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Short Communication: DNA barcodes and phylogenetic of striped snakehead and ocellated snakehead fish from South Sumatra, Indonesia

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Abstract. *Syaifudin M, Wijayanti M, Dwinanti SH, Muslim, Mahendra M, Marliana S. 2020. DNA barcodes and phylogenetic of striped snakehead and ocellated snakehead fish from South Sumatra, Indonesia. Biodiversitas 21: 1227-1235.* This research aimed to identify the sequences of cytochrome c oxidase subunit I gene mitochondrial DNA (COI mtDNA), to construct a phylogenetic tree of striped snakehead (*Channa striata*) and ocellated snakehead (*Channa pleuropthalma*), and to measure water quality of Kelekar River, Indralaya, Ogan Ilir Distr and Danau Burung Besar River, Penukal Abab Lematang Ilir (PALI) District in South Sumatra, Indonesia. The research proce ures consisted of DNA isolation, amplification by PCR (Polymerase Chain Reaction) and sequencing of fragment COI mtDNA. The length of nucleotide was 604 bp for striped snakehead and 587-604 bp for ocellated snakehead. Optimum annealing temperature was 50^{0}C r 15 seconds with 30 cycles. The result of BLAST analysis showed that striped snakehead from Kelekar and Danau Burung Besar River had 100% identity to striped snakehead from Java-Bali and furthest (97%) with striped snakehead from India. Ocellated snakehead had 100% similarity with the same species from Musi Banyuasin and Banjarmasin; and furthest (83%) with *Channa limbata* from Myanmar. Water quality in Kelekar River were temperature 31-31.6^{0}C, pH 4.76-4.96, dissolved oxygen 2.7-3.0 mg/L, ammonia <0.009 mg/L, total alkalinity 20 mg/L, and turbidity 62.5-63 cm. Meanwhile in Danau Burung Besar River showed temperature (29.3-30.7^{0}C), pH (3.6-6.7), dissolved oxygen (1.31-3.76 mg/L), ammonia (0.17-0.20 mg/L), and turbidity (50-90 cm).

Keywords: COI, ocellated snakehead, phylogenetics, striped snakehead

INTRODUCTION

Striped snakehead (*Channa striata*) is economically important fish in Sumatra, Indonesia. There are some popular foods made from this fish, for instance, pempek, model, tekwan and cracker. Recently, domestication and artificial breeding of this fish have already been done, however genetic authentication of this fish is still limited in Indonesia. Growing this fish is also not successful until commercial size. Furthermore, decreasing water quality due to land use change, pollution and overfishing lead to decreasing in fish production in the wild. Therefore, fish genetic research, reproduction, and habitat characteristics in the wild are beneficial for initial attempt in aquaculture. Channidae has 2 genera i.e *Channa* and *Parachanna*. *Channa* is originally from Asia, while *Parachanna* is native to Africa. All these fish in this family are known as *snakehead*s (Courtenay and Williams 2004). There are four species of *Channa* in Kelekar River (Indralaya sub-district, Ogan Ilir District) i.e toman (*Channa micropeltes*), serandang (*Channa pleuropthalma*), gabus (*Channa striata*), and bujuk (*Channa lucius*) (Muslim, 2013). Ocellated snakehead, a native species in Musi River, Batanghari and Barito, has economic value for consumption in Sumatra and Kalimantan (Kotellat et al. 1993).

There are 37 species in family Channidae globally, which consisted of 34 species are *Channa* (Asia) and three species are *Parachanna* (Africa) (Froese and Pauly, 2018). Therefore, robust identification for species is important, especially for comparison between wild and cultured populati dan rehabilitation program (Rajiv and Chauhan, 2010). Mitochondrial DNA can be used to investigate evolutionary process with high resolution (Brown et al. 1979). Sequencing of this region has been widely used to discriminate species level (Nagl et al. 2001) and population study (Rognon and Guyomard 1997; D'Amato et al. 2007). Cytochrome C Oxidase subunit I is one of mtDNA genes, which is conserved, used for distinguishing species and population.

