootstrap value of 100% in the Neighbor-Joining and Maximum Likelihood, as well as a posterior probability value of 1.00 on Bayesian Inference. The first clade included GBL, GBA, and GBS populations, and the second included GBB and GBO populations. This clades formation was consistent with the haplotype networking result which separated GBL, GBA, and GBS populations (H1, H2, and H3) from GBB and GBO populations (H4 and H5). The separation of these two distinct clades was representative of two geographical regions in Indonesia, namely, the western part of Indonesia (Sumatera) and the central to the eastern part of Indonesia (Kalimantan, West Nusa Tenggara, and Papua). Both regions also acted as geographical barriers between wild populations of *C. striata*, thus limiting gene flow for each population. The grouping of haplotype 1 (H1) which consisted of the central and eastern part of Indonesian populations was likely formed due to human intervention, possibly through fish trading from Kalimantan which is one of the native habitats for *C. striata* in Indonesia. This result indirectly showed the high potential of *C. striata* to adapt to a newly colonized region.

The result of this study suggested that the striped snakehead in Indonesia consisted of two different groups, i.e., the western part of Indonesia (Sumatera) and the central to the eastern part of Indonesia (Kalimantan, West Nusa Tenggara, and Papua).

CONCLUSION

This study revealed that 18 samples of striped snakeheads had 98.85-99.51% similarity to *Channa striata* samples with accession number KU986899 from GenBank. The 489 bp mtDNA *16S* sequence revealed 5 haplotypes with 5 variable sites and 4 parsimony informative sites. The mean of haplotype diversity and nucleotide diversity were 0.706 ± 0.088 and 0.00325 ± 0.00052 respectively with genetic divergence ranging from 0% to 0.73%. The phylogenetic tree exhibited two separate clades, which were supported by a bootstrap value of 100% (based on Neighbour Joining, NJ and Maximum Likelihood, ML method) and a posterior probability value of 1.00. (BI). This result indicated that there is intraspecific genetic variation (high haplotypes diversity but low nucleotide diversity) of wild *C. striata* populations in Indonesia, with two distinct groups consisting of the western part of Indonesia (Sumatera) and the central to the eastern part of Indonesia (Kalimantan, West Nusa Tenggara, and Papua). This finding will be helpful in efforts to conserve and cultivate the striped snakehead through breeding programs in Indonesia.

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