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Reviewer A:

Review of “Genetically continuous populations of Striped Snakehead (*Channa striata*) in the Cingcingguling River fragmented
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Reviewer A:

The article presents original basic research within scope of the Biodivers
in the Cingcinggiilling River fragmented by Sempor Reservoir, Central Ja

4. Line 236: The Sempor reservoir did not cause genetic fragmentatio
5. Line 239: Please provide proof, data etc or supporting references r

Overall, authors used clear, relatively unambiguous English language th

Experimental design

Research question are well defined and relevant. There is also a confus

Genetically continuous populations of Striped Snakehead (*Channa striata*) in the Cingcingguiling River fragmented by Sempor Reservoir, Central Java, Indonesia

Abstract. Cingcingguling River, located in Kebumen Regency Central Java, Indonesia. It is fragmented by the Sempor Reservoir. The pPrevious study proved the negative impact of the reservoir on positive rheotaxis fish, mainly in genetic constituent between the reservoir and river populations. However, research has not been conducted on the negative rheotaxis fish, such as *Channa striata*. Assessing population genetic and taxonomic validity study of Striped Snakehead in the Cingcingguling River is an essential effort. Both studies could be done using cytochrome c oxidase 1 gene. Therefore, this research aims to determine taxonomic status and evaluate the population genetic of C. striata in the Cingcingguling River. The samples were collected at eight sites inside and outside the reservoir. The used marker was sequenced from 53 individuals, and all specimens showed high (98.67% to 100%) and low genetic distances (0.00 to 0.01) to *C. stirata* (KU692421, KU852443, and MG438366). Those values proved that all samples were genetically identified to as *Channa striata*. The vertical genetic distribution analysis proved that *C. striata* populations are genetically not different along the river. Unlike rheotaxis positive fish phenomena, the reservoir's existence does not cause genetic fragmentation and leads to continuous striped snakehead populations.

Key words: genetic diversity, reservoir, rheotaxis, striped snakehead

Abbreviations (if any): AMOVA= analysis of molecular variance, DRPM BRIN = Direktorat Riset dan Pengabdian Kepada Masyarakat Badan Riset dan Inovasi Nasional

Running title: Genetically continuous populations of *Channa striata*

INTRODUCTION

Striped snakehead (*Channa striata*) is an essential important freshwater fish species in several Asian countries. In Indonesia, it is particularly found in the main islands of the Sunda Shelf, including Sumatra, Java, and Borneo (Froese & Pauly, 2021). Currently, this species is also found in the lesser Sunda Island, such as Bali (Yudha et al. 2018), and has been introduced to the Wallacea Regions (Irmawati et al., 2017). *C. stirata* is primarily discovered in stagnant or swampy water ecosystems (Amilhat & Lorenzen 2005; Muflikhah 2007; Listyanto & Andriyanto 2009), therefore, it is a negative rheotaxis fish species. Nevertheless, it lives in a wide range of habitats, such as swamps, stagnant rivers, river flood plain, and dams or reservoirs (Iskandar & Dahiyat, 2012; Nuryanto et al., 2012; Roema, 2013; Nuryanto et al., 2015). This species is also reported found both inside and outside Sempor Reservoir in the Cingcingguling River, Central Java, Indonesia (Setyaningrum et al., 2020, 2021).

Sempor Reservoir was built approximately 51 years ago and has caused the Cingcingguling River to be fragmented into two2 extremely different habitats. These include completely stagnant and running water bodies located underneath the reservoir (Hedianto et al., 2014). The rReservoir is a physical barrier for gene flow and causes significant genetic differences among its populations (Heggenes & Roed 2006). However, the available data concerning the reservoir's negative impact on river populations was only available for the positive rheotaxis fish species (Wibowo et al., 2012; Bahiyah et al., 2013; Barasa et al. 2014; Plavova et al. 2017). Meanwhile, there is no recorded information about the reservoir's genetic effect on negative rheotaxis fish species. Therefore, it is essential to research the genetic impact of Sempor Reservoir on the *C. striata* population in the Cingcingguling River.

The genetic impact of a reservoir on the fish population could be assessed with a molecular tool, such as the cytochrome c oxidase 1 (COI) gene (Nuryanto et al. 2019). Previous research reported that it was used as a powerful robust marker for population genetic analysis of *C. striata* in Perak State situated in Malaysia (Jammaluddin et al. 2011, Tan et al. 2012, 2015). Nevertheless, population genetic research tends to be carried out when the taxonomic status of the

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analyzed organisms is valid. In the case of *C. striata*, it was reported that the morphological identification of samples obtained from different regions showed inconsistent diagnostic characters (Zhu et al. 2013; Arma et al. 2014; Khan et al. 2019; Muslimin et al. 2020). Taxonomic validity could also be determined using cytochrome c oxidase 1 (COI) gene (Ko et al., 2013; Nuryanto et al., 2017; 2019; 2020). Furthermore, it was reliable for species delineation of *C. striata* from Sumatra (Muchlisin et al. 2013; Dahruddin et al. 2016; Irmawati et al. 2017; Syaifudin et al. 2020) and is used to validate morphological identification (Nuryanto et al. 2021). Therefore, this research wa~~i~~s aimed to validate and assess the taxonomic status and genetic population of *C. striata* in Sempor Reservoir Central Java using cytochrome c oxidase 1 gene.

MATERIALS AND METHODS

Research location and sampling sites

Striped snakehead specimens were collected from 8 different sites, four~~4~~ of them were situated inside the reservoir, while the remaining were located downstream (Figure 1). These were collected using traps and lines with the help of fisher~~srmen~~. Tinny tissue samples were chopped from the pectoral fin of each specimen and preserved in ethanol 96%.

Figure 1. Research location with five~~5~~ sampling sites along the river. Four ~~and 4~~ of them located inside the reservoir.

Procedures

Genomic DNA extraction and Marker polymerization

The total genome was extracted from the pectoral fin tissue using the Quick-DNA™ Miniprep Plus kit adopted from Zymo's research. Extraction procedures were carried out based on the company's manual, and its success was tested using 1% agarose electrophoresis. Subsequently, the COI gene target fragments were reproduced using FishF2 and FishR2 primers (Ward et al. 2005) in Primus 25 Peqlab Thermocycler. Meanwhile, 50 µl of amplified reactions consisted of 1x buffer PCR, 2 mM MgCl2, 0.2 mM of each primer, 0.2 mM dNTP mix, 1 U Taq polymerase, and 2.0 ng / µl template DNA. Furthermore, the final volume of 50 µl was adjusted by adding DNA-RNA-free water. Thermal cycles were pre-denatured at 95°C for 4 minutes and were repeated 35 times. The denaturation steps lasted for 30 seconds at 95°C, 2 minutes at 53°C, and 1 minute at 72°C for primer annealing and chain elongation. Additionally, a final extension terminated the cycles after 5 minutes, at 72°C. The PCR products were stained using ethidium bromide and 1.5% agarose gel and placed under ultraviolet light. Gel documentation was further performed using the GelDoc apparatus (BioRad).

Marker sequencing and editing

The PCR products of the marker were shipped to 1st BASE Malaysia for sequencing, while that the sequencing process was performed using the Sanger method. Consensus and multiple sequences alignment were obtained by assembling the forward and reverse sequences using ClustalW ver.1.4 in Bioedit (Hall 2011). Haplotype data was obtained from its generating process in DnaSP 5 (Rozas et al. 2017).

Data analysis

The striped snakehead specimens' taxonomic status was validated through a sequence identity test carried out using a basic local alignment search tool (BLAST) closest to the taxa in GenBank. Genetic distance was also used to support the identity data. Haplotypes (h) and nucleotide (π) diversities were calculated using Arlequin 3.5, while neutral evolution of the COI marker was estimated using Fu's Fs and Tajima D test (Excoffier & Lischer 2010). Population differentiation was calculated using Fst and variance analysis (AMOVA) ~~carried out~~ in Arlequin 3.5 (Excoffier & Lischer 2010). It was also estimated using a shared haplotype, which was observed in its network. This was reconstructed using the median-joining method in NETWORK software (Bandelt et al. 1999). The phylogenetic relationship of *C. striata* in Cincingguling River was estimated using Neighbor-Joining (NJ) and Maximum Likelihood algorithms in MEGA X (Kumar et al. 2018) with 1000 bootstraps replications. Also, the topological stability tree wa~~i~~s obtained from the out-group comparison (*Channa gacua* MK599522; *Channa micropeltes* JN024962; *Channa Lucius* KJ937433).

RESULTS AND DISCUSSION

Taxonomic status

Approximately 50 individuals of *Channa* specimens were successfully sequenced, resulting in fragments within the range of 596 bp to 689 bp lengths. Sequence identity test showed that the samples were genetically similar to the top 10 hits closest taxa in the GenBank, all identified as *C. striata* (KU692421, KU852443, and MG438366). However, their percentages were between 98.67% and 100%, with the expected value being 0.0. The samples showed varied genetic distances ~~in accordance with~~following Kimura 2 parameter (K2P) from 0.000 % to 1.019, indicating low genetic distances to their closest related taxa in GenBank ~~, as shown in~~(Table 1).

Table 1. Sample code, expect value, percent identity, genetic distances, and closest taxa in GenBank

Sample code	E-value	Percent Identity (%)	Genetic distance (%)	Closest Taxa in GenBank
KW 1	0.0	100.0	0.000	Channa striata KU692421
KW 2	0.0	100.0	0.000	Channa striata KU692421
KW3	0.0	98.67	1.019	Channa striata KU852443
KW 4	0.0	100.0	0.000	Channa striata KU692421
KW 5	0.0	100.0	0.000	Channa striata KU692421
KW 6	0.0	100.0	0.000	Channa striata KU692421
KW 7	0.0	100.0	0.000	Channa striata KU692421
KW 8	0.0	100.0	0.000	Channa striata KU692421
KW 9	0.0	100.0	0.000	Channa striata KU692421
KW 10	0.0	100.0	0.000	Channa striata KU692421
BK 1	0.0	100.0	0.000	Channa striata KU692421
BK 2	0.0	100.0	0.000	Channa striata KU692421
BK 3	0.0	100.0	0.000	Channa striata KU692421
BK 4	0.0	100.0	0.000	Channa striata KU692421
BK 5	0.0	100.0	0.000	Channa striata KU692421
BK 6	0.0	100.0	0.000	Channa striata KU692421
KA 1	0.0	100.0	0.000	Channa striata KU692421
KA 2	0.0	100.0	0.000	Channa striata KU692421
KA 3	0.0	100.0	0.000	Channa striata KU692421
KA 4	0.0	100.0	0.000	Channa striata KU692421
KA 5	0.0	100.0	0.000	Channa striata MG438366
KA6	0.0	99.20	0.169	Channa striata KU692421
KA7	0.0	99.54	0.000	Channa striata KU692421
WO 1	0.0	99.68	0.508	Channa striata KU692421
WO 2	0.0	99.84	0.000	Channa striata KU692421
WO 3	0.0	99.35	0.848	Channa striata MG438366
WO 4	0.0	99.69	0.000	Channa striata KU692421
KS 1	0.0	99.07	0.678	Channa striata KU692421

Sample code	E-value	Percent Identity (%)	Genetic distance (%)	Closest Taxa in GenBank
KS 2	0.0	100.0	0.000	Channa striata KU692421
KS 3	0.0	100.0	0.000	Channa striata KU692421
KS 4	0.0	100.0	0.000	Channa striata KU692421
KS 5	0.0	100.0	0.000	Channa striata KU692421
KS 6	0.0	100.0	0.000	Channa striata KU692421
KS 7	0.0	98.77	0.849	Channa striata KU692421
KS 8	0.0	100.0	0.000	Channa striata KU692421
KS 9	0.0	100.0	0.000	Channa striata KU692421
KS 10	0.0	100.0	0.000	Channa striata KU692421
PW 1	0.0	99.84	0.000	Channa striata KU692421
PW 2	0.0	100.0	0.000	Channa striata KU692421
KB1	0.0	100.0	0.000	Channa striata KU692421
KB2	0.0	100.0	0.000	Channa striata KU692421
KB3	0.0	100.0	0.000	Channa striata KU692421
KB4	0.0	100.0	0.000	Channa striata KU692421
KB5	0.0	100.0	0.000	Channa striata KU692421
KB6	0.0	100.0	0.000	Channa striata KU692421
KB7	0.0	100.0	0.000	Channa striata KU692421
KB8	0.0	100.0	0.000	Channa striata KU692421
KB9	0.0	99.84	0.000	Channa striata KU692421
KB10	0.0	99.84	0.000	Channa striata KU692421
BA 1	0.0	100.0	0.000	Channa striata KU692421
BA 2	0.0	100.0	0.000	Channa striata KU692421
BA 3	0.0	100.0	0.000	Channa striata KU692421
BA 4	0.0	100.0	0.000	Channa striata KU692421

KW: Kedungwringin, BK: Bangkong, KA: Kalianget, WO: Waduk outlet, KS: Sempor, PW: Purbowangi, BA: Buayan, KB: Karang Bolong

This research delineated the samples ~~to~~ as *C. striata* because their high genetic identities (above 97%) and genetic distance were less than 3%, respectively, to their conspecific. According to Ratnasingham and Hebert (2013), this is the standard identity threshold for animal species determination. Simultaneously, a distance of 3% is acceptable for threshold species determination in fish barcoding (Ranasingham & Hebert 2007; Hubert et al. 2010; Candek & Kuntner 2015). Even though a higher threshold of approximately 4% and 5% is allowed, other factors need to be considered (Higashi et al., 2011; Jeffrey et al., 2011; Candek & Kuntner, 2015).