The COI gene is one of the molecular markers used to identify a species (Ward et al. 2005) that has conservative nucleotide base sequences and has little variation deletion, and insertion (Hebert et al. 2003). This technology (DNA barcoding) relies on the observation that the 'barcode' sequence divergence within species is typically much lower than the divergence exhibited between species (Hebert et al. 2003). This technique has been widely used for barcoding DNA for shark (Peloa et al. 2015), baung *Hemibagrus nemurus* (Syaifudin et al. 2017), Pangasius *Pangasius hypophthalmus* (Pratama et al. 2017), wild stock and the first generation (F1) of *Channa* spp. (Irmawati et al. 2017), tilapia (Syaifudin et al. 2019), snakeskin gourami and blue gourami (Syaifudin et al. 2019). This research aimed to obtain nucleotide sequences of COI gene of *C.*

striata and *C. plurophthalma*, construct a phylogenetic tree and characterize water quality of fish habitat in the Kelekar and Danau Burung Besar River in South Sumatra.

MATERIALS AND METHODS

Biological materials

Four individuals each species of striped snakehead and ocellated snakehead were collected from Kelekar River in Indralaya, Ogan Ilir District and Danau Burung Besar River, Penukal Abab Lematang Ilir (PALI) District at South Sumatra Provinces, Indonesia (Fig. 1). Fish were captured in the field with the help of local fishermen in the research area. Approximately 3 cm of a segment of the caudal fin was snipped and preserved in 99% ethanol (1:10 w:v), then stored in individual Eppendorf tubes at-20°C until required.

DNA extraction

A total of 8 fin clips from two species have been used in genomic DNA extraction. Total genomic DNA was extracted based on Geneaid DNA Extraction kit (GT 20) Geneaid Biotech Ltd. Taiwan) as outlined in the manufacturer's protocol. An RNAse incubation step was included to minimize RNA contamination. DNA samples were further stored in freezer (-20°C) until required. Those samples that showed no observable RNA and comprising predominantly high molecular weight DNA were selected for PCR (Polymerase Chain Reaction).

DNA amplification

In order to amplify 650 bp fragment, the DNA of stripped snakehead and ocellated snakehead were used in PCR with primer pairs of FishF2-5' TCGACTAATCATAAAGATATCGGCAC 3' and FishR2-5' ACTTCAGGGTGACCGAAGAATCAGAA 3' (Ward et al. 2005). PCR was performed in 40 μl final volumes using KAPA HiFi HotStart ReadyMixPCR kit (Kapa Biosystems, Massachusetts, United States). Each reaction contained 1.6 μl of 10 μM each primer, 14.8 μl of nuclease-free water, 20 μl of 2x KAPA HiFi HotStart ReadyMix and 2 μl of DNA template. The thermal cycling protocol was as follows: initial denaturation at 95°C for 3 min followed by 30 cycles of 98°C for 20 sec, annealing at 50°C for 15 sec, extension at 72°C for 30 sec and a final extension at 72°C for 1 min. Optimation of PCR resulted in an annealing temperature of 50°C (temperature melting/Tm of primer is 54.5°C). The temperature of annealing can be used in the range of *(Tm-5°C)* to *(Tm+5°C)*. Amplified fragments were run in electrophoresis 1% agarose gel at 75 V for 40 min with 1 Kb marker and visualized using GelDoc. PCR products were described in Figure 2.

