BIODIVERSITAS
Volume 25, Number 9, September 2024
Pages: 2959-2966

ISSN: 1412-033X
E-ISSN: 2085-4722
DOI: 10.13057/biodiv/d250916

Small scale genetic structure of striped snakehead, *Channa striata* in the river and swampy areas of the south region of Central Java, Indonesia

NUNING SETYANINGRUM♥, W. LESTARI, AGUS NURYANTO
Faculty of Biology, Universitas Jenderal Soedirman. Jl. Dr. Soeparno 63 Purwokerto 53122, Central Java, Indonesia. Tel.: +62-281-638794,
Fax.: +62-281-631700, ♥email: nuning.setyaningrum@unsoed.ac.id

Manuscript received: 4 June 2024. Revision accepted: 10 September 2024.

Abstract. *Setyaningrum N, Lestari W, Nuryanto A. 2024. Small scale genetic structure of striped snakehead,* Channa striata *in the river and swampy areas of the south region of Central Java, Indonesia. Biodiversitas 25: 2959-2966.* This study focuses on the exploration and characterization of *Channa striata* population in swampy areas located in south-central Java, Indonesia, specifically in the regencies of Purworejo, Kebumen, Banyumas, and Cilacap. These regions are known for their diverse and unique ecosystems, making them ideal sites for ecological research. Fragmented populations of striped snakehead (*Channa striata*) are observed in the aquatic ecosystems of south-central Java, Indonesia. However, research on *C. striata* in swampy areas of south-central Java has not been conducted yet. Evaluating the genetic structure of *C. striata*is a crucial endeavor that can be accomplished through the analysis of the cytochrome c oxidase 1 gene. Therefore, this research aims to estimate the genetic diversity and differentiation among *C. striata* populations. This study analyzed 74 specimens of *C. striata* collected from Keburuan, Karangbolong, Jatijajar, Sumpiuh, and Kedungreja in south-central Java. The used marker has moderate haplotype diversity (0.541±0.065) but low nucleotide diversity (0.0025±0.0017). Haplotype diversity within the population ranges from low (0.151±0.093) to high (0.833±0.222), while all populations showed low nucleotide diversity (0.0006±0.0008 to 0.0037±0.0030). Through the analysis of genetic markers, *C. striata* populations can be categorized into three distinct groups. The findings revealed that population fragmentation has resulted in reduced genetic diversity and localized population structuring in the river and swampy areas of south-central Java. These results highlight the importance of separate management strategies for each population to ensure their conservation and sustainable management.

Keywords: Fragmentation, genetic diversity, haplotype, snakehead, southern Java

INTRODUCTION

Previous studies have demonstrated that animal populations exhibit varying patterns of geographic structure across their ranges (Nuryanto et al. 2019; Tisthammer et al. 2020). Some species have been found to display genetic homogeneity among populations that are geographically distant (Barasa et al. 2014; Viret et al. 2018), while others exhibit significant genetic differences between distantly separated populations (Dohna et al. 2015; Tan et al. 2015; Basviar et al. 2018; Supmee et al. 2021). Additionally, certain species have been observed to exhibit small-scale significant genetic structure among populations (Tim et al. 2017; Coleman et al. 2018; Leyton 2020).

The striped snakehead (*Channa striata*) is potamodromous fish species with limited dispersal capability in the habitat, a maximum of 500 m (Amilhat and Lorenzen 2005; Froese and Pauly 2024). It is an economically valuable fisheries commodity in Asia. This species is widely distributed in Asia. In Indonesia, it is distributed in large Sunda Island, namely Sumatra, Java, and Borneo (Coad et al. 2016; Ansyari et al. 2020; Djumanto et al. 2020; Muslimin et al. 2020). This species has been introduced to Bali (Yudha et al. 2018) and the eastern regions, such as Sulawesi and Maluku (Irmawati et al. 2017). *C. striata* is known to inhabit a diverse range of habitats, with a preference for swamps, stagnant rivers, river flood plains, and dams or reservoirs (Galib et al. 2016; Chan et al. 2017, 2020; Gumiri et al. 2018). Previous studies have also documented the presence of *C. striata* in various freshwater ecosystems in the southern regions of Central Java, Indonesia (Nuryanto et al. 2015; Setyaningrum et al. 2021).