The low genetic distance among individuals of *C. striata* was reportedly occurred in the wild population found in Lake Towuti, South Sulawesi, with ~~the~~ values between 0.043 and 0.309% and (Irmawati et al., 2017). Similar values were reported in China (Zhu et al. 2013), using 5 *C. striata* populations, which showed that the intraspecific genetic distances were ranged from 0.002% to 0.027%. In contrast, it was approximately 8 to 45 fold higher than among the species (0.091% to 0.219%). As observed in this research, the minimum (98%) and maximum (1.019%) values of genetic identity and distance, respectively, were reliably to determine the species status of the striped snakehead samples from Cingcingguling Rivers. The present result is consistent with previous research carried out by Aquilino et al. (2011) and Irmawati et al. (2017) that DNA barcoding is a powerful technique for species-level identification of snakehead fish.

Furthermore, the K2P phylogenetic tree was reconstructed by considering neighbor-joining and maximum likelihood (Figure 2). Both algorithms produced a similar topology and were supported by high bootstrap values (ML=100; NJ=100). *C. striata* samples formed a monophyletic clade with their conspecific reference (Figure 2). According to Xu et al. (2015) and Kusbiyanto et al. (2021), monophyly is also reliable data for species determination. Figure 2 shows that the striped snakehead samples and their conspecific were had a smaller branch scale than the predetermined scale of 0.02. This information strongly indicates that the samples belong to the same species as their closest related taxa (*C. striata*). Monophyly of *C. striata* was also detected between the natural and cultivated population in Vietnam (Nguyen & Duong 2015).

This research also indicates that the CO1 gene is a reliable marker for species identification. Its reliability serves as a barcode because ~~of~~ this varies among species due to its high mutation rate (Sachithanandam et al., 2012). Due to its variability, the CO1 gene is a suitable marker for unambiguous species identification (Balkhis et al., 2011; Winarni et al., 2021). This is congruent with previous research in several locations in Indonesia (Muchlisin et al. 2013; Irmawati et al. 2017; Pramono et al. 2017) and other countries (Aquilino et al. al. 2011; Triantafyllidis et al. 2011), including Lake Greece.

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134
135 **Figure 2.** Phylogenetic tree showing monophyly between samples and their conspecific references. left: ML value, right: NJ value
136

Historical demography and genetic diversity

Overall, Tajima's D value was -2.564~~;~~, meanwhile, this significant result proved that the neutral hypothesis of marker evolution was rejected, thereby leading to ~~the occurrence of~~ selection pressure. However, the negative sign rejected the assumption on selection pressure and indicated a recent population bottleneck (Tajima1989; Jong et al. 2011). The negative signs and insignificant Fus' Fs supported the neutral marker and population bottleneck assumption, as shown in Table 2. According to Jong et al. (2011) and Mohammed et al. (2021), Tajimas' D and Fus' Fs values are calculated based on haplotype and nucleotide variations, respectively. This simply signifies that Fus' Fs values are more sensitive than Tajimas' D in terms of using it for neutral theory testing of the marker.

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Table 2. Population, number of individuals (N), number of haplotypes (nhp), haplotype diversity (h), nucleotide diversity (μ), Tajima'D, and Fu's Fs

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Population	N	Genetic Diversity			Neutrality Test			
		nhp	h (x±SD?)	π (x±SD?%)	D	P	Fs	P
Overall	53	6	0.181±0.071	0.108±0.095	-2.564***	0.000	-2.360ns	0.070
SR	27	4	0.214±0.103	4.195±3.617	-2.226***	0.001	-0.913ns	0.220
KS	10	3	0.378±0.181	14.568±9.702	-1.901ns	0.006	1.726ns	0.831
PW	2	1	0.000±0.000	0.000±0.000	0.000ns	1.000	-	-
KB	10	1	0.000±0.000	0.000±0.000	0.000	1.000	-	-
BA	4	1	0.000±0.000	0.000±0.000	0.000	1.000	-	-

p> 0.05 = ns, 0.05>p>0.01= significant, p<0.01= highly significant, ns= non-significant, ***= highly significant

This research analyzed a 593 bp COI gene fragment length of 53 individual *C. striata* collected from eight sampling sites. Furthermore, it was reported that 17 out of 593 bp were polymorphic, resulting in 6 haplotypes. Overall haplotype and nucleotide diversities were 0.181 ± 0.071, and 0.108% ± 0.095%, respectively. This indicates that *C. striata* populations in the Cingcingguling River have low genetic diversity, and this is due to 2 reasons. First, it is caused by small population sizes due to the recent bottleneck. Besides, this has been proven by negative and positive insignificant Tajimas' D and Fus' Fs values, respectively, as shown in Table 2. According to Zanella et al. (2016) and Doublet et al. (2019), a small population shows low genetic diversity due to inbreeding depression. Second, it is caused by limited ancestors, and this was proven by the haplotype network, which showed that the *C. striata* population in the Cingcingguling River had evolved from a common ancestor, as indicated in Figure 3. It was previously stated that limited maternal ancestors lead to the low genetic diversity of the offspring population because of the drift effect (Zanella et al., 2016). Besides, this attribute in *C. striata* populations was also observed in Malaysia (Jamaluddin et al., 2011).

The present result is inconsistent with the previous research carried out in India (Baisvar et al. 2018), stating that *C. striata* populations exhibited a complex pattern of genetic diversity~~.~~; ~~H~~however, this implies that it is a common phenomenon. Meanwhile, several fish species have reported high and low haplotype genetic diversity (Sukmanomon et al., 2012; Song et al., 2013; Barasa et al., 2014; Nuryanto et al., 2020). This indicates that environmental factors have exhibited different evolutionary forces on their populations, which needs further analysis.

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The within-population evaluation indicates that the haplotype diversity of the *C. striata* population in the Cingcingguling River ranges from 0.000±0.000 to 0.378±0.181. The values prove that the striped snakehead population had low genetic diversity. The majority of the populations underneath the reservoir (PW, KB, and BA) are genetically homogenous. Moreover, two subpopulations (KA and KP) had low genetic diversity. This indicates that river subpopulations show a complex genetic diversity pattern. The obtained values were lower than the previously reported results (Boonkusol & Tongbai 2016; Baisvar et al. 2018; 2019). The exploitation of *C. Striata* causes low genetic diversity, as indicated by the bottleneck effect shown by Tajimas' D and Fus Fs values in Table 2, which caused minor population size and an opportunity for inbreeding to occur. According to Hauser et al. (2002) and Tan et al. (2012), fishing pressure reduces genetic diversity in fish species. Meanwhile, low genetic diversity caused by exploitation also occurs in various aquatic organisms and several regions (Wibowo 2012; Tan et al. 2015; Barasa et al. 2014; Baisvar. et al. 2019; Kochzius & Nuryanto 2008).

Table 2 shows that the nucleotide diversity ranges from 0.00±0.000% to 14.568±9.703%, and these values indicate that *C. striata* in the Cingcingguling River have both low and high nucleotide diversities. According to Kochzius & Nuryanto (2008) and Nuryanto et al. (2019), when this attribute is greater than 1%, it is ~~regarded as~~ highly diversc~~ity~~. Moreover, high nucleotide diversity was detected in reservoir populations of fish species in Victoria Lake (Barasa et al., 2014).

Population connectivity

~~In accordance with~~Per the reservoir population, there was no difference in the genetic analysis of the four~~4~~ subpopulations. Therefore, this research focused on differentiating the reservoir and river populations. The amova results proved that genetic variations of -4.27% were mainly observed within the population (104.27%, Table 3)~~, as shown in Table 3~~. It was assumed that no genetic differences occurred between reservoir and river populations along the Cingcingguling River, and this was supported by a negative fixation index (-0.043) and p-values of 0.115. The data proved

that *C. striata* populations in the Cingcingguling River formed a genetically continuous population. An interesting finding was that the Sempor Reservoir did not lead to fragmentation, as proven by the genetic similarities among the river populations. The phenomenon is related to the ecological characteristics of striped snakehead as negative rheotaxis fish, which ~~are more~~ prefer stagnant water ecosystems. The alteration of running water into a static ecosystem due to the presence of Sempor Reservoir did not significantly affect the genetics of *C. striata* both inside and outside. According to Froese and Pauly (2021), this species primarily lives in a swampy ecosystem with stagnant water.

Table 3. Variance and Fst analysis among *C. striata* subpopulation

Source of variation	d.f.	Sum of square	Variance components	Percentage of variation	Fixation index (FST)	p-Value
Among subpopulations	4	0.239	-0.004 Va^{ns}	-4.27	-0.043^{ns}	0.115±0.003
Within subpopulations	48	4.478	0.093 Vb	104.27		
Total	52	4.717	0.089			

p> 0.05 = non-significant (ns), 0.05>p>0.01= significant, p<0.01= highly significant.

This result is inconsistent with ~~a~~ previous research carried out by Song et al. (2013), that significant genetic structure was found among the *C. striata* population in Malaysia. However, this is due to differences in the research locations. This research examined *C. striata* in only one~~1~~ river but fragmented by the reservoir. In contrast, Song et al. (2013) researched different Malaysian river systems. It was previously reported that the river is a closed ecosystem and the freshwater populations tend to show significant genetic differences (Hughes 2009). Conversely, Kano et al. (2011) stated ~~that there are~~ solid genetic structures without physical barriers among tributaries within a river system. However, the different research locations caused an imbalance comparison between the present research and that carried out by Kano et al. (2011).

These findings were also inconsistent with the research on *Barbonymus balleroides* in the Serayu River conducted by Bahiyah et al. (2013). According to this research, the significant genetic structures between the reservoir and river population in the Serayu were observed. However, this research evaluated the population genetics of positive rheotaxis species (*B. balleroides*) whose primary habitat is running water. Therefore, the presence of reservoirs in Serayu River altered the habitat of *B. balleroides* from running to stagnant water, which became an evolutionary factor causing genetic changes in its reservoir populations. Therefore, a significant genetic structure was observed in between *B. balleroides* population inside and outside the reservoir. Furthermore, barriers, such as reservoirs, tend to cause genetic differences among the river populations (Tan et al., 2012; Adamson et al., 2012; Barasa et al., 2014). In contrast, this study observed that the Sempor Reservoir did not cause genetic fragmentation of *C. striata* because it did not alter its habitat.