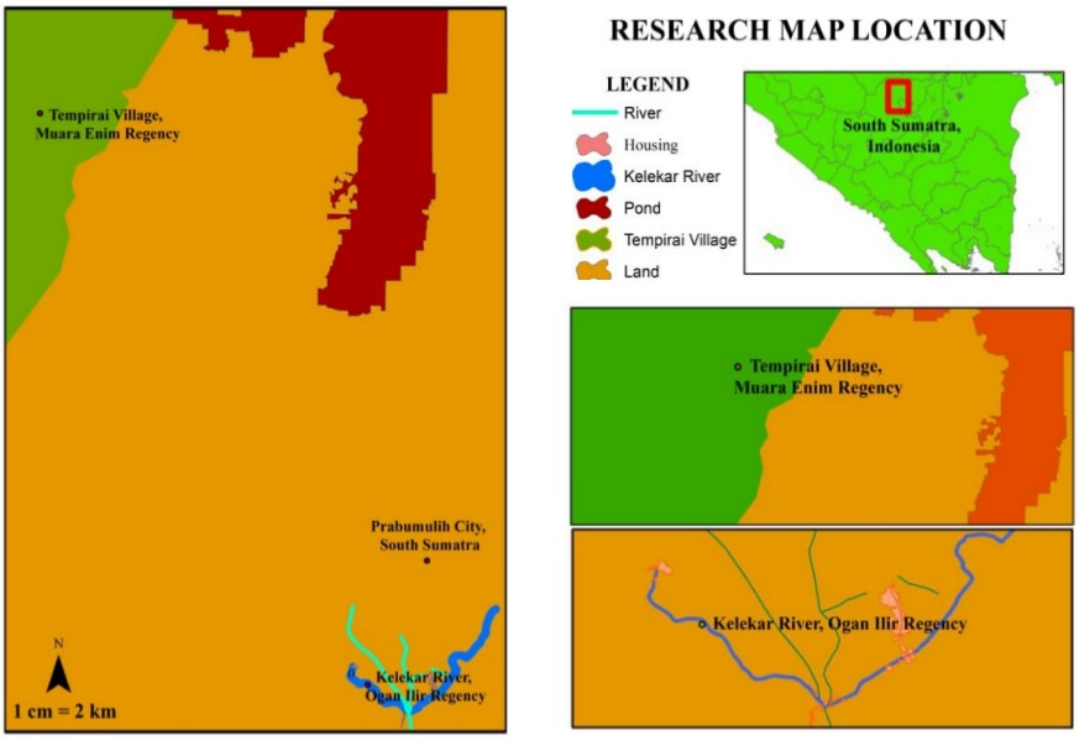

Figure 1. Sampling site of striped snakehead and ocellated snakehead collected from Kelekar River in Indralaya, Ogan Ilir District and Danau Burung Besar River, Penukal Abab Lematang Ilir (PALI) District at South Sumatra Provinces, Indonesia

COI gene sequencing

Eight DNA samples derived from two species that were successfully amplified using PCR were then bi-direction sequenced at the target area of the COI gene in Singapore through the services of the Genetica Science Institute in Jakarta.

Water quality

The physical and chemical water qualities were characterized during sample collection in Kelekar and Danau Burung Besar River. The physical characteristics i.e turbidity (cm) was measured using Secchi disc, while temperature (°C) using a thermometer. The chemical water qualities i.e pH was measured using pH meter, dissolved oxygen ($mg.L^{-1}$) using DO meter, ammonia ($mg.L^{-1}$) using spectrophotometer and total alkalinity ($mg.L^{-1}$ $CaCO_3$) using titrimetric method.

Data analysis

Four COI sequences of each species in Fasta format were aligned using MEGA 7.0 software (Kumar et al. 2016) for trimming process. It was then blasted using BLASTn (Basic Local Alignment Search Tool-nucleotide) in GenBank NCBI (National Center for Biotechnology Information) on the sequence database http://www.ncbi.nlm.nih.gov to compare the similarity of the *COI* gene between the Channidae to those in the GenBank database. Each sequence from each individual was put together in the alignment file from Mega7 software. The polymorphic were determined by aligning the sequence to know nucleotide variations within and between species. The nucleotide sequence was translated to the amino acid sequence using software from http://web.expasy.org/translate. The sequence data of this study (size 587-604 bp) have been reported to GenBank with accession number for *C. striata* (MN992966-MN992969) and *C. pleurophthalma* (MN992962-MN992965). For sequence comparisons, pairwise genetic distances were quantified based on the Kimura 2-parameter (K2P) distance model (Kimura et al. 1980) using MEGA, version 7.0 (Kumar et al. 2016). Voucher sequences from GenBank, and consensus sequences of each species generated from this study were compared and aligned using the CLUSTALW program. Furthermore, all sequences were used to analyze the phylogenetic tree. The phylogenetic tree between species of striped snakehead and ocellated snakehead was constructed using the Neighbor-Joining (NJ) method in the MEGA 7.0. *Oreochromis niloticus* was also used as species outgroup in the analysis.

RESULTS AND DISCUSSION

Species identity and phylogenetics

The analysis through BLASTn on the NCBI showed the percentage of identity with the other sequences in GenBank, which is presented in Tables 1 and 2.

