BIODIVERSITAS
Volume 23, Number 1, January 2022
Pages: 222-230

ISSN: 1412-033X
E-ISSN: 2085-4722
DOI: 10.13057/biodiv/d230128

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

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Manuscript received: 20 November 2021. Revision accepted: 23 December 2021.

Abstract. *Setyaningrum N, Lestari W, Nuryanto A. 2022. Genetically continuous populations of Striped Snakehead (*Channa striata*) in the Cingcingguling River fragmented by Sempor Reservoir, Central Java, Indonesia. Biodiversitas 23: 222-230.* Cingcingguling River, located in Kebumen District, Central Java Province, 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 identity (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

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). The specimens 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%.

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).

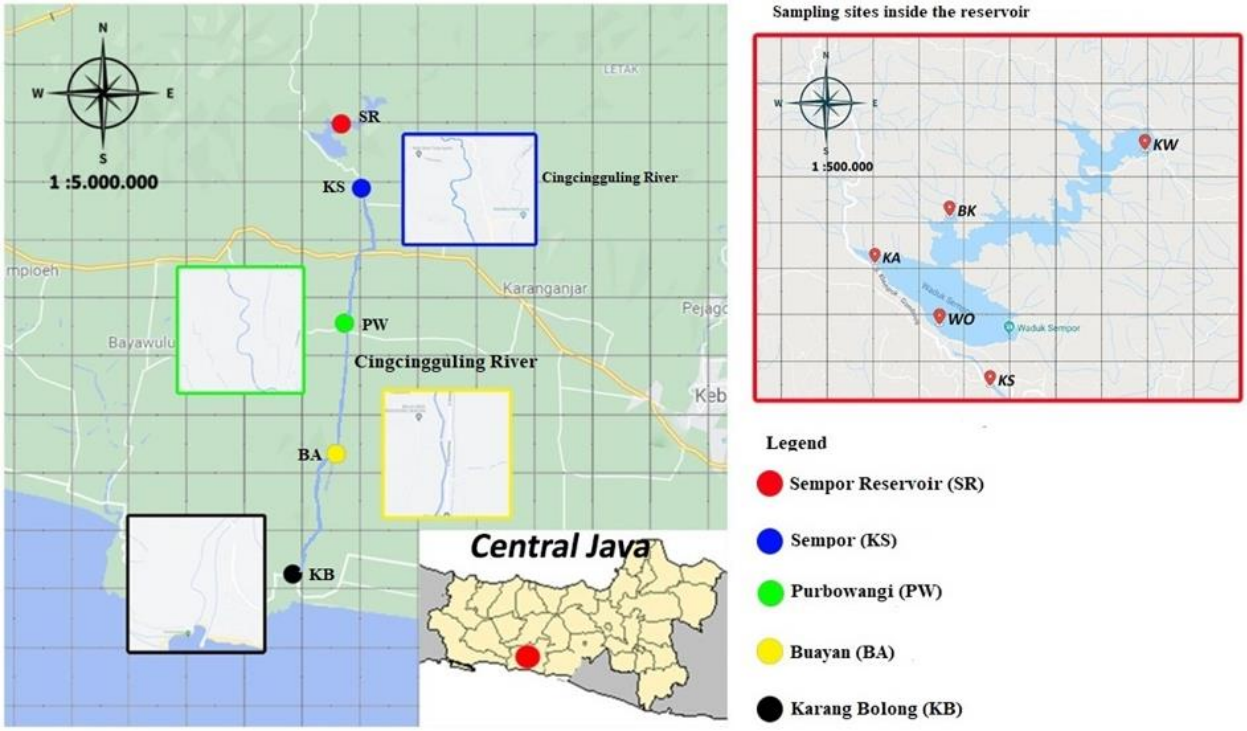

Figure 1. Research location with five sampling sites along the river in in Cingcingguling River, Central Java, Indonesia. Four subsampling sites are located inside the reservoir

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

The COI gene of 53 *Channa* specimens was 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).

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).

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
KW 3	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
KA 6	0.0	99.20	0.169	Channa striata KU692421
KA 7	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
KB 1	0.0	100.0	0.000	Channa striata KU692421
KB 2	0.0	100.0	0.000	Channa striata KU692421
KB 3	0.0	100.0	0.000	Channa striata KU692421
KB 4	0.0	100.0	0.000	Channa striata KU692421
KB 5	0.0	100.0	0.000	Channa striata KU692421
KB 6	0.0	100.0	0.000	Channa striata KU692421
KB 7	0.0	100.0	0.000	Channa striata KU692421
KB 8	0.0	100.0	0.000	Channa striata KU692421
KB 9	0.0	99.84	0.000	Channa striata KU692421
KB 10	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

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

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.

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 (Tajima 1989; 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.