A detailed analysis of the within-population showed that the inner part of the reservoir and below the Sempor River subpopulations showed higher genetic variability than that in the downstream~~s~~ (Table 2). This indicates that the upper stream subpopulations evolve faster than the lowland river regions. However, due to the age of the reservoir, which is approximately 51 years old (Hedianto et al. 2014), higher genetic variability in the upper stream subpopulations did not cause significant genetic differentiation. Similar phenomena were reported in *Chilata lopis* (Wibowo et al. 2012) and African catfish (Barasa et al. 2014).

Meanwhile, 6 COI haplotypes were observed in *the Channa striata* populations ~~found~~ in the Cingcingguling River Central Java, Indonesia. Median-joining analysis proved that haplotype 1 was dominant and found in all subpopulations~~,~~ ~~as shown in~~ (Figure 3). The phenomenon strengthens the result of AMOVA that genetic homogeneity occurred along the Cingcingciguling River, and the reservoir did not cause genetic fragmentation in the *C. striata* population.

Commented [MOU13]: Typically?

Commented [MOU14]: Please clarify about Song et al (2013). Put in the correct context of different Malaysian river systems with previously reported that the river is a closed ecosystem. It reads confusing

Commented [MOU15]: Explain comparison in what aspect?

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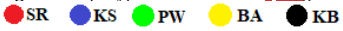
Figure 3. Haplotype network indicates the genetic homogeneity of C. striata in the Cingcingguling River.



The sStar-like haplotype network in (Figure 3) showed that *C. striata* populations in the Cingcingguling River evolved from a single maternal ancestor (H1). The network proved that H1 is the most primitive haplotype, which is characterized by high abundance and wide distribution in most populations. Similar phenomena were reported in preliminary research on the *Channa* in several regions (Balkhis et al. 2011; Song et al. 2012; Adamson et al. 2012; Basvar et al. 2018; 2019) and other fish groups (Barasa et al. 2014; Abila et al. 2004).

The snakehead fish that lives in the Cingcingguling River was genetically identified as *Channa striata* and had low genetic diversity. Also, there were no genetic differences between the reservoir and river populations, which simply means that *C. striata* formed a genetically homogenous population. This indicates that *C. striata* needs to be treated as a single genetic conservation unit.

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REFERENCES

Abila R, Barluenga M, Engelken J, Meyer A, Salzburger W. 2004. Population structure and genetic diversity in a haplochromine fish cichlid of a satellite lake of Lake Victoria. Molecular Ecology 13(9): 2589-2602.

Adamson EAS, Hurwood DA, Mather PB. 2012. Insights into historical drainage evolution based on the phylogeography of the chevron snakehead fish (*Channa striata*) in the Mekong Basin. Freshwater Biology https://doi.org/10.1111/j.1365-2427.2012.02864.x

Amilhat E, Lorenzen K. 2005. Habitat use, migration pattern and population dynamics of chevron snakehead Channa striata in a rainfed rice farming landscape. Journal of Fish Biology 67(SUPPL. B): 23–34. https://doi.org/10.1111/j.0022-1112.2005.00927.x

Aquilino SVL, Tango JM, Fontanilla IKC, Pagulayan RC, Basiao ZU, Ong PS, Quilang JP. 2011. DNA barcoding of the ichthyofauna of Taal Lake, Philippines. Molecular Ecology Resources 11(4): 612–619. https://doi.org/10.1111/j.1755-0998.2011.03000.x

Arma NR, Illijas MA, Irmawati MA. 2014) Morphometric and meristic characteristics of snakehead fish for hatchery production. Proceeding of the 3rd International and National Seminar on Fisheries and Marine Science: Strengthening Science and Technology towards the Development of Blue Economy, Faculty of Fisheries and Marine Science University of Riau, Riau, (October), 198–201.

Astuti P. 2018. Dampak sosial ekonomi pembangunan waduk sempor bagi masyarakat kebumen tahun 1958-1978. Jurnal Prodi Ilmu Sejarah 3(5): 1–15.

Bahiyah, Solihin DD, Affandi R. 2013. Variasi genetik populasi ikan brek (*Barbonymus balleroides* Val. 1842) sebagai dampak fragmentasi habitat di Sungai Serayu. Jurnal Iktiologi Indonesia 13(2): 175–186.

Baisvar VS, Kumar R, Singh M, Kushwaha B. 2019. Cytochrome c oxidase i gene-based genetic divergence and molecular phylogeny among the species of fish Genus *Channa*. Proceedings of the National Academy of Sciences India Section B - Biological Sciences 89(4): 1455–1463. https://doi.org/10.1007/s40011-018-01070-w

Baisvar VS, Singh M, Kumar R. 2018. Population structuring of *Channa striata* from Indian waters using control region of mtDNA. Mitochondrial DNA Part A 30(3): 414–423. https://doi.org/10.1080/24701394.2018.1532416

Balkhis ABS, Firdaus A, Jamsari J, Hwai TS, Yasin Z. 2011. Evidence of geographical structuring in the Malaysian Snakehead, *Channa striata* based on a partial segment of the CO1 gene. Genetics and Molecular Biology 34(3): 520-523.

Bandelt HJ, Foster P, Rohl A. 1999. Median-joining networks for inferring intraspecific phylogenies. Molecular Biology and Evolution 16(1): 37-48.

Barasa JE, Abila R, Grobler JP, Dangasuk OG, Njahira MN. 2014. Genetic diversity and gene flow in *Clarias gariepinus* from Lakes Victoria and Kanyaboli, Kenya. African Journal of Aquatic Science 39(3): 287-293. DOI: 10.2989/16085914.2014.933734

Boonkusol D, Tongbai W. 2016. Genetic variation of striped snakehead fish (Channa striata) in the river basin of central Thailand inferred from mtDNA COI gene sequences analysis. Journal of Biological Sciences 16(1): 37–43. https://doi.org/10.3923/jbs.2016.37.43

Candek K, Kuntner M. 2015. DNA barcoding gap: Reliable species identification over morphological and geographical scales. Molecular Ecology Resources 15 (2): 268-277.

Cia WOC, Asriyana, Halili. 2018. Mortality and exploitation rate of striped snakehead (*Channa striata*) in Aopa Watumohai Swamp , District of Anga. Jurnal Manajemen Sumber Daya Perairan 3(3): 223–231. [Indonesia]

Dahruddin H, Hutama A, Busson F, Sauri S, Hanner R, Keith P, Hadiaty R, Hubert N. 2016. Revisiting the ichthyodiversity of Java and Bali through DNA barcodes: taxonomic coverage, identification accuracy, cryptic diversity and identification of exotic species. Molecular Ecology Resources DOI: 10.1111/1755-0998.12528

Doublet AC, Croiseau P, Fritz S, Michenet A, Hoze C, Danchin-Burge C, Laloe D, Restoux G. 2019. The impact of genomic selection on genetic diversity and genetic gain in three French dairy cattle breeds. Genetics Selection Evolution 51: 52 (13pp). https://doi.org/10.1186/s12711-019-0495-1

Estradivari, Handayani CNN, Daniel D, Mustofa A. 2017. Marine protected area network design: A case study of Maluku Province. Coastal and Ocean Journal 1(2): 135-146.

Excoffier L, Lischer HEL. 2010. Arlequin suite ver 3.5: A new series of programs to perform population genetics analyses under Linux and Windows. Molecular Ecology Resources 10(3): 564–567. https://doi.org/10.1111/j.1755-0998.2010.02847.x

Fahmi Z, Nurdawati S, Supriyadi F. 2013. Growth and exploitation status (*Channa striata* Bloch, 1793) in Lubuk Lampam Floodplains, South Sumatera. Ind. Fish. Res. J. 19(1): 1–7.

Froese R, Pauly D. (eds). 2021. Fishbase. World Wide Web Electronic Publication. www.fishbase.org, version (06/2021)

Hall T. 2011. BioEdit: An important software for molecular biology. GERF Bulletin of Biosciences 2(1): 60–61. https://doi.org/10.1017/S0317167100012865

Hanif MA, Siddik MAB, Chaklader MR, Nahar A, Mahmud S. 2015. Raznolikost ihtiofaune južnih obalnih voda bangladeša: Postojeće stanje, prijetnje i mogućnosti očuvanja. Ribarstvo, Croatian Journal of Fisheries, 73(4), 148–161. https://doi.org/10.14798/73.4.848

Hauser L, Adcock GJ, Smith PJ, Ramírez JHB, Carvalho GR. 2002. Loss of microsatellite diversity and low effective population size in an overexploited population of New Zealand snapper (*Pagrus auratus*). Proceedings of the National Academy of Sciences of the United States of America, 99(18), 11742–11747. https://doi.org/10.1073/pnas.172242899

Hedianto DA, Purnomo K, Kartamihardja ES, Warsa A. 2014. Population parameters of three spot cichlid (*Cichlasoma trimaculatum*, Günther 1867) in Sempor Reservoir, Central Java. Jurnal Penelitian Perikanan Indonesia 20(2): 81-88. [Indonesia]

Heggenes J, Røed KH. 2006. Do dams increase genetic diversity in brown trout (Salmo trutta)? Microgeographic differentiation in a fragmented river. Ecology of Freshwater Fish 15(4): 366–375. https://doi.org/10.1111/j.1600-0633.2006.00146.x

Higashi R, Tsukagoshi A, Kimura H, Kato K. 2011. Male dimorphism in a new interstitial species of the genus *Microloxoconcha* (Podocopida: Ostracoda). Journal of Crustacean Biology 31(1): 142-152.

Hughes JM, Schmidt DJ, Finn DS. 2009. Genes in streams: using DNA to understand the movement of freshwater fauna and their riverine habitat. BioScience 59: 573-583.

Hubert N, Delieu-Trottin E, Irisson JO, Meyer C, Planes S. 2010. Identifying coral reef fish larvae through DNA barcoding: A test case with the families Acanthuridae and Holocentridae. Molecular Phylogenetics and Evolution 55: 1195-1203.

Irmawati Tresnati J, Nadiarti, Fachruddin L, Arma NR. 2017. Identification of wild stock and the first generation (F1) of domesticated snakehead fish , Chann. Jurnal Iktiologi Indonesia 17(2): 165–173. [Indonesia]

Iskandar J, Dahiyat Y. 2012. Keanekaragaman ikan di Sungai Siak Riau. Bionatura 14(1): 51–58.

Jamaluddin JAF, Pau TM, Siti-Azizah MN. 2011. Genetic structure of the snakehead murrel, Channa striata (Channidae) based on the cytochrome c oxidase subunit I gene: Influence of historical and geomorphological factors. Genetics and Molecular Biology 34(1): 152–160. https://doi.org/10.1590/S1415-47572011000100026

Jeffery NW, Elias-Guttierrez M, Adamowicz SJ. 2011. Species diversity and phylogeographical affinities of the Branchiopoda (Crustacea) of Churchill, Manitoba, Canada. PLoS ONE 6 (5): e18364.

Joadder MAR, Galib SM, Haque SMM, Chaki N. (2015). Fishes of the river Padma, Bangladesh: Current trend and conservation status. Journal of Fisheries 3(2): 259. https://doi.org/10.17017/jfish.v3i2.2015.111

Jong MAde, Wahlberg N, van Eijk M, Brakefield PM, Zwaan BJ. 2011. Mitochondrial DNA Signature for Range-Wide Populations of Bicyclus anynana Suggests a Rapid Expansion from Recent Refugia. PLoS ONE 6(6): e21385. doi:10.1371/journal.pone.0021385

Kano Y, Vokoun JC, Letcher BH. 2011. Fine-scale population structure and riverscape genetics of brook trout (Salvelinus fontinalis) distributed continuously along headwater channel networks. Molecular Ecology 20: 3711-3729.