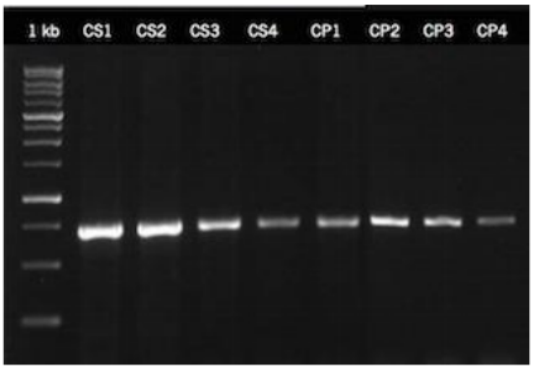

Figure 2. Visualization of PCR products of COI gene on stripped snakehead and ocellated snakehead. 1 kb = DNA *ladder* (bp) : 250, 500, 750, 1500, 2000, 2500, 3000, 4000, 5000, 6000, 8000, 10000. CS1-CS4 = stripped snakehead; CP1-CP4 = ocellated snakehead

The BLASTn analysis showed that COI sequences in this study conceded with those in the GenBank database. *C. striata* from Kelekar and Danau Burung Besar River, South Sumatra had the highest percentage of identity (100%) to the same species from Java and Bali, Indonesia (Accession code KU692420), but showed lower percentage of identity (97%) to the same species from India (Accession code MG675617 and KJ936901). Table 2 indicated that *C. pleurophthalma* had 100% identity to the same species from Musi Banyuasin (Accession code KM213041) and Banjarmasin (KJ937345) but denoted lower identity (87-83%) to *C. diplogramme*, *C. micropeltes*, *C. gachua*, and *C. limbata*.

Irmawati et al. (2017) reported COI gene nucleotides of snakehead fish from Towuti Lake, Sorowako in South Sulawesi indicated 99% homology with *Channa striata* from Cigede, Tasikmalaya, West Java (Accession code KU692418), and Rawa Pening Lake, Ambarawa, Central Java (KU692421). Wallace (1876) in Woodruff (2010) divided Asia into the Indochinese, Sundaic and Philipines zoogeographic subregions, meanwhile the Wallacean, lies to the east, has more similar to Australian biota. This study showed that either *C. striata* or *C. pleurophthalma* was concordance to Wallace statement where the diverse communities within each subregion in Asia share a common biogeographic history and many genera and families of plants and animals.

Genetic distance at this study was also used to determine the genetic relationship between *C. striata* and *C. pleurophthalma* (Table 3). The value of genetic distance within *C. striata* based on the COI gene ranged from 0.002 to 0.100, however, all *C. pleurophthalma* samples were identical. The two species had a genetic distance ranged from 0.110 to 0.116. This distance was very small, because the value did not reach 1, which means the kinship was very close. However, the data denoted that within the species barcode variation was low compared to the

sequence variation between *C. striata* and *C. pleurophthalma*. This phenomenon can be said as a monophyletic kinship which mean a group of taxa originating from the same ancestor. Various studies also proved that the genetic distance within the genus is lower than between the genus (Avise et al. 1987; Hebert et al. 2003; Khan et al. 2010). The results of genetic distance analysis will affect the reconstruction of phylogenetic trees.

Phylogenetics

The phylogenetic tree showed that *C. striata* and *C. pleurophthalma* were clustered in separate clade with a scale of 0.02 (Figure 2). As prediction, fishes from the same species were clustered closely into a single clade.

The phylogenetic tree indicated 2 different clades (monophyletic groups) i.e *C. striata* and *C. pleurophthalma* with *O. niloticus* as an outgroup species (Fig. 2). The first clade showed two different clusters, where *C. striata* from India were separated to the same species from Vietnam, China, the Philippines, Brunei Darussalam, Thailand, Malaysia, Indonesia (West and Central Kalimantan), USA and Indonesia (PALI, Indralaya, Java, and Bali) (bootstrap value/bv = 100). However, those species also make a different sub-clusters within species, for instance, sample of this study were in different sub-clusters from Indonesia (West and Central Kalimantan), USA (bv=96) and also another sub-clusters of Vietnam, China, the Philippines, Brunei Darussalam, Thailand, and Malaysia (bv=89). Robert et al. (2018) reported there were two reciprocally monophyletic genomes of *C. striata* were identified along the west and east coast that may have been separated by mountain upthrusts throughout the central region of Sabah, Malaysia.