Four regencies reside in the southern region of Central Java Province, Indonesia, i.e., Purworejo, Kebumen, Banyumas, and Cilacap. There are rivers and swampy areas found in each district. Cincingguling is among the rivers in Kebumen District that have been fragmented by Sempor Reservoir since approximately 60 years ago (Setyaningrum et al. 2022a). Keburuhan is a river in the Kutoarjo Sub-district, Kebumen District, with its estuary known as the fish auction center. Several swampy areas are also found in the Kebumen, Banyumas, and Cilacap Regencies. The swampy areas in question are geographically separated from each other by physical barriers such as land masses, resulting in fragmentation. This isolation likely originated during the formation of Java Island (Setijadji et al. 2006).

Habitat fragmentation can restrict genetic exchange and result in notable genetic structure among populations (Underwood et al. 2015; Basviar et al. 2018; Coleman et al. 2018). A study focusing on *Channa argus* demonstrated substantial genetic regional subdivision, both among rivers and within sampling sites within river systems, indicating the role of physical barriers (Yan et al. 2017). The genetic structure of regionally subdivided populations can be investigated using mitochondrial DNA, such as the cytochrome c oxidase 1 (COI) gene, even in the absence of physical barriers (Liu et al. 2020). Previous research has confirmed the reliability of this gene fragment as a genetic marker for studying population genetics in various animal populations (Henriques et al. 2016; Nuryanto et al. 2019; Padmavathi and Srinu 2019; Fernandez-Alias et al. 2022). Additionally, a study reported significant genetic differentiation among *C. striata* populations in Indian rivers (Basviar et al. 2018).

In contrast, a recent study conducted in the Cicingguling River in Central Java revealed a genetically homogeneous population of *C. striata*, despite being fragmented by a reservoir (Setyaningrum et al. 2022a). However, the study by Setyaningrum et al. (2002a) was focused only on a single river and could not provide a comprehensive understanding of the population genetics of *C. striata* in south-central Java. To address this gap, our research aims to assess the genetic diversity and population structure of *C. striata* in the rivers and swampy areas of southern Central Java, Indonesia.

MATERIALS AND METHODS

Research location and sampling sites

Channa striata samples were collected from six different sampling sites in Central Java Province, Indonesia, i.e. (i) Sempor Reservoir (7°33’13”S-7°34’01”S and 109°29’05”E-109°29’16”E) of Kebumen District, as well as other rivers in: (ii) Jatijajar (7°40'3.8"S; 109°25'36.5"E) and (iii) Karangbolong (7°45'33.4"S; 109° 27'57"E) of Kebumen District, Central Java; (iv) Kedungreja (7°34'19.6"S; 108°48'36.8"E) of Cilacap District; (v) Sumpiuh (7°37'9.5"S; 109°17'3.7"E) of Banyumas District, and (vi) Keburuan (7°49'41.9"S; 109°53'18.9"E) of Purworejo District, respectively (Figure 1). *C. striata*specimens were collected using traditional traps made from bamboo and lines with the help of fishermen. Tinny fragments of tissue 74 samples were cut off from the pectoral fin of each individual and put in new Eppendorf tubes filled with 96% ethanol. The fish bodies were preserved in 70% ethanol for morphological studies and stored at the Aquatic Biology Laboratory, Faculty of Biology, Universitas Jenderal Soedirman, Purwokerto Utara, Banyumas, Central Java, Indonesia.

Procedures

DNA isolation and marker polymerization

The DNA genome was isolated from the pectoral fin tissue using the Quick-DNA™ Miniprep Plus kit adopted from Zymo’s research. This study employed DNA extraction procedures following the Kit’s manual, and the success of the extraction was verified through 1% agarose electrophoresis. The target fragments of the COI gene were then amplified using FishF2-5’TCGACTAATCATAAAGATATCGGCAC3’ and FishR2-5’ACTTCAGGGTGACCGAAGAATCAGAA3’ primers with PCR products approximately 655 bp (Ward et al. 2005). For each 25 µL PCR mixture, the volume of each chemical reagent was as follows: 1 µL of KOD FX Neo, 13.5 µL of 2X PCR Buffer KOD FX Neo, 1 µL of 2 mM dNTPs, 1 µL of each primer at 10 pmol/µL, 1 µL of template DNA, and 6 µL of ddH_2O. All DNA analysis procedures were conducted at the Genetika Laboratory (PT. Genetika Science Indonesia).