Khan AM, Khan S, Miyan K. 2019. Stock identification of the Channa striata Inhabiting the Gangetic River System using Truss Morphometry. Russian Journal of Ecology 50(4): 391–396. https://doi.org/10.1134/S1067413619040106

Ko HL, Wang YT, Chiu TS, Lee MA, Leu MY, Chang KZ … Shao KT. 2013. Evaluating the Accuracy of Morphological Identification of Larval Fishes by Applying DNA Barcoding. PLoS ONE 8(1): 3–9. https://doi.org/10.1371/journal.pone.0053451

Kochzius M, Nuryanto A. 2008. Strong genetic population structure in the boring giant clam, Tridacna crocea, across the Indo-Malay Archipelago : implications related to evolutionary processes and connectivity. Molecular Ecology 17: 3775–3787. https://doi.org/10.1111/j.1365-294X.2008.03803.x

Kumar S, Stecher G, Li M, Knyaz C, Tamura K. 2018. MEGA X: Molecular evolutionary genetics analysis across computing platforms. Molecular Biology and Evolution 35(6): 1547–1549. https://doi.org/10.1093/molbev/msy096

Kusbiyanto, Bhagawati D, Nuryanto A. 2020. DNA barcoding of crustacean larvae in Segara Anakan, Cilacap, Central Java, Indonesia using cytochrome c oxidase gene. Biodiversitas 21(10): 4878-4887.

Listyanto N, Andriyanto S. 2009. Ikan Gabus (Channa striata) Manfaat Pengembangan Dan Alternatif Teknik Budidayanya. Media Akuakultur 4(1): 18–25.

Mohammed MA, Nuryanto A, Kusmintarsih ES. 2021. Genetic differentiation of dengue vector Aedes aegypti in the small geographical scale of Banyumas District, Indonesia based on Cytochrome Oxidase I. Biodiversitas 22(2): 675-683

Muchlisin ZA, Thomy Z, Fadli N, Sarong MA, Siti-Azizah MN. 2013. DNA barcoding of freshwater fishes from Lake Laut Tawar, Aceh Province, Indonesia. Acta Ichthyologica et Piscatoria 43(1): 21–29. https://doi.org/10.3750/AIP2013.43.1.04

Muslimin B, Rustadi R, Hardaningsih H, Retnoaji B. 2020. Morphometric variation of cork fish (Channa striata Bloch, 1793) from nine populations in Sumatra island, Indonesia. Iranian Journal of Ichthyology 7(3): 209–221. https://doi.org/10.22034/iji.v7i3.480

Nguyen, T., & Duong, Y. (2015). Morphological and genetic differences between cultured and wild populations of Channa striata in Viet Nam and its phylogenetic relationship with other Channa species. Songklanakarin Journal of Science and Technology SJST, 0147.R3.

Muflikhah N. 2007. Domestikasi ikan gabus (Channa striata). Bawal 1(5): 169–175. [Indonesia]

Nurdawati S, Fahmi Z, Supriyadi F. 2019. Parameter Populasi Ikan Betok (Anabas testudineus Bloch, 1792) Di Ekosistem Paparan Banjir Sungai Musi, Lubuk Lapam. Berita Biologi Jurnal Ilmu-Ilmu Hayati 18(1): 25–35.

Nuryanto A, Kusbiyanto K, Bhagawati D. 2021. Molecular barcoding of marine ornamental fish from the southern coast of West Java validates conventional identification. E3S Web of Conferences 322: 01004. https://doi.org/10.1051/e3sconf/202132201004
Nuryanto A, Baghawati D, Husein MOH, Rachmawati FNUR. 2020. Molecular Characterization of Anguilla from Cibereum and Sapuregel Rivers Segara Anakan Watersheds Cilacap, Central Java, 8(2): 145–156.
Nuryanto A, Komalawati N, Sugiharto. 2019. Genetic diversity assessment of Hemibagrus nemurus from rivers in Java Island, Indonesia using COI gene. Biodiversitas 20(9): 2707–2717. https://doi.org/10.13057/biodiv/d200936
Nuryanto A, Pramono H, Sastranegara MH. 2017. Molecular identification of fish larvae from East Plawangan of Segara Anakan, Cilacap, Central Java, Indonesia. Biosaintifika 9(1): 33-40.
Nuryanto A, Bhagawati D, Abulias MA, Indarmawan. 2015. Fauna ikan di Sungai Cikawung Kabupaten Cilacap Jawa Tengah. Jurnal Iktiologi Indonesia 15(1): 25-37. [Indonesia]
Nuryanto A, Bhagawati D, Abulias MA, Indarmawan. 2012. Fish diversity at Cileumeuh River in District of Majenang, Cilacap Regency, Central Java. Jurnal Iktiologi Indonesia 12(2): 147-153.
Pavlova A, Beheregaray LB, Coleman R, Gilligan D, Harrisson KA, Ingram BA, Kearns J, Lamb AM, Lintermans M, Lyon J, Nguyen TTT, Sasai M, Tonkin Z, Yen JDL, Sunnucks P. 2017. Severe consequences of habitat fragmentation on genetic diversity of an endangered Australian freshwater fish: A call for assisted gene flow. Evolutionary Applications 10: 531-550
Pramono TB, Arfiati D, Widodo MS, Yanuhar U. 2017. Identifikasi Ikan Genus Mystus Dengan. Jurnal Sumberdaya Akuatik Indopasifik 1(2): 123–132.
Firlianty, Pratasik SB. 2018. The Potential *of Channa micropeltes* and *C. striata* pudding for healing acceleration of the white rat. Jurnal Agribisnis Perikanan, 11(2), 65–69. https://doi.org/10.29239/j.agrikan.11.2.65-69. [Indonesia]
Ratnasingham S, Hebert PDN. 2013 A DNA-Based Registry for All Animal Species: The Barcode Index Number (BIN) System. PLoS ONE 8(8): e66213. doi:10.1371/journal.pone.0066213
Ratnasingham R, Hebert PDN. 2007. BOLD: The Barcode of life data system (www.barcodinglife.org). Molecular Ecology Notes 7: 355-364.
Roesma DI. 2013. Evaluasi Keanekaragaman Spesies Ikan Danau Maninjau. Prosiding Semirata FMIPA Universitas Lampung, 197–204.
Rozas J, Ferrer-Mata A, Sanchez-DelBarrio JC, Guirao-Rico S, Librado P, Ramos-Osins SE, Sanchez-Gracia A. 2017. DnaSP 6: DNA sequence polymorphism analysis of large data sets. Mol Biol Evol 34 (12): 3299-3302. DOI: 10.1093/molbev/msx248.
Sachithanandam V, Mohan PM, Muruganandam N, Chaaithanya IK, Dhivya P, Baskaaran R. 2012. DNA barcoding, phylogenetic study of *Epinephelus* spp. from Andaman coastal region, India. Indian Journal of Geo-Marine Sciences 41(4): 203-211.
Setyaningrum N, Sugiharto S, Susatyo P. 2020. Species richness and status guilds of fish in Sempor Reservoir Central Java. Depok 9(3): 411-420. [Indonesia]
Sofarini D, Mahmudi M, Hertika AMS, Herawati EY. 2018. Population dynamic of snakehead fish (*Channa striata*) at Danau Panggang Swamp, South Kalimantan. EnviroScienteae 14(1): 16-20.
Song LM, Munian K, Rashid ZA, Bhassu S. 2013. Characterisation of Asian snakehead murrel *Channa striata* (Channidae) in Malaysia: An insight into molecular data and morphological approach. The Scientific World Journal: 917506. https://doi.org/10.1155/2013/917506
Sukmanomon S, Senanan W, Kapuscinski AR. 2012. Genetic diversity of feral populations of Nile Tilapia (*Oreochromis niloticus*) in Thailand and evidence of genetic introgression. Kasetsart Journal Natural Sciences 46(2): 200–216.
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(3): 1227-1235.
Tajima F. 1989. Statistical method for testing then mutation hypothesis by DNA polymorphism. Genetics 123: 585-595.
Tan MP, Jamsari AFJ, Muchlisin ZA, Siti-Azizah MN. 2015. Mitochondrial genetic variation and population structure of the striped snakehead, *Channa striata* in Malaysia and Sumatra, Indonesia. Biochemical Systematics and Ecology 60: 99-105. https://doi.org/10.1016/j.bse.2015.04.006
Tan MP, Jamsari AFJ, Siti-Azizah MN. 2012. Phylogeographic pattern of the striped snakehead, *Channa striata* in Sundaland : Ancient river connectivity, geographical, and anthropogenic signatures. PLoS ONE 7(12): e52089. https://doi.org/10.1371/journal.pone.0052089
Triantafyllidis A, Bobori D, Koliamitra C, Gbandi E, Mpanti M, Petriki O, Karaiskou N. 2011. DNA barcoding analysis of fish species diversity in four north Greek lakes. Mitochondrial DNA 22(SUPPL. 1): 37–42. https://doi.org/10.3109/19401736.2010.542242
Ward RD, Zemlak TS, Innes BH, Last PR, Hebert PDN. 2005. DNA barcoding Australia's fish species. Philosophical Transactions of the Royal Society B: Biological Sciences 360(1462): 1847–1857. https://doi.org/10.1098/rstb.2005.1716
Wibowo A. 2012a. Struktur genetik populasi ikan belida *(Chitala lopis*, Bleeker 1851) di Waduk Kutopanjang. Bawal 4(308): 53–58.
Wibowo A. 2012b. Genetic diversity of masheer *Tor tambroides* Bleeker 1854) in Manna River Bengkulu and Semangka River Lampung. Bawal 4(2): 105–112. [Indonesia]
Winarni ET, Kusbiyanto, Nuryanto A. 2021 Estimating Crustacean Species Utilize Segara Anakan Estuary Cilacap, Indonesia as Nursery Ground through DNA Barcoding. Journal of Hunan University (Natural Sciences) 48(10): 275-282.
Xu X, Liu F, Chen J, Li D, Kuntner M. 2015. Integrative taxonomy of the primitively segmented spider genus Ganthela (Araneae: Mesothelae: Liphistiidae): DNA barcoding gap agrees with morphology. Zool J Linnean Soc 175(2): 288-306. DOI: 10.1111/zoj.12280.
Yudha IGNAP, Arya IW, Suryani SAMP. 2018. Studi identifikasi keragaman jenis, feeding habit dan food habit ikan demersal pada bagian hilir sungai yeh sungi, Tabanan, Bali. Gema Agro 23(2): 114-123.
Zanella R, Peixoto JO, Cardoso FF, Cardoso LL, Biegelmeyer P, Cantao ME, Otaviano A, Freitas MS, Caetano AR, Ledur MC. 2016. Genetic diversity analysis of two commercial breeds of pigs using genomic and pedigree data. Genetics Selection and Evolution 48: 24.
Zhu SR, Fu JJ, Wang Q, Li JL. 2013. Identification of Channa species using the partial cytochrome c oxidase subunit I (COI) gene as a DNA barcoding marker. Biochemical Systematics and Ecology 51: 117–122. https://doi.org/10.1016/j.bse.2013.08.011

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Genetically continuous populations of Striped Snakehead (*Channa striata*) in the Cingcingguling River fragmented by Sempor Reservoir, Central Java, Indonesia

Abstract. Cingcingguling River, located in Kebumen Regency Central Java, Indonesia. The Sempor Reservoir fragments it. The previous study proved the negative impact of the reservoir on positive rheotaxis fish, mainly in genetic constituents between the reservoir and river populations. However, research has not been conducted on the negative rheotaxis fish, such as *Channa striata*. Assessing population genetic and taxonomic validity study of Striped Snakehead in the Cingcingguling River is an essential effort. Both studies could be done using the cytochrome c oxidase 1 gene. Therefore, this research aims to determine taxonomic status and evaluate the population genetic of C. striata in the Cingcingguling River. The samples were collected at eight sites inside and outside the reservoir. The used marker was sequenced from 53 individuals, and all specimens showed high (98.67% to 100%) and low genetic distances (0.00 to 0.01) to *C. striata* (KU692421, KU852443, and MG438366). Those values proved that all samples were genetically identified as *Channa striata*. The vertical genetic distribution analysis demonstrated that *C. striata* populations are genetically not different along the river. Unlike rheotaxis positive fish phenomena, the reservoir's existence does not cause genetic fragmentation and leads to continuous striped snakehead populations.