The second clade consisted of all *C. pleurophthalma*, *C. diplogramma*, *C. micopeltes* and a paraphyletic group of *C. gachua* (Vietnam and Laos) and *C. limbata* (Myanmar) (bv = 80). *C. pleurophthalma* from PALI and Indralaya were in the same cluster to the same species from Musi Banyuasin (KM213041) and Banjarmasin (KJ937345) (bv = 100), which are separated from *C. diplogramma* (India) and *C. micropeltes* (Indonesia and Malaysia) (bv = 81). The clade differences between *C. striata* and *C. pleurophthalma* are based on difference of nucleotide. There were 82 of nucleotide difference between species, but seven nucleotides caused a change in amino acids (Table 4). Five nucleotides were different within *C. striata*, however only one nucleotide at position 165 indicated an amino acid change, however, there was no difference in the nucleotide within species of *C. pleurophthalma*.

Table 1. The top hit match in the GenBank database of COI nucleotide of striped snakehead (*C. striata*)

Species	Identity (%)	Accession no.	Origin
Channa striata	100%	KU692420	Java and Bali, Indonesia
Channa striata	99%	HM345931	Aceh, Indonesia
Channa striata	99%	MF496960	Central Kalimantan, Indonesia
Channa striata	99%	JQ661364	Nakornsawan, Thailand
Channa striata	99%	JF781203	Kuala Lumpur, Malaysia
Channa striata	98%	MF496954	West Kalimantan, Indonesia
Channa striata	98%	KT001935	Can Tho, Vietnam
Channa striata	98%	KC819606	Shanghai, China
Channa striata	98%	HQ654692	Batangas, Filipina
Channa striata	98%	MF496939	Brunei Darusalam
Channa striata	98%	KJ937425	USA
Channa striata	97%	MG675617	Andhra Pradesh, India
Channa striata	97%	KJ936901	Tripura, India

Table 2. The top hit match in the GenBank database of COI nucleotide of ocellated snakehead (*C.pleurophthalma*)

Species	Identity (%)	Accession no.	Origin
Channa pleuropthalma	100%	KM213041	Musi Banyuasin, Indonesia
Channa pleuropthalma	100%	KJ937345	Banjarmasin, Indonesia
Channa diplogramme	87%	KJ937448	Tamil Nadu, India
Channa diplogramme	87%	KY750711	Kerala, India
Channa micropeltes	86%	KT001052	Kuala Lumpur, Malaysia
Channa micropeltes	86%	KM213040	South Sumatra, Indonesia
Channa micropeltes	85%	MF496862	Perak, Malaysia
Channa gachua	84%	MF496766	Khammouan, Laos
Channa gachua	84%	MF496738	Dak Lak, Vietnam
Channa limbata	83%	LC190116	Shan State, Myanmar

Tabel 3. Genetic distance between striped snakehead (*C. striata*) and ocellated snakehead (*C. pleurophthalma*) based on COI gene