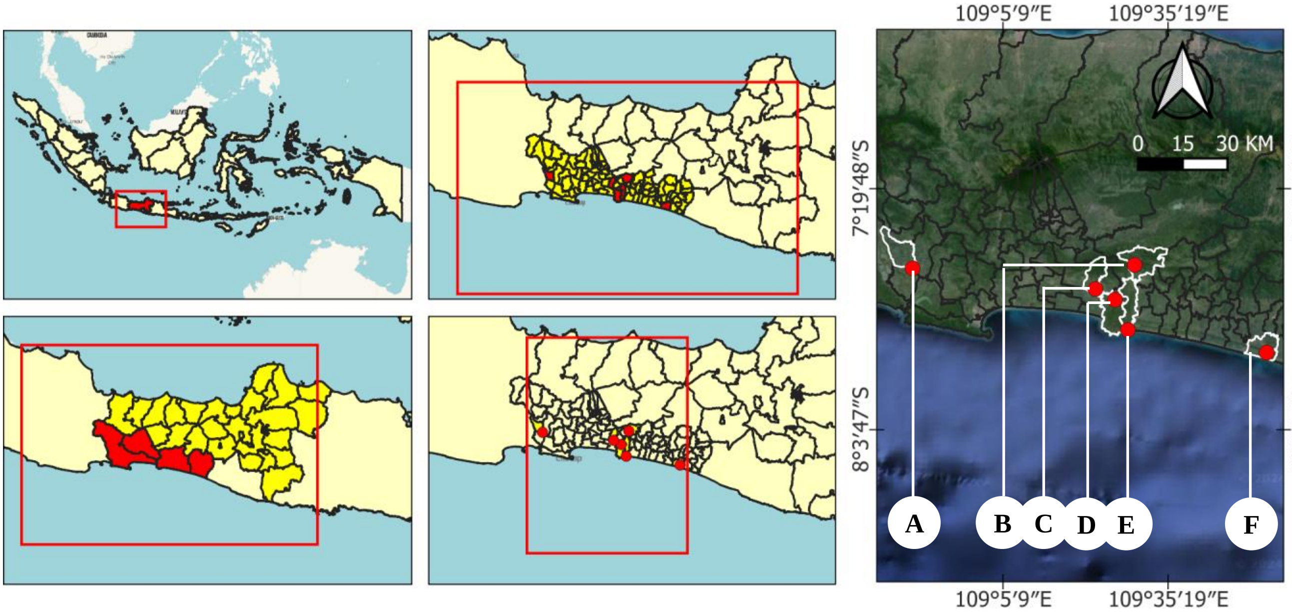

Figure 1. Research location of *Channa striata* in reservoir and rivers in the south region of Central Java, Indonesia. A. Kedungreja, B. Sempor Reservoir, C. Sumpiuh, D. Jatijajar, E. Karangbolong, F. Keburuan

The thermal cycling conditions consisted of an initial denaturation at 95°C for 4 minutes, followed by 35 cycles of denaturation at 95°C for 30 seconds, annealing at 53°C for 2 minutes, and extension at 72°C for 1 minute. The final extension step was performed at 72°C for 5 minutes to complete the cycles. The PCR products were visualized by staining with ethidium bromide and electrophoresed on a 1.5% agarose gel, followed by examination under ultraviolet light. Gel documentation was carried out using the GelDoc apparatus (BioRad).

The cytochrome oxidase 1 sequencing and editing

The 21 PCR products amplified from the COI marker were subjected to sequencing using the standard Sanger method in both the forward and reverse directions at 1st BASE Malaysia (haplotype accession number OQ852690-OQ852694). Additional of 53 sequences were obtained from GenBank (haplotype accession number OQ852682-OQ852687). The sequences were edited in BioEdit (Hall 2017). Multiple sequence alignment (MSA) was conducted using ClustalW ver.1.4, implemented in BioEdit (Hall 2017), which yielded a 564 bp fragment of the COI gene from 74 individuals of *C. striata*. Haplotype information was derived from the analysis using DnaSP 6 (Rozas et al. 2017).