Keywords: genetic diversity, reservoir, rheotaxis, striped snakehead

Abbreviations (if any): AMOVA= analysis of molecular variance, DRPM BRIN = Direktorat Riset dan Pengabdian Kepada Masyarakat, Badan Riset dan Inovasi Nasional

Running title: Genetically continuous populations of *Channa striata*

INTRODUCTION

Striped snakehead (*Channa striata*) is an important freshwater fish species in several Asian countries. In Indonesia, it is mainly found in the main islands of the Sunda Shelf, including Sumatra, Java, and Borneo (Adamson et al. 2010; Lakra et al. 2010; Bezinger et al. 2011; Coad et al. 2016). Currently, this species is also found in the lesser Sunda Island, such as Bali (Yudha et al. 2018), and introduced to the Wallacea Regions (Irmawati et al., 2017). *C. striata* is primarily discovered in stagnant or swampy water ecosystems (Amilhat and Lorenzen 2005; Muflikhah 2007; Listyanto and Andriyanto 2009). This species was also found both inside and outside Sempor Reservoir in the Cingcingguling River, Central Java, Indonesia (Setyaningrum et al. 2020, 2021).

Aquatic organisms are able to move in response to water currents, known as rheotaxis (Baker and Montgomery 1999; Kanter and Coombs 2006; Enders et al. 2009). Fish that actively swim against the water current is referred to as positive rheotaxis fish (Suli et al. 2012; Back-Coleman et al. 2015; Oteiza et al. 2017). In the case of *C. striata*, previous studies had reported that *C. striata* also lives in the river, but it could only be found in the parts of the river with stagnant water, river flood plain, and reservoir. It seems that *C. striata* tended to avoid water current (Iskandar and Dahiyat 2012; Nuryanto et al. 2012; Roesma 2013; Nuryanto et al. 2015). Therefore, *C. striata* could be grouped into negative rheotaxis fish. Fish species that tend to avoid water current are negative rheotaxis fish (Enders et al. 2009; Febrina 2016).

Sempor Reservoir was built approximately 51 years ago and has caused the Cingcingguling River to be fragmented into two extremely different habitats. These include entirely stagnant and running water bodies located underneath the reservoir (Hedianto et al. 2014). The reservoir is a physical barrier for gene flow and causes significant genetic differences among its populations (Heggenes and Roed 2006). However, the available data concerning the reservoir's negative impact on river populations was only available for the positive rheotaxis fish species (Wibowo et al. 2012; Bahiyah et al. 2013; Barasa et al. 2014; Plavova et al. 2017). Meanwhile, there is no recorded information about the reservoir's genetic effect on

negative rheotaxis fish species. Therefore, it is essential to research the genetic impact of Sempor Reservoir on the *C. striata* population in the Cingcingguling River.

The genetic impact of a reservoir on the fish population could be assessed with a molecular tool, such as the cytochrome c oxidase 1 (COI) gene (Nuryanto et al. 2019). Previous research reported that it was used as a robust marker for population genetic analysis of *C. striata* in Perak State situated in Malaysia (Jammaluddin et al. 2011, Tan et al. 2012, 2015). Nevertheless, population genetic research tends to be carried out when the taxonomic status of the analyzed organisms is valid. In the case of *C. striata*, it was reported that the morphological identification of samples obtained from different regions showed inconsistent diagnostic characters (Zhu et al. 2013; Arma et al. 2014; Khan et al. 2019; Muslimin et al. 2020). Taxonomic validity could also be determined using the cytochrome c oxidase 1 (COI) gene (Ko et al. 2013; Nuryanto et al. 2017; 2019; 2020). Furthermore, it was reliable for species delineation of *C. striata* from Sumatra (Muchlisin et al. 2013; Dahruddin et al. 2016; Irmawati et al. 2017; Syaifudin et al. 2020) and is used to validate morphological identification (Nuryanto et al. 2021). Therefore, this research was aimed to validate and assess the taxonomic status and genetic population of *C. striata* in Sempor Reservoir Central Java using cytochrome c oxidase 1 gene.

MATERIALS AND METHODS

Research location and sampling sites

Striped snakehead specimens were collected from eight different sites, four of them were situated inside the reservoir, while the remaining were located downstream (Figure 1). These were collected using traps and lines with the help of fishers. Tinny tissue samples were chopped from the pectoral fin of each specimen and preserved in ethanol 96%.

Figure 1. Research location with five sampling sites along the river. Four subsampling sites are located inside the reservoir.

Procedures

Genomic DNA extraction and Marker polymerization

The total genome was extracted from the pectoral fin tissue using the Quick-DNA™ Miniprep Plus kit adopted from Zymo's research. Extraction procedures were carried out based on the company's manual, and its success was tested using 1% agarose electrophoresis. Subsequently, the COI gene target fragments were reproduced using FishF2 and FishR2 primers (Ward et al. 2005) in Primus 25 Peqlab Thermocycler. Meanwhile, 50 µl of amplified reactions consisted of 1x buffer PCR, 2 mM MgCl2, 0.2 mM of each primer, 0.2 mM dNTP mix, 1 U Taq polymerase, and 2.0 ng / µl template DNA. Furthermore, the final volume of 50 µl was adjusted by adding DNA-RNA-free water. Thermal cycles were pre-

denatured at 95°C for 4 minutes and were repeated 35 times. The denaturation steps lasted for 30 seconds at 95°C, 2 minutes at 53°C, and 1 minute at 72°C for primer annealing and chain elongation. Additionally, a final extension terminated the cycles after 5 minutes, at 72°C. The PCR products were stained using ethidium bromide and 1.5% agarose gel and placed under ultraviolet light. Gel documentation was further performed using the GelDoc apparatus (BioRad).

Marker sequencing and editing

The PCR products of the marker were shipped to 1st BASE Malaysia for sequencing, while that the sequencing process was performed using the Sanger method. Consensus and multiple sequences alignment were obtained by assembling the forward and reverse sequences using ClustalW ver.1.4 in Bioedit (Hall 2011). Haplotype data was obtained from its generating process in DnaSP 5 (Rozas et al., 2017).

Data analysis

The striped snakehead specimens' taxonomic status was validated through a sequence identity test carried out using a basic local alignment search tool (BLAST) closest to the taxa in GenBank. Genetic distance was also used to support the identity data. Haplotypes (h) and nucleotide (π) diversities were calculated using Arlequin 3.5, while neutral evolution of the COI marker was estimated using Fu's Fs and Tajima D test (Excoffier and Lischer 2010). Population differentiation was calculated using Fst and variance analysis (AMOVA) in Arlequin 3.5 (Excoffier and Lischer 2010). It was also estimated using a shared haplotype observed in its network. The network was reconstructed using the median-joining method in NETWORK software (Bandelt et al. 1999). The phylogenetic relationship of *C. striata* in Cingcingguling River was estimated using Neighbor-Joining (NJ) and Maximum Likelihood algorithms in MEGA X (Kumar et al. 2018) with 1000 bootstraps replications. Also, the topological stability tree was obtained from the out-group comparison (*Channa gacua* MK599522; *Channa micropeltes* JN024962; *Channa Lucius* KJ937433).

RESULTS AND DISCUSSION

Taxonomic status

Approximately 50 individuals of *Channa* specimens were successfully sequenced, resulting in fragments within the range of 596 bp to 689 bp lengths. Sequence identity test showed that the samples were genetically similar to the top 10 hits closest taxa in the GenBank, all identified as *C. striata* (KU692421, KU852443, and MG438366). However, their percentages were between 98.67% and 100%, with the expected value being 0.0. The samples showed varied genetic distances following Kimura 2 parameter (K2P) from 0.000 % to 1.019, indicating low genetic distances to their closest related taxa in GenBank (Table 1).

Table 1. Sample code, expect value, percent identity, genetic distances, and closest taxa in GenBank

Sample code	E-value	Percent Identity (%)	Genetic distance (%)	Closest Taxa in GenBank
KW 1	0.0	100.0	0.000	Channa striata KU692421
KW 2	0.0	100.0	0.000	Channa striata KU692421
KW3	0.0	98.67	1.019	Channa striata KU852443
KW 4	0.0	100.0	0.000	Channa striata KU692421
KW 5	0.0	100.0	0.000	Channa striata KU692421
KW 6	0.0	100.0	0.000	Channa striata KU692421
KW 7	0.0	100.0	0.000	Channa striata KU692421
KW 8	0.0	100.0	0.000	Channa striata KU692421
KW 9	0.0	100.0	0.000	Channa striata KU692421
KW 10	0.0	100.0	0.000	Channa striata KU692421
BK 1	0.0	100.0	0.000	Channa striata KU692421
BK 2	0.0	100.0	0.000	Channa striata KU692421
BK 3	0.0	100.0	0.000	Channa striata KU692421
BK 4	0.0	100.0	0.000	Channa striata KU692421
BK 5	0.0	100.0	0.000	Channa striata KU692421
BK 6	0.0	100.0	0.000	Channa striata KU692421
KA 1	0.0	100.0	0.000	Channa striata KU692421
KA 2	0.0	100.0	0.000	Channa striata KU692421
KA 3	0.0	100.0	0.000	Channa striata KU692421
KA 4	0.0	100.0	0.000	Channa striata KU692421
KA 5	0.0	100.0	0.000	Channa striata MG438366
KA6	0.0	99.20	0.169	Channa striata KU692421

Sample code	E-value	Percent Identity (%)	Genetic distance (%)	Closest Taxa in GenBank
KA7	0.0	99.54	0.000	Channa striata KU692421
WO 1	0.0	99.68	0.508	Channa striata KU692421
WO 2	0.0	99.84	0.000	Channa striata KU692421
WO 3	0.0	99.35	0.848	Channa striata MG438366
WO 4	0.0	99.69	0.000	Channa striata KU692421
KS 1	0.0	99.07	0.678	Channa striata KU692421
KS 2	0.0	100.0	0.000	Channa striata KU692421
KS 3	0.0	100.0	0.000	Channa striata KU692421
KS 4	0.0	100.0	0.000	Channa striata KU692421
KS 5	0.0	100.0	0.000	Channa striata KU692421
KS 6	0.0	100.0	0.000	Channa striata KU692421
KS 7	0.0	98.77	0.849	Channa striata KU692421
KS 8	0.0	100.0	0.000	Channa striata KU692421
KS 9	0.0	100.0	0.000	Channa striata KU692421
KS 10	0.0	100.0	0.000	Channa striata KU692421
PW 1	0.0	99.84	0.000	Channa striata KU692421
PW 2	0.0	100.0	0.000	Channa striata KU692421
KB1	0.0	100.0	0.000	Channa striata KU692421
KB2	0.0	100.0	0.000	Channa striata KU692421
KB3	0.0	100.0	0.000	Channa striata KU692421
KB4	0.0	100.0	0.000	Channa striata KU692421
KB5	0.0	100.0	0.000	Channa striata KU692421
KB6	0.0	100.0	0.000	Channa striata KU692421
KB7	0.0	100.0	0.000	Channa striata KU692421
KB8	0.0	100.0	0.000	Channa striata KU692421
KB9	0.0	99.84	0.000	Channa striata KU692421
KB10	0.0	99.84	0.000	Channa striata KU692421
BA 1	0.0	100.0	0.000	Channa striata KU692421
BA 2	0.0	100.0	0.000	Channa striata KU692421
BA 3	0.0	100.0	0.000	Channa striata KU692421
BA 4	0.0	100.0	0.000	Channa striata KU692421

KW: Kedungwringin, BK: Bangkong, KA: Kalianget, WO: Waduk outlet, KS: Sempor, PW: Purbowangi, BA: Buayan, KB: Karang Bolong

This research delineated the samples as *C. striata* because their high genetic identities (above 97%) and genetic distance were less than 3%, respectively, to their conspecific. According to Ratnasingham and Hebert (2013), this is the standard identity threshold for animal species determination. Simultaneously, a distance of 3% is acceptable for threshold species determination in fish barcoding (Ratnasingham and Hebert 2007; Hubert et al. 2010; Candek and Kuntner 2015). Even though a higher threshold of approximately 4% and 5% is allowed, other factors need to be considered (Higashi et al. 2011; Jeffrey et al. 2011; Candek and Kuntner 2015).