No.	Species	Genetic Distance																								
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26
1	*C. striata*_VNM_(Dak_Lak)																										
2	*C. striata*_VNM_(Can_Tho)	0.002																									
3	*C. striata*_CHN_(Shanghai)	0.003	0.003																								
4	*C. striata*_USA	0.010	0.010	0.011																							
5	*C. striata*_BRN	0.001	0.001	0.002	0.009																						
6	*C. striata*_MLY_(Kuala_ Lumpur)	0.005	0.005	0.006	0.007	0.004																					
7	*C. striata*_THA_(Phang_ Nga)	0.001	0.001	0.002	0.009	0.000	0.004																				
8	*C. striata*_THA_(Nakornsawan)	0.003	0.003	0.004	0.007	0.002	0.002	0.002																			
9	*C. striata*_PHL_ (Nueva_Ecija)	0.001	0.001	0.002	0.009	0.000	0.004	0.000	0.002																		
10	*C. striata*_PHL_ (Laguna)	0.001	0.001	0.002	0.009	0.000	0.004	0.000	0.002	0.000																	
11	*C. striata*_PHL_ (Batangas)	0.004	0.004	0.005	0.012	0.003	0.006	0.003	0.005	0.003	0.003																
12	*C. striata*_IND_(Tripura)	0.018	0.018	0.017	0.017	0.017	0.015	0.017	0.015	0.017	0.017	0.020															
13	*C. striata*_IND_(Andhra_ Pradesh)	0.019	0.019	0.018	0.018	0.018	0.016	0.018	0.016	0.018	0.018	0.021	0.005														
14	*C. striata*_ IDN_(PALI1')	0.009	0.009	0.010	0.010	0.008	0.006	0.008	0.006	0.008	0.008	0.011	0.018	0.017													
15	*C. striata*_IDN_(PALI2')	0.009	0.009	0.010	0.010	0.008	0.006	0.008	0.006	0.008	0.008	0.011	0.018	0.017	0.000												
16	*C. striata*_ IDN_(Central_Kalimantan)	0.007	0.007	0.008	0.003	0.006	0.005	0.006	0.005	0.006	0.006	0.009	0.014	0.015	0.007	0.007											
17	*C. striata*_IDN_(West_ Kalimantan)	0.010	0.010	0.011	0.000	0.009	0.007	0.009	0.007	0.009	0.009	0.012	0.017	0.018	0.010	0.010	0.003										
18	*C. striata*_IDN_(Aceh)	0.005	0.005	0.006	0.009	0.004	0.002	0.004	0.002	0.004	0.004	0.006	0.017	0.018	0.006	0.006	0.006	0.009									
19	*C. striata*_IDN_(Java_ Bali)	0.009	0.009	0.010	0.010	0.008	0.006	0.008	0.006	0.008	0.008	0.011	0.018	0.017	0.000	0.000	0.007	0.010	0.006								
20	*C. striata*_IDN_(Indralaya 1')	0.010	0.010	0.011	0.011	0.009	0.007	0.009	0.007	0.009	0.009	0.012	0.019	0.018	0.001	0.001	0.008	0.011	0.007	0.001							
21	*C. striata*_IDN_(Indralaya2')	0.010	0.010	0.011	0.011	0.009	0.007	0.009	0.007	0.009	0.009	0.012	0.019	0.018	0.001	0.001	0.008	0.011	0.007	0.001	0.000						
22	*C. pleurophthalma*_ IDN_(South_ Sumatra)	0.112	0.115	0.114	0.110	0.114	0.108	0.114	0.111	0.114	0.114	0.116	0.113	0.113	0.113	0.113	0.110	0.110	0.111	0.113	0.114	0.114					
23	*C. pleuropthalma*_ IDN_(PALI 1')	0.112	0.115	0.114	0.110	0.114	0.108	0.114	0.111	0.114	0.114	0.116	0.113	0.113	0.113	0.113	0.110	0.110	0.111	0.114	0.114	0.114	0.000				
24	*C. pleuropthalma*_ IDN_(PALI2')	0.112	0.115	0.114	0.110	0.114	0.108	0.114	0.111	0.114	0.114	0.116	0.113	0.113	0.113	0.113	0.110	0.110	0.111	0.115	0.114	0.114	0.000	0.000			
25	*C. pleuropthalma*_IDN_(1ndralaya1*)	0.112	0.115	0.114	0.110	0.114	0.108	0.114	0.111	0.114	0.114	0.116	0.113	0.113	0.113	0.113	0.110	0.110	0.111	0.116	0.114	0.114	0.000	0.000	0.000		
26	*C. pleuropthalma*_IDN_(1ndralaya2*)	0.112	0.115	0.114	0.110	0.114	0.108	0.114	0.111	0.114	0.114	0.116	0.113	0.113	0.113	0.113	0.110	0.110	0.111	0.117	0.114	0.114	0.000	0.000	0.000	0.000	
27	*C. pleurophthalma*_ION_(Banjarmasin)	0.112	0.115	0.114	0.110	0.114	0.108	0.114	0.111	0.114	0.114	0.116	0.113	0.113	0.113	0.113	0.110	0.110	0.111	0.118	0.114	0.114	0.000	0.000	0.000	0.000	0.000

According to Avise (1994), the use of mitochondrial DNA sequences can clarify the relationship between species in evolution that was blurred due to morphological variations. Furthermore, Zhu et al. (2013) stated that identification of this species based on morphological characters showed very erratic results because of the high diversity of *C. striata*. Song et al. (2013) observed that genetic diversity within *Channa striata* was lower than that of other species and did not correlate to morphological variations. Phylogenetic relationships illustrated the possibility of genetic mixing among populations (Laudien et al. 2003), caused by several factors such as genetic flow (gene flow) and introduced activities by humans. *C. striata* are very widely distributed in Asia from Pakistan, India and Sri Lanka, all parts of the country of Myanmar, Thailand, Cambodia, Vietnam, Malaysia and Indonesia (Grand et al. 2017). The mainland of Peninsular Malaysia, the islands of Borneo, Thailand, Indo-China, and Sumatra were once part around 250,000 years ago. Because of the effects of maximum seawater declining, the mainland of Peninsular Malaysia and the island of Borneo were once connected by lowlands traversed by rivers, which was rich in diversity of fish because fish flowed out the east coast of Peninsular Malaysia, the west coast of Kalimantan and Sumatra. This condition allows the migration of species *C. striata* between regions (Benziger et al. 2011).