Data analysis

This study used sequence identity of 97% and 3% genetic distance between the sample and reference species available as the species border. These values were also utilized in previous studies (Nuryanto et al. 2022; Winarni et al. 2023). All specimens were subjected to molecular identification using the COI barcode method. Taxonomic status was determined based on sequence comparison to conspecific sequence in GenBank.

The COI fragment was tested for its neutrality using Tajima’s D (Tajima 1989) and Fu’s Fs tests (Fu 1997) with 10,000 permutations as implemented in Arlequin 3.5 (Excoffier and Lischer 2015). These tests were carried out to ensure that the used COI fragment is a neutral marker suitable for population genetic analysis. Mismatch distribution analysis and sudden population expansion model (Rogers 1995) were also performed to ensure the result of the neutrality test. The investigation was conducted for overall samples without separating the populations. The calculations were performed in Arlequin 3.5 (Excoffier and Lischer 2015). Genetic diversity and genetic structure analysis of *C. striata* were conducted in five populations. Overall and within the population, genetic diversities were estimated using haplotype diversity h and nucleotide diversity π (Nei and Jin 1989), calculated in Arlequin 3.5 (Excoffier and Lischer 2015). Significant population differentiation was calculated using pairwise Fst values and molecular variance (AMOVA) analysis carried out in Arlequin 3.5 (Excoffier and Lischer 2015). The significance of genetic structure was also estimated using a shared haplotype among populations observed in the haplotype network (Figure 2). The network was reconstructed using the median-joining method in NETWORK software (Bandelt et al. 1999).

RESULTS AND DISCUSSION

Taxonomic status

The sequence identity test revealed that the *C. striata*samples in this study exhibited a sequence similarity ranging from 98.67% to 100% with the top ten closest taxa in the GenBank. All of these taxa were identified as *Channa striata* (KU692421, KU852443, and MG438366), with coverages ranging from 99% to 100% and an expect value of 0.0.

The samples exhibited low genetic distances to *C. striata* in GenBank, ranging from 0.000% to 1.019%. These observed genetic similarities and distances were found to be below the predetermined genetic threshold of 97% similarity and 3% genetic distance, respectively. Thus, this study confirms that all samples belong to *C. striata*. Previous studies have established that a genetic similarity of 97% and genetic distance of 3% are acceptable thresholds for species determination in fish barcoding (Candek and Kuntner 2015; Karanovic 2015; Kusbiyanto et al. 2020; Nuryanto et al. 2022; Winarni et al. 2023). Furthermore, considering ecological factors and geographic distance among samples and available conspecific references, a genetic threshold of up to 5% is also considered acceptable for species differentiation (Candek and Kuntner 2015; Karanovic 2015).

Historical demography

Tajima's D value of the used COI marker was -2.053, with a significant value (p=0.000; Table 1). 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 of selection pressure and indicated a recent population bottleneck (Tajima 1989; Mohammed et al. 2021). The negative signs and significant Fu’s Fs (Fs= -3.808; 0.05>p>0.01) supported the neutral marker and population bottleneck assumption, as shown in Table 1. The neutrality of the marker was confirmed by the non-significant SSD and HRI values, suggesting stability in population demography, as indicated by the negative value of Fu's Fs (Roger 1995). These results (Fs, SSD, and HRI) indicate that the COI marker utilized in this study is neutral and suitable for population genetics analysis of *C. striata* in the southern region of Central Java. Furthermore, the present study observed marker neutrality across all studied populations (Table 1). Previous studies have also reported the neutrality of the COI gene in other investigations (Mohammed et al. 2021; Setyaningrum et al. 2022a).