The low genetic distance among individuals of *C. striata* was reportedly occurred in the wild population found in Lake Towuti, South Sulawesi, with values between 0.043 and 0.309% (Irmawati et al. 2017). Similar values were reported in China (Zhu et al. 2013), using 5 *C. striata* populations, which showed that the intraspecific genetic distances were ranged from 0.002% to 0.027%. In contrast, it was approximately 8 to 45 fold higher than among the species (0.091% to 0.219%). As observed in this research, the minimum (98%) and maximum (1.019%) values of genetic identity and distance, respectively, were reliable to determine the species status of the striped snakehead samples from Cingcingguling Rivers. The present result is consistent with previous research conducted by Aquilino et al. (2011) and Irmawati et al. (2017) that DNA barcoding is a powerful technique for species-level identification of snakehead fish.

Furthermore, the K2P phylogenetic tree was reconstructed by considering neighbor-joining and maximum likelihood (Figure 2). Both algorithms produced a similar topology supported by high bootstrap values (ML=100; NJ=100). *C. striata* samples formed a monophyletic clade with their conspecific reference (Figure 2). According to Xu et al. (2015) and Kusbiyanto et al. (2021), monophyly is also reliable data for species determination. Figure 2 shows that the striped snakehead samples and their conspecific were had a smaller branch scale than the predetermined scale of 0.02. This information strongly indicates that the samples belong to the same species as their closest related taxa (*C. striata*). Monophyly of *C. striata* was also detected between the natural and cultivated population in Vietnam (Nguyen and Duong 2015).

This research also indicates that the CO1 gene is a reliable marker for species identification. Its reliability serves as a barcode because this gene varies among species due to its high mutation rate (Sachithanandam et al. 2012). Due to its

variability, the CO1 gene is a suitable marker for unambiguous species identification (Balkhis et al. 2011; Winarni et al. 2021). This result is congruent with previous research in several locations in Indonesia (Muchlisin et al. 2013; Irmawati et al. 2017; Pramono et al. 2017) and other countries (Aquilino et al. al. 2011; Triantafyllidis et al. 2011), including Lake Greece.

Figure 2. Phylogenetic tree showing monophyly between samples and their conspecific references. left: ML value, right: NJ value

Historical demography and genetic diversity

Overall, Tajima's D value was -2.564; meanwhile, this significant result proved that the neutral hypothesis of marker evolution was rejected, thereby leading to selection pressure. However, the negative sign rejected the assumption on selection pressure and indicated a recent population bottleneck (Tajima1989; Jong et al. 2011). The negative signs and insignificant Fus' Fs supported the neutral marker and population bottleneck assumption, as shown in Table 2. According to Jong et al. (2011) and Mohammed et al. (2021), Tajimas' D and Fus' Fs values are calculated based on haplotype and nucleotide variations, respectively. This difference in the data used simply signifies that Fus' Fs values are more sensitive than Tajimas' D in terms of using it for neutral theory testing of the marker.

Table 2. Genetic diversity value and neutrality test for the used marker

Population	N	Genetic Diversity			Neutrality Test			
		nhp	h (x±SD)	π (x±SD%)	D	P	Fs	P
Overall	53	6	0.181±0.071	0.108±0.095	-2.564***	0.000	-2.360ns	0.070
SR	27	4	0.214±0.103	4.195±3.617	-2.226***	0.001	-0.913ns	0.220
KS	10	3	0.378±0.181	14.568±9.702	-1.901ns	0.006	1.726ns	0.831
PW	2	1	0.000±0.000	0.000±0.000	0.000ns	1.000	-	-
KB	10	1	0.000±0.000	0.000±0.000	0.000	1.000	-	-
BA	4	1	0.000±0.000	0.000±0.000	0.000	1.000	-	-

p> 0.05 = ns, 0.05>p>0.01= significant, p<0.01= highly significant, ns= non-significant, ***= highly significant

This research analyzed a 593 bp COI gene fragment length of 53 individual *C. striata* collected from eight sampling sites. Furthermore, it was reported that 17 out of 593 bp were polymorphic, resulting in 6 haplotypes. Overall haplotype and nucleotide diversities were 0.181 ± 0.071, and 0.108% ± 0.095%, respectively. The data indicate that *C. striata* populations in the Cingcingguling River have low genetic diversity, and this is due to 2 reasons. First, it is caused by small population sizes due to the recent bottleneck. Besides, this has been proven by negative and positive insignificant Tajimas' D and Fus' Fs values, respectively, as shown in Table 2. According to Zanella et al. (2016) and Doublet et al. (2019), a small population shows low genetic diversity due to inbreeding depression. Second, it is caused by limited ancestors. This condition was proven by the haplotype network, which showed that the *C. striata* population in the Cingcingguling River had evolved from a common ancestor, as indicated in Figure 3. It was previously stated that limited maternal ancestors lead to the low genetic diversity of the offspring population because of the drift effect (Zanella et al. 2016). Besides, this attribute in *C. striata* populations was also observed in Malaysia (Jamaluddin et al. 2011).

The present result is inconsistent with the previous research carried out in India (Baisvar et al. 2018), stating that *C. striata* populations exhibited a complex pattern of genetic diversity. However, this implies that it is a common phenomenon. Meanwhile, several fish species have reported high and low haplotype genetic diversity (Sukmanomon et al. 2012; Song et al. 2013; Barasa et al. 2014; Nuryanto et al. 2020). This complex pattern of genetic diversity of *C. striata* populations indicates that environmental factors have exhibited different evolutionary forces on their populations, which needs further analysis.

The within-population evaluation indicates that the haplotype diversity of the *C. striata* population in the Cingcingguling River ranges from 0.000±0.000 to 0.378±0.181. The values prove that the striped snakehead population had low genetic diversity. The majority of the populations underneath the reservoir (PW, KB, and BA) are genetically homogenous. Moreover, two subpopulations (KA and KP) had low genetic diversity. This data indicates that river subpopulations show a complex genetic diversity pattern. The obtained values were lower than the previously reported results (Boonkusol and Tongbai 2016; Baisvar et al. 2018; 2019). The exploitation of *C. Striata* causes low genetic diversity, as indicated by the bottleneck effect shown by Tajimas' D and Fus Fs values in Table 2, which caused minor population size and an opportunity for inbreeding to occur. According to Hauser et al. (2002) and Tan et al. (2012), fishing pressure reduces genetic diversity in fish species. Meanwhile, low genetic diversity caused by exploitation also occurs in various aquatic organisms and several regions (Kochzius and Nuryanto 2008; Wibowo 2012; Barasa et al. 2014; Tan et al. 2015; Baisvar. et al. 2019).

Table 2 shows that the nucleotide diversity ranges from 0.00±0.000% to 14.568±9.703%, and these values indicate that *C. striata* in the Cingcingguling River have both low and high nucleotide diversities. According to Kochzius and Nuryanto (2008) and Nuryanto et al. (2019), when this attribute is greater than 1%, it is highly diverse. Moreover, high nucleotide diversity was detected in reservoir populations of fish species in Victoria Lake (Barasa et al. 2014).

Population connectivity

Per the reservoir population, there was no genetic difference of the four subpopulations. Therefore, this research focused on differentiating the reservoir and river populations. The amova results demonstrated that genetic variations of -

4.27% were mainly observed within the population (104.27%, Table 3). It was assumed that no genetic differences occurred between reservoir and river populations along the Cingcingguling River, and this was supported by a negative fixation index (-0.043) and p-values of 0.115. The data proved that *C. striata* populations in the Cingcingguling River formed a genetically continuous population. An interesting finding was that the Sempor Reservoir did not lead to fragmentation, as proven by the genetic similarities among the river populations. The phenomenon is related to the ecological characteristics of striped snakehead as negative rheotaxis fish, which prefer stagnant water ecosystems. Alteration of running water into a static ecosystem due to Sempor Reservoir did not significantly affect the genetics of *C. striata* both inside and outside. Previous studies reported that *C. striata* typically lives in a swampy ecosystem with stagnant water (Amilhat and Lorenzen 2005; Muflikhah 2007; Listyanto and Andriyanto 2009).

Table 3. Variance and Fst analysis among *C. striata* subpopulation

Source of variation	d.f.	Sum of square	Variance components	Percentage of variation	Fixation index (FST)	p-Value
Among subpopulations	4	0.239	-0.004 Va^{ns}	-4.27	-0.043^{ns}	0.115±0.003
Within subpopulations	48	4.478	0.093 Vb	104.27		
Total	52	4.717	0.089			

p> 0.05 = non-significant (ns), 0.05>p>0.01= significant, p<0.01= highly significant.

The present result is inconsistent with previous research carried out by Song et al. (2013), that significant genetic structure was found among rivers of the *C. striata* in Malaysia. However, this is due to differences in the research locations. This research examined *C. striata* in only one river but fragmented by the reservoir. In contrast, Song et al. (2013) researched different Malaysian river systems. It was previously reported that the river is a closed ecosystem and the freshwater populations tend to show significant genetic differences (Hughes 2009). Therefore, it was reasonable that Song et al. (2013) observed significant genetic differences among rivers. Even Kano et al. (2011) stated solid genetic structures could be observed among tributaries within a river system without physical barriers. However, the different research locations caused an imbalance comparison about genetic differentiation between the present research and that carried out by Kano et al. (2011).

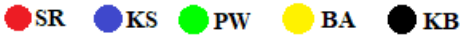
These findings were also inconsistent with the research on *Barbonymus balleroides* in the Serayu River conducted by Bahiyah et al. (2013). According to this research, the significant genetic structures between the reservoir and river population in the Serayu were observed. However, this research evaluated the population genetics of positive rheotaxis species (*B. balleroides*) whose primary habitat is running water. Therefore, the presence of reservoirs in the Serayu River altered the habitat of *B. balleroides* from running to stagnant water, which became an evolutionary factor causing genetic changes in its reservoir populations. Therefore, a significant genetic structure was observed in between *B. balleroides* population inside and outside the reservoir.

It was previously reported, the presence of the Sempor Reservoir has altered upstream areas of Cingcinggguling River to become flooded ecosystems or stagnant water ecosystems (Hedianto et al. 2014). Nevertheless, ecosystems alteration in the Cingcingguling River did not change the typical habitat of *C. striata*. It means that *C. striata* collected inside and outside the reservoir live in similar habitat types. Therefore, identical habitat types inside and outside the Sempor Reservoir did not cause genetic fragmentation of *C. striata*.

A detailed analysis of the within-population showed that WO and KS subpopulations showed slightly higher genetic variability than downstream (Table 2). This difference in genetic diversity level might indicate that both subpopulations are less exploited than the lowland river regions. The assumption arises because both subpopulations reside close to reservoirs' outlets with strong outflow. The fishers are prohibited from collecting fish near the outlet because it is hazardous (Setyaningrum et al. 2020). However, due to the age of the reservoir, which is approximately 51 years old (Hedianto et al. 2014), higher genetic variability in the upper stream subpopulations did not cause significant genetic differentiation. Similar phenomena were reported in *Chilata lopis* (Wibowo et al. 2012) and African catfish (Barasa et al. 2014).

Meanwhile, 6 COI haplotypes were observed in *the Channa striata* populations in the Cingcingguling River Central Java, Indonesia. Median-joining analysis proved that haplotype 1 was dominant and found in all subpopulations (Figure 3). The phenomenon strengthens the result of AMOVA that genetic homogeneity occurred along the Cingcingciguling River, and the reservoir did not cause genetic fragmentation in the *C. striata* population.

Figure 3. Haplotype network indicates the genetic homogeneity of *C. striata* in the Cingcingguling River.



The star-like haplotype network (Figure 3) showed that *C. striata* populations in the Cingcingguling River evolved from a single maternal ancestor (H1). The network proved that H1 is the most primitive haplotype, characterized by high abundance and wide distribution in most populations. Similar phenomena were reported in preliminary research on the *Channa* in several regions (Balkhis et al. 2011; Song et al. 2012; Adamson et al. 2012; Basvar et al. 2018; 2019) and other fish groups (Barasa et al. 2014; Abila et al. 2004).