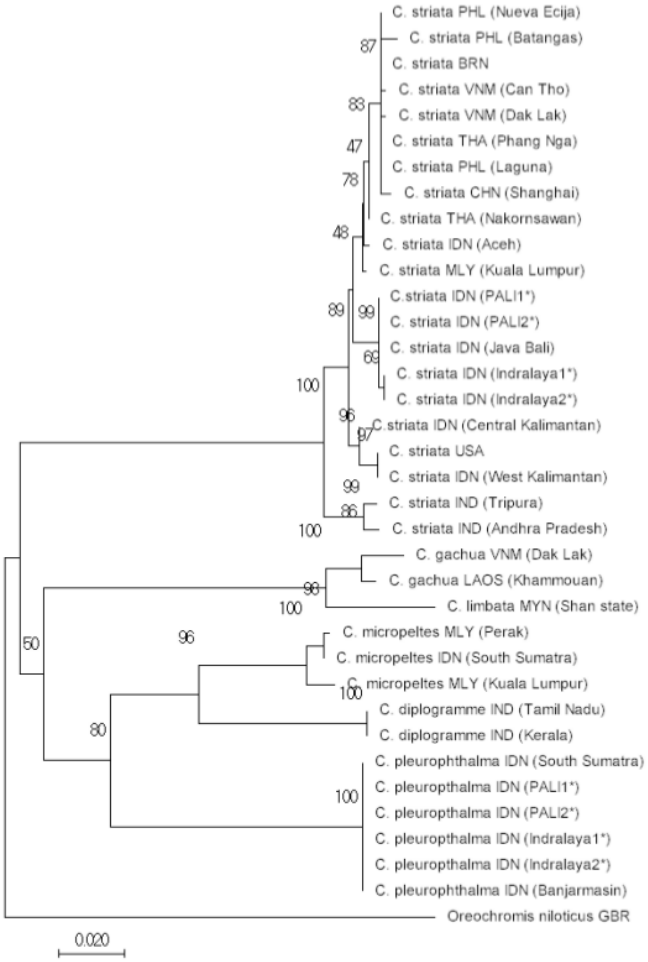

Figure 2. Phylogenetic tree of striped snakehead (*C. striata*) and ocellated snakehead (*C. pleurophthalma*) was constructed using Neighbor-Joining (NJ) method. *): specimen of current study. *C. striata* : Indonesia (KU692420, HM345931, MF496960, MF496954), Thailand (JQ661364), Malaysia (JF781203), Vietnam (KT001935), China (KC819606), Filipina (HQ654692), Brunei (MF496939), USA (KJ937425) and India (MG675617, KJ936901). *C. pleurophthalma* (Indonesia: KM213041, KJ937345), *C. diplogramme* (India: KJ937448, KY750711), *C. micropeltes* (Malaysia : KT001052, MF496862; Indonesia: KM213040), *C. gachua* (Vietnam: MF496766, MF496738), *C. limbata* (Myanmar: LC190116).

Table 4. Polymorphic site of nucleotide and amino acids in *COI* gene between *C. striata* and *C. pleurophthalma*