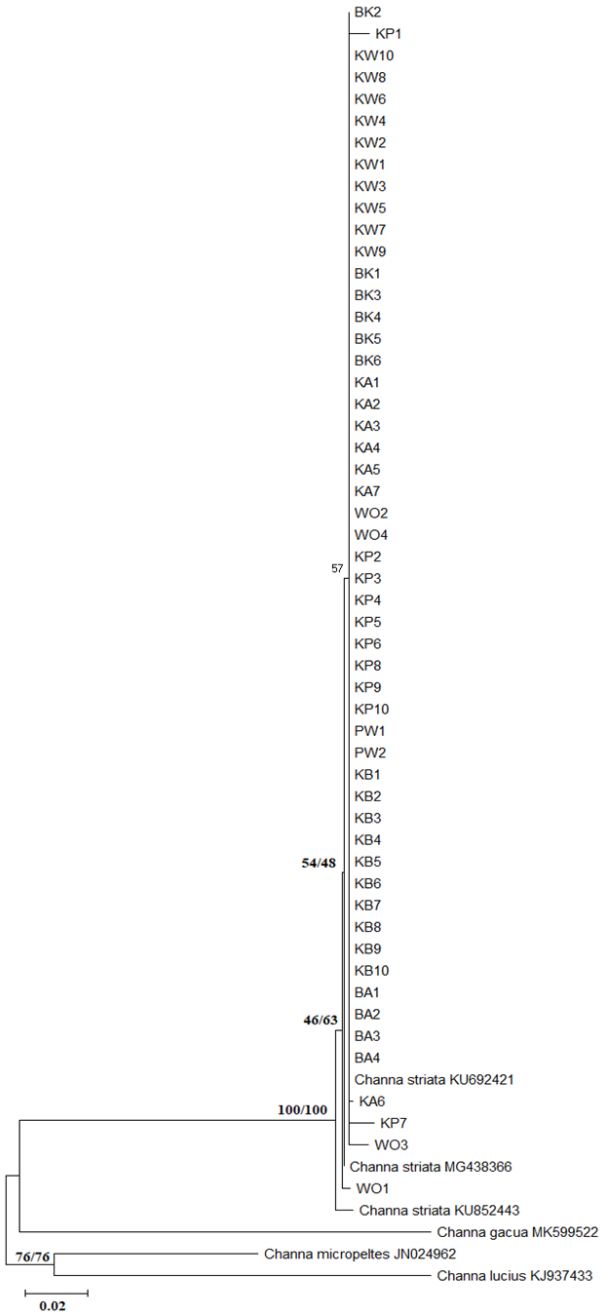

Figure 2. Phylogenetic tree showing monophyly between samples and their conspecific references. left: ML value, right: NJ value

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 between the four subpopulations. Therefore, this research focused on differentiating the reservoir and river populations. The amova results demonstrated that genetic variations 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 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	-	-

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

Table 3. Variance and Fst analysis among *Channa 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			

Note: 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 *Chitala 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.

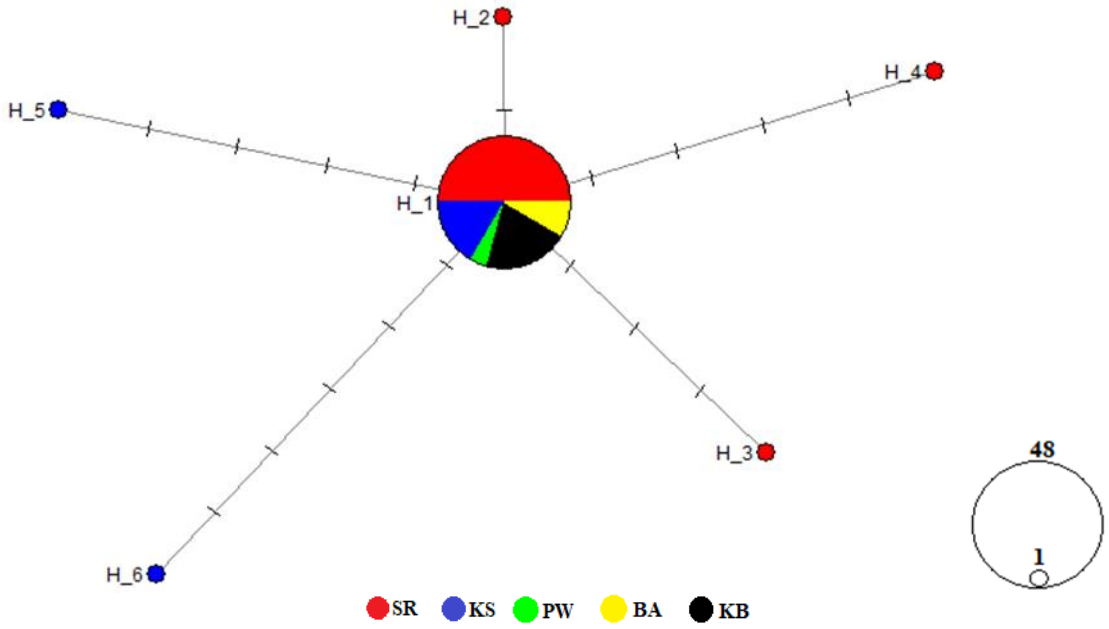

Figure 3. Haplotype network indicates the genetic homogeneity of *Channa striata* in Cingcingguling River, Central Java, Indonesia

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.

ACKNOWLEDGEMENTS

The authors are grateful to DRPM BRIN for funding this research and to the Faculty of Biology, Universitas Jenderal Soedirman (Unsoed), Indonesia for allowing the use of their facilities.

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