The snakehead fish in the Cingcingguling River was genetically identified as *Channa striata* and had low genetic diversity. The upper-stream subpopulations had higher genetic diversity than the downstream subpopulations. Also, there were no genetic differences between the reservoir and river populations, which simply means that *C. striata* formed a genetically homogenous population. These data indicate that *C. striata* need to be treated as a single genetic conservation unit.

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REFERENCES

Abila R, Barluenga M, Engelken J, Meyer A, Salzburger W. 2004. Population structure and genetic diversity in a haplochromine fish cichlid of a satellite lake of Lake Victoria. Molecular Ecology 13(9): 2589-2602.

Adamson EAS, Hurwood DA, Mather PB. 2010. A reappraisal of the evolution of Asian snakehead fishes (Pisces, Channidae) using molecular data from multiple genes and fossil calibration. Molecular Phylogenetics and Evolution 56: 707-717.

Adamson EAS, Hurwood DA, Mather PB. 2012. Insights into historical drainage evolution based on the phylogeography of the chevron snakehead fish (*Channa striata*) in the Mekong Basin. Freshwater Biology https://doi.org/10.1111/j.1365-2427.2012.02864.x

Amilhat E, Lorenzen K. 2005. Habitat use, migration pattern and population dynamics of chevron snakehead Channa striata in a rainfed rice farming landscape. Journal of Fish Biology 67(SUPPL. B): 23–34. https://doi.org/10.1111/j.0022-1112.2005.00927.x

Aquilino SVL, Tango JM, Fontanilla IKC, Pagulayan RC, Basiao ZU, Ong PS, Quilang JP. 2011. DNA barcoding of the ichthyofauna of Taal Lake, Philippines. Molecular Ecology Resources 11(4): 612–619. https://doi.org/10.1111/j.1755-0998.2011.03000.x

Arma NR, Illijas MA, Irmawati MA. 2014) Morphometric and meristic characteristics of snakehead fish for hatchery production. Proceeding of the 3rd International and National Seminar on Fisheries and Marine Science: Strengthening Science and Technology towards the Development of Blue Economy, Faculty of Fisheries and Marine Science University of Riau, Riau, (October), 198–201.

Astuti P. 2018. Dampak sosial ekonomi pembangunan waduk sempor bagi masyarakat kebumen tahun 1958-1978. Jurnal Prodi Ilmu Sejarah 3(5): 1–15.

Back-Coleman J, Smith D, Coombs S. 2015. Going with, then against the flow: evidence against the optomotor hypothesis of fish rheotaxis. Animal Behaviour 107: 7-17.

Bahiyah, Solihin DD, Affandi R. 2013. Variasi genetik populasi ikan brek (*Barbonymus balleroides* Val. 1842) sebagai dampak fragmentasi habitat di Sungai Serayu. Jurnal Iktiologi Indonesia 13(2): 175–186.

Baisvar VS, Kumar R, Singh M, Kushwaha B. 2019. Cytochrome c oxidase I gene-based genetic divergence and molecular phylogeny among the species of fish Genus *Channa*. Proceedings of the National Academy of Sciences India Section B - Biological Sciences 89(4): 1455–1463. https://doi.org/10.1007/s40011-018-01070-w

Baisvar VS, Singh M, Kumar R. 2018. Population structuring of *Channa striata* from Indian waters using control region of mtDNA. Mitochondrial DNA Part A 30(3): 414–423. https://doi.org/10.1080/24701394.2018.1532416

Baker and Montgomery 1999. The sensory basis of rheotaxis in the blind Mexican cave fish, *Astyanax fasciatus*. Journal of Comparative Physiology A 184: 519-527.

Balkhis ABS, Firdaus A, Jamsari J, Hwai TS, Yasin Z. 2011. Evidence of geographical structuring in the Malaysian Snakehead, *Channa striata* based on a partial segment of the CO1 gene. Genetics and Molecular Biology 34(3): 520-523.

Bandelt HJ, Foster P, Rohl A. 1999. Median-joining networks for inferring intraspecific phylogenies. Molecular Biology and Evolution 16(1): 37-48.

Barasa JE, Abila R, Grobler JP, Dangasuk OG, Njahira MN. 2014. Genetic diversity and gene flow in *Clarias gariepinus* from Lakes Victoria and Kanyaboli, Kenya. African Journal of Aquatic Science 39(3): 287-293. DOI: 10.2989/16085914.2014.933734

Benziger A, , Philip S, Raghavan R, Ali PHA, Sukumaran M, Tharian JC, Dahanukar N, Baby F, Peter R, Devi KR, Radhakrishnan KV, Haniffa MA, Britz R, Antunes A. 2011. Unraveling a 146 years old taxonomic puzzle: validation of malabar snakehead, species-status and its relevance for channid systematics and evolution. PLoS ONE 6(6): e21272. doi:10.1371/journal.pone.0021272

Boonkusol D, Tongbai W. 2016. Genetic variation of striped snakehead fish (*Channa striata*) in the river basin of central Thailand inferred from mtDNA COI gene sequences analysis. Journal of Biological Sciences 16(1): 37–43. https://doi.org/10.3923/jbs.2016.37.43

Candek K, Kuntner M. 2015. DNA barcoding gap: Reliable species identification over morphological and geographical scales. Molecular Ecology Resources 15 (2): 268-277.

Cia WOC, Asriyana, Halili. 2018. Mortality and exploitation rate of striped snakehead (*Channa striata*) in Aopa Watumohai Swamp , District of Anga. Jurnal Manajemen Sumber Daya Perairan 3(3): 223–231. [Indonesia]

Coad BW. 2016. Contribution to the knowledge of the snakeheads of Iran (Family Channidae). Iranian Journal of Ichthyology 3(1): 65-72.

Dahruddin H, Hutama A, Busson F, Sauri S, Hanner R, Keith P, Hadiaty R, Hubert N. 2016. Revisiting the ichthyodiversity of Java and Bali through DNA barcodes: taxonomic coverage, identification accuracy, cryptic diversity and identification of exotic species. Molecular Ecology Resources DOI: 10.1111/1755-0998.12528

Doublet AC, Croiseau P, Fritz S, Michenet A, Hoze C, Danchin-Burge C, Laloe D, Restoux G. 2019. The impact of genomic selection on genetic diversity and genetic gain in three French dairy cattle breeds. Genetics Selection Evolution 51: 52 (13pp). https://doi.org/10.1186/s12711-019-0495-1

Enders EC, Gessel MH, Williams JG. 2009. Development of successful fish passage structures for downstream migrants requires knowledge of their behavioural response to accelerating flow. Canadian Journal of Fisheries and Aquatic Sciences 66: 2109-2117.

Estradivari, Handayani CNN, Daniel D, Mustofa A. 2017. Marine protected area network design: A case study of Maluku Province. Coastal and Ocean Journal 1(2): 135-146.

Excoffier L, Lischer HEL. 2010. Arlequin suite ver 3.5: A new series of programs to perform population genetics analyses under Linux and Windows. Molecular Ecology Resources 10(3): 564–567. https://doi.org/10.1111/j.1755-0998.2010.02847.x

Fahmi Z, Nurdawati S, Supriyadi F. 2013. Growth and exploitation status (*Channa striata* Bloch, 1793) in Lubuk Lampam Floodplains, South Sumatera. Ind. Fish. Res. J. 19(1): 1–7.

Febrina R. 2016. Modeling rheotaxis based on preference to predict fish migration behavior in a river. Media Komunikasi Teknik Sipil 22(1): 23-34.

Hall T. 2011. BioEdit: An important software for molecular biology. GERF Bulletin of Biosciences 2(1): 60–61. https://doi.org/10.1017/S0317167100012865

Hanif MA, Siddik MAB, Chaklader MR, Nahar A, Mahmud S. 2015. Raznolikost ihtiofaune južnih obalnih voda bangladeša: Postojeće stanje, prijetnje i mogućnosti očuvanja. Ribarstvo, Croatian Journal of Fisheries, 73(4), 148–161. https://doi.org/10.14798/73.4.848

Hauser L, Adcock GJ, Smith PJ, Ramírez JHB, Carvalho GR. 2002. Loss of microsatellite diversity and low effective population size in an overexploited population of New Zealand snapper (*Pagrus auratus*). Proceedings of the National Academy of Sciences of the United States of America, 99(18), 11742–11747. https://doi.org/10.1073/pnas.172242899

Hedianto DA, Purnomo K, Kartamihardja ES, Warsa A. 2014. Population parameters of three spot cichlid (*Cichlasoma trimaculatum*, Günther 1867) in Sempor Reservoir, Central Java. Jurnal Penelitian Perikanan Indonesia 20(2): 81-88. [Indonesia]

Heggenes J, Røed KH. 2006. Do dams increase genetic diversity in brown trout (Salmo trutta)? Microgeographic differentiation in a fragmented river. Ecology of Freshwater Fish 15(4): 366–375. https://doi.org/10.1111/j.1600-0633.2006.00146.x

Higashi R, Tsukagoshi A, Kimura H, Kato K. 2011. Male dimorphism in a new interstitial species of the genus *Microloxoconcha* (Podocopida: Ostracoda). Journal of Crustacean Biology 31(1): 142-152.

Hughes JM, Schmidt DJ, Finn DS. 2009. Genes in streams: using DNA to understand the movement of freshwater fauna and their riverine habitat. BioScience 59: 573-583.

Hubert N, Delieu-Trottin E, Irisson JO, Meyer C, Planes S. 2010. Identifying coral reef fish larvae through DNA barcoding: A test case with the families Acanthuridae and Holocentridae. Molecular Phylogenetics and Evolution 55: 1195-1203.

Irmawati Tresnati J, Nadiarti, Fachruddin L, Arma NR. 2017. Identification of wild stock and the first generation (F1) of domesticated snakehead fish, Channa striata. Jurnal Iktiologi Indonesia 17(2): 165–173. [Indonesia]

Iskandar J, Dahiyat Y. 2012. Keanekaragaman ikan di Sungai Siak Riau. Bionatura 14(1): 51–58.

Jamaluddin JAF, Pau TM, Siti-Azizah MN. 2011. Genetic structure of the snakehead murrel, Channa striata (Channidae) based on the cytochrome c oxidase subunit I gene: Influence of historical and geomorphological factors. Genetics and Molecular Biology 34(1): 152–160. https://doi.org/10.1590/S1415-47572011000100026

Jeffery NW, Elias-Guttierrez M, Adamowicz SJ. 2011. Species diversity and phylogeographical affinities of the Branchiopoda (Crustacea) of Churchill, Manitoba, Canada. PLoS ONE 6 (5): e18364.

Joadder MAR, Galib SM, Haque SMM, Chaki N. (2015). Fishes of the river Padma, Bangladesh: Current trend and conservation status. Journal of Fisheries 3(2): 259. https://doi.org/10.17017/jfish.v3i2.2015.111

Jong MAde, Wahlberg N, van Eijk M, Brakefield PM, Zwaan BJ. 2011. Mitochondrial DNA Signature for Range-Wide Populations of Bicyclus anynana Suggests a Rapid Expansion from Recent Refugia. PLoS ONE 6(6): e21385. doi:10.1371/journal.pone.0021385

Kano Y, Vokoun JC, Letcher BH. 2011. Fine-scale population structure and riverscape genetics of brook trout (Salvelinus fontinalis) distributed continuously along headwater channel networks. Molecular Ecology 20: 3711-3729.

Kanter MJ, Coombs S. 2006. Rheotaxis and prey detection in uniform currents by Lake Michigan mottled sculpin (*Cottus bairdi*). The Journal of Experimental Biology 206: 59-70.