No	Nucleotide	Position	Amino acid	Origin of species
Channa striata				
1	T	57	no change	Kelekar River and Pali (current study), Java and Bali, Andrha_Pradesh
	C	57	no change	Others sample
2	C	60	no change	Kelekar River and Pali (current study), Java and Bali, Andrha_Pradesh
	T	60	no change	Others sample
3	A	165	I	Kelekar River and Pali (current study), Java and Bali, Andrha_Pradesh
	G	165	M	Others sample
4	T	342	no change	Kelekar River and Pali (current study), Java and Bali, Andrha_Pradesh
	A	342	no change	Others sample
5	C	429	no change	Kelekar River and Pali (current study) others sample
	T		no change	Others sample
Channa pleurophthalma				
There was no difference of nucleotide and amino acids within species in this study and GenBank database				
Channa striata* vs *C. pleurophthalma*				
1	A	120	I	*C. pleurophthalma*
	G		M	*C. striata*
2	G	129	M	*C. pleurophthalma*
	A		I	*C. striata*
3	G	150	W	*C. pleurophthalma*
	A		Deletion	*C. striata*
4	A	183	I	*C. pleurophthalma*
	G		M	*C. striata*
5	G	216	W	*C. pleurophthalma*
	A		Deletion	*C. striata*
6	G	420	M	*C. pleurophthalma*
	A		I	*C. striata*
7	A	465	Deletion	*C. pleurophthalma*
	G		W	*C. striata*

Note: There were 82 different nucleotides between *C. striata* and *C. pleurophthalma*, however only 7 were different in amino acids.

Table 5. Water qualities of Kelekar and Danau Burung Besar River

Parameter	River	
	Kelekar	Danau Burung Besar
Temperature (°C)	31-31.6	29.3-30.7
pH (Unit)	4.76-4.96	4.6-6.7
DO (mg.L^{-1})	2.7-3.0	1.31-3.76
Ammonia (mg.L^{-1})	<0.009	0.17-0.20
Alkalinity (mg.L^{-1} $CaCO_3$)	20	30
Turbidity (cm)	62.5-63	50-90

Water quality

The water qualities during fish collection were presented in Table 5. Based on measurements in both of river, the temperature in Kelekar (31-31.6°C) and Danau Burung Besar (29.3-30.7°C) were still in good condition for the life of striped snakehead and ocellated snakehead. Striped snakehead can live with water temperature ranging between 26.5-31.5°C (Syafei et al. 1995), while ocellated snakehead can live at a temperature of 27-30°C (Said, 2007). The pH value of the rivers ranged between 4.76-6.7. This condition was still supported for growth and survival of two species. These fish are able to live and grow at a fairly wide pH susceptibility, which ranges from 4-7 for striped snakehead (Balai Perikanan Budidaya Air Tawar Mandiangin, 2014) and 5-6.5 for ocellated snakehead (Said, 2007).

According to Muslim (2017), striped snakehead was mostly found in swamps, living in shallow waters. Dissolved oxygen levels in the Kelekar River ranged from 2.7 to 3.0 mg.L^{-1}, while in the Danau Burung Besar River was 1.31-3.76 mg.L^{-1}. This parameter was less appropriate, however, striped snakehead has an additional breathing device, diverticula to support fish life (Kottelat et al. 1993). Tropical fish species can grow well in dissolved oxygen content > 5 mg.L^{-1}, with lethal concentrations <0.3 mg. L^{-1} (Zweig et al. 1999). Ammonia value in the Kelekar and Danau Burung Besar River was <0.009 and 0.17-0.20 mg.L^{-1}, respectively. Effendi (2003) stated that a maximum of ammonia level to support fish life is no more than 0.2 mg.L^{-1}. These also suit for striped snakehead (Balai Perikanan Budidaya Air Tawar Mandiangin, 2014). The alkalinity was 20 mg.L^{-1} in the Kelekar River and 30 mg.L^{-1} in the Danau Burung Besar River. These values were still in optimum conditions for striped snakehead and ocellated snakehead. Total alkalinity in natural freshwater systems ranges from 5 to 500 mg.L^{-1} (Lawson 1995). The turbidity value of the Kelekar River ranged from 62.5 to 63 cm, while at Danau Brurung Besar was 50-90 cm. This

turbidity level was still in tolerance for the life of striped snakehead and ocellated snakehead. According to Ageriyanto (2012), striped snakehead can still be found and grown well in waters with values ranging from 65-145 cm. In general, the productivity of aquatic organisms increases at turbidity level between 30-65 cm (Boyd dan Licthkoppler 1979).

In conclusion, *C. striata* and *C. pleurophthalma* from Indralaya, and PALI, South Sumatra have been successfully barcoded, where the two species were clustered in separate branches. Water quality of Kelekar River indicated that temperature was 31-31.6°C, turbidity (62.5-63 cm), pH (4.76-4.79), dissolved oxygen (2.7-3.0 mg/L), ammonia (<0,009 mg/L), and alkalinity total was 20 mg/L, were still in tolerance for both species.

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