Khan AM, Khan S, Miyan K. 2019. Stock identification of the Channa striata Inhabiting the Gangetic River System using Truss Morphometry. Russian Journal of Ecology 50(4): 391–396. https://doi.org/10.1134/S1067413619040106

Ko HL, Wang YT, Chiu TS, Lee MA, Leu MY, Chang KZ … Shao KT. 2013. Evaluating the Accuracy of Morphological Identification of Larval Fishes by Applying DNA Barcoding. PLoS ONE 8(1): 3–9. https://doi.org/10.1371/journal.pone.0053451

Kochzius M, Nuryanto A. 2008. Strong genetic population structure in the boring giant clam, Tridacna crocea, across the Indo-Malay Archipelago : implications related to evolutionary processes and connectivity. Molecular Ecology 17: 3775–3787. https://doi.org/10.1111/j.1365-294X.2008.03803.x

Kottelat M, Whitten AJ, Kartikasari SN, Wirjoatmodjo S (1993) Freshwater Fishes of Western Indonesia and Sulawesi. Hong Kong: Periplus Editions (HK) Ltd. 293p.
Kumar S, Stecher G, Li M, Knyaz C, Tamura K. 2018. MEGA X: Molecular evolutionary genetics analysis across computing platforms. Molecular Biology and Evolution 35(6): 1547–1549. https://doi.org/10.1093/molbev/msy096
Kusbiyanto, Bhagawati D, Nuryanto A. 2020. DNA barcoding of crustacean larvae in Segara Anakan, Cilacap, Central Java, Indonesia using cytochrome c oxidase gene. Biodiversitas 21(10): 4878-4887.
Lakra WS, Goswani M, Gopalakrishnan A, Singh DP, Singh A, Nagpure NS. 2010. Genetic relatedness among fish species of Genus *Channa* using mitochondrial DNA genes. Biochemical Systematics and Ecology 38: 1212-1219.
Listyanto N, Andriyanto S. 2009. Ikan Gabus (Channa striata) Manfaat Pengembangan Dan Alternatif Teknik Budidayanya. Media Akuakultur 4(1): 18–25.
Mohammed MA, Nuryanto A, Kusmintarsih ES. 2021. Genetic differentiation of dengue vector Aedes aegypti in the small geographical scale of Banyumas District, Indonesia based on Cytochrome Oxidase I. Biodiversitas 22(2): 675-683
Muchlisin ZA, Thomy Z, Fadli N, Sarong MA, Siti-Azizah MN. 2013. DNA barcoding of freshwater fishes from Lake Laut Tawar, Aceh Province, Indonesia. Acta Ichthyologica et Piscatoria 43(1): 21–29. https://doi.org/10.3750/AIP2013.43.1.04
Muslimin B, Rustadi R, Hardaningsih H, Retnoaji B. 2020. Morphometric variation of cork fish (Channa striata Bloch, 1793) from nine populations in Sumatra island, Indonesia. Iranian Journal of Ichthyology 7(3): 209–221. https://doi.org/10.22034/iji.v7i3.480
Nguyen, T., & Duong, Y. (2015). Morphological and genetic differences between cultured and wild populations of Channa striata in Viet Nam and its phylogenetic relationship with other Channa species. Songklanakarin Journal of Science and Technology SJST, 0147.R3.
Muflikhah N. 2007. Domestikasi ikan gabus (Channa striata). Bawal 1(5): 169–175. [Indonesia]
Nurdawati S, Fahmi Z, Supriyadi F. 2019. Parameter Populasi Ikan Betok (Anabas testudineus Bloch, 1792) Di Ekosistem Paparan Banjir Sungai Musi, Lubuk Lapam. Berita Biologi Jurnal Ilmu-Ilmu Hayati 18(1): 25–35.
Nuryanto A, Kusbiyanto K, Bhagawati D. 2021. Molecular barcoding of marine ornamental fish from the southern coast of West Java validates conventional identification. E3S Web of Conferences 322: 01004. https://doi.org/10.1051/e3sconf/202132201004
Nuryanto A, Baghawati D, Husein MOH, Rachmawati FNUR. 2020. Molecular Characterization of Anguilla from Cibereum and Sapuregel Rivers Segara Anakan Watersheds Cilacap, Central Java, 8(2): 145–156.
Nuryanto A, Komalawati N, Sugiharto. 2019. Genetic diversity assessment of Hemibagrus nemurus from rivers in Java Island, Indonesia using COI gene. Biodiversitas 20(9): 2707–2717. https://doi.org/10.13057/biodiv/d200936
Nuryanto A, Pramono H, Sastranegara MH. 2017. Molecular identification of fish larvae from East Plawangan of Segara Anakan, Cilacap, Central Java, Indonesia. Biosaintifika 9(1): 33-40.
Nuryanto A, Bhagawati D, Abulias MA, Indarmawan. 2015. Fauna ikan di Sungai Cikawung Kabupaten Cilacap Jawa Tengah. Jurnal Iktiologi Indonesia 15(1): 25-37. [Indonesia]
Nuryanto A, Bhagawati D, Abulias MA, Indarmawan. 2012. Fish diversity at Cileumeuh River in District of Majenang, Cilacap Regency, Central Java. Jurnal Iktiologi Indonesia 12(2): 147-153.
Oteiza P, Odstrcil I, Lauder G, Portugues R, Engert F. 2017. A novel mechanism for mechanosensory-based rheotaxis in larval zebrafish. Nature 547(7664): 445-448.
Pavlova A, Beheregaray LB, Coleman R, Gilligan D, Harrisson KA, Ingram BA, Kearns J, Lamb AM, Lintermans M, Lyon J, Nguyen TTT, Sasai M, Tonkin Z, Yen JDL, Sunnucks P. 2017. Severe consequences of habitat fragmentation on genetic diversity of an endangered Australian freshwater fish: A call for assisted gene flow. Evolutionary Applications 10: 531-550
Pramono TB, Arfiati D, Widodo MS, Yanuhar U. 2017. Identifikasi Ikan Genus Mystus Dengan. Jurnal Sumberdaya Akuatik Indopasifik 1(2): 123–132.
Firlianty, Pratasik SB. 2018. The Potential *of Channa micropeltes* and *C. striata* pudding for healing acceleration of the white rat. Jurnal Agribisnis Perikanan, 11(2), 65–69. https://doi.org/10.29239/j.agrikan.11.2.65-69. [Indonesia]
Ratnasingham S, Hebert PDN. 2013 A DNA-Based Registry for All Animal Species: The Barcode Index Number (BIN) System. PLoS ONE 8(8): e66213. doi:10.1371/journal.pone.0066213
Ratnasingham R, Hebert PDN. 2007. BOLD: The Barcode of life data system (www.barcodinglife.org). Molecular Ecology Notes 7: 355-364.
Roesma DI. 2013. Evaluasi Keanekaragaman Spesies Ikan Danau Maninjau. Prosiding Semirata FMIPA Universitas Lampung, 197–204.
Rozas J, Ferrer-Mata A, Sanchez-DelBarrio JC, Guirao-Rico S, Librado P, Ramos-Osins SE, Sanchez-Gracia A. 2017. DnaSP 6: DNA sequence polymorphism analysis of large data sets. Mol Biol Evol 34 (12): 3299-3302. DOI: 10.1093/molbev/msx248.
Sachithanandam V, Mohan PM, Muruganandam N, Chaaithanya IK, Dhivya P, Baskaaran R. 2012. DNA barcoding, phylogenetic study of *Epinephelus* spp. from Andaman coastal region, India. Indian Journal of Geo-Marine Sciences 41(4): 203-211.
Setyaningrum N, Sugiharto S, Susatyo P. 2020. Species richness and status guilds of fish in Sempor Reservoir Central Java. Depok 9(3): 411-420. [Indonesia]
Setyaningrum et al. 2021. Spatial distribution and abundance of *Channa striata* Bloch1793 in Sempor Reservoir, Kebumen Central Java. E3S Web of Conferences 322: 01029. https://doi.org/10.1051/e3sconf/202132201029.
Sofarini D, Mahmudi M, Hertika AMS, Herawati EY. 2018. Population dynamic of snakehead fish (*Channa striata*) at Danau Panggang Swamp, South Kalimantan. EnviroScienteae 14(1): 16-20.
Song LM, Munian K, Rashid ZA, Bhassu S. 2013. Characterisation of Asian snakehead murrel *Channa striata* (Channidae) in Malaysia: An insight into molecular data and morphological approach. The Scientific World Journal: 917506. https://doi.org/10.1155/2013/917506
Sukmanomon S, Senanan W, Kapuscinski AR. 2012. Genetic diversity of feral populations of Nile Tilapia (*Oreochromis niloticus*) in Thailand and evidence of genetic introgression. Kasetsart Journal Natural Sciences 46(2): 200–216.
Suli A, Watson GM, Rubel EW, Raible DW. 2012. Rheotaxis in larval zebrafish is mediated by lateral line mechanosensory hair cells. PLoS ONE 7(2): e29727. doi:10.1371/journal.pone.0029727.
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(3): 1227-1235.
Tajima F. 1989. Statistical method for testing then mutation hypothesis by DNA polymorphism. Genetics 123: 585-595.
Tan MP, Jamsari AFJ, Muchlisin ZA, Siti-Azizah MN. 2015. Mitochondrial genetic variation and population structure of the striped snakehead, *Channa striata* in Malaysia and Sumatra, Indonesia. Biochemical Systematics and Ecology 60: 99-105. https://doi.org/10.1016/j.bse.2015.04.006
Tan MP, Jamsari AFJ, Siti-Azizah MN. 2012. Phylogeographic pattern of the striped snakehead, *Channa striata* in Sundaland : Ancient river connectivity, geographical, and anthropogenic signatures. PLoS ONE 7(12): e52089. https://doi.org/10.1371/journal.pone.0052089
Triantafyllidis A, Bobori D, Koliamitra C, Gbandi E, Mpanti M, Petriki O, Karaiskou N. 2011. DNA barcoding analysis of fish species diversity in four north Greek lakes. Mitochondrial DNA 22(SUPPL. 1): 37–42. https://doi.org/10.3109/19401736.2010.542242
Ward RD, Zemlak TS, Innes BH, Last PR, Hebert PDN. 2005. DNA barcoding Australia's fish species. Philosophical Transactions of the Royal Society B: Biological Sciences 360(1462): 1847–1857. https://doi.org/10.1098/rstb.2005.1716
Wibowo A. 2012a. Struktur genetik populasi ikan belida *(Chitala lopis*, Bleeker 1851) di Waduk Kutopanjang. Bawal 4(308): 53–58.
Wibowo A. 2012b. Genetic diversity of masheer *Tor tambroides* Bleeker 1854) in Manna River Bengkulu and Semangka River Lampung. Bawal 4(2): 105–112. [Indonesia]

Winarni ET, Kusbiyanto, Nuryanto A. 2021 Estimating Crustacean Species Utilize Segara Anakan Estuary Cilacap, Indonesia as Nursery Ground through DNA Barcoding. Journal of Hunan University (Natural Sciences) 48(10): 275-282.

Xu X, Liu F, Chen J, Li D, Kuntner M. 2015. Integrative taxonomy of the primitively segmented spider genus Ganthela (Araneae: Mesothelae: Liphistiidae): DNA barcoding gap agrees with morphology. Zool J Linnean Soc 175(2): 288-306. DOI: 10.1111/zoj.12280.

Yudha IGNAP, Arya IW, Suryani SAMP. 2018. Studi identifikasi keragaman jenis, feeding habit dan food habit ikan demersal pada bagian hilir sungai yeh sungi, Tabanan, Bali. Gema Agro 23(2): 114-123.

Zanella R, Peixoto JO, Cardoso FF, Cardoso LL, Biegelmeyer P, Cantao ME, Otaviano A, Freitas MS, Caetano AR, Ledur MC. 2016. Genetic diversity analysis of two commercial breeds of pigs using genomic and pedigree data. Genetics Selection and Evolution 48: 24.

Zhu SR, Fu JJ, Wang Q, Li JL. 2013. Identification of Channa species using the partial cytochrome c oxidase subunit I (COI) gene as a DNA barcoding marker. Biochemical Systematics and Ecology 51: 117–122. https://doi.org/10.1016/j.bse.2013.08.011