Genetic diversity

The current study analyzed genetic diversity based on 564 bp fragments of the COI gene of 74 individuals of *C. striata* collected from six sampling sites. Overall haplotype diversity was 0.541±0.065, which indicates medium gene diversity. Nucleotide diversity was 0.0025±0.0017, which showed low diversity. The low genetic diversity level could be due to recent population expansion, as proven by non-significant SSD and HRI values (Roger 1995). The phenomenon occurred after the bottleneck event, as it has been confirmed by negative Tajimas' D and insignificant Fu's Fs values, respectively, as presented in Table 1. Recent population expansion after a bottleneck event might result in low genetic diversity due to a small population leading to inbreeding depression and drift effect (Zanella et al. 2016; Doublet et al. 2019). However, referring Zanella et al. (2016) and Doublet et al. (2019) were not wholly congruent since those previous studies were in mammals, while this study was in fish (*C. striata*). Nevertheless, low genetic diversity in *C. striata* populations was also reported in Cincingguling River (Setyaningrum et al. 2022a).

This study added information about the complex pattern (low, moderate, and high) of the genetic diversity level of the *C. striata* population in its geographic ranges. It has been reported in India and Malaysia that *C. striata* populations showed a complex pattern of genetic diversity (Baisvar et al. 2018). The phenomena were also reported to occur in various fish species. A high and low haplotype genetic diversity was common, such as in An*guilla bicolor* (Nuryanto et al. 2022), *Clarias gariepinus* (Barasa et al. 2014), *Anguilla bicolor* (Nuryanto et al. 2020) and *C. striata* in Cingcingguling River (Setyaningrum et al. 2022a). The phenomena did not relate to the sample size. Nuryanto et al. (2020) and Setyaningrum et al. (2022a) observed moderate (h=0.849) and low (h=0.181) genetic diversities in their species even though the sample size were similar (55 and 53, respectively). The phenomena proved that ecological parameters and species had exhibited variable evolutionary fates of the populations.

The within-population evaluation indicates that the haplotype diversity of the *C. striata* population in the river and swampy areas of the southern region of Central Java ranges from 0.151±0.093 in the Karangbolong population to 0.833±0.222 in the Sumpiuh population. The Karangbolong population had the lowest genetic diversity. The result was consistent with a previous report by Setyaningrum et al. (2022a) that showed low genetic diversity of *C. striata* populations along the Cingcingguling River, which was assumed due to overexploitation (Setyaningrum et al. 2022b). The obtained values were comparable to the previously reported (Boonkusol and Tongbai 2016; Baisvar et al. 2018, 2019). However, the comparison to Baisvar et al. (2018) was not hundred percent congruent because the present study used the COI gene, while Baisvar et al. (2018) used mtDNA D-loop as a genetic marker.

According to Righi et al. (2020) and Petit-Marty et al. (2022), fish species experience a decrease in genetic diversity due to high fishing pressure. The exploitation of *C. striata* has resulted in low genetic diversity, as evidenced by the bottleneck effect indicated by Tajima's D and Fu's Fs values presented in Table 1. This reduction in genetic diversity is attributed to a small population size, which creates opportunities for genetic drift to occur. Previous studies have also reported similar findings of low genetic diversity resulting from the overexploitation of various aquatic organisms in different regions (Barasa et al. 2014; Tan et al. 2015; Baisvar et al. 2019; Nuryanto et al. 2019).

Table 1 presents the nucleotide diversity values ranging from 0.0006±0.0008 to 0.0037±0.0030, indicating that *C. striata* populations in the southern region of Central Java exhibit low nucleotide diversities. Nucleotide diversity below 0.01 is considered low diversity (Nuryanto et al. 2019). A study also reported this condition in the *C. striata* population in the Cingcingguling River Central Java (Setyaningrum et al. 2022a).

Genetic structure

Variance components (0.191) of the AMOVA result showed highly significant differences among populations (p=0.000). This result indicated that *C. striata* populations in the southern region of Central Java were genetically different among populations (Table 2).

The difference among the population was supported by a high fixation index (F_{ST}=0.594) with p-values of 0.000. The data provide evidence that the fragmented and separated ecosystem in south-central Java has resulted in population structuring of *C. striata*. An interesting finding is that even fine-scale geographical distances have contributed to this population structuring. This phenomenon can be explained by the assumption that the studied populations are completely isolated, which is a common occurrence in freshwater ecosystems due to physical barriers, such as land masses. A previous study has reported significant genetic structure within a single river system, even in the absence of a physical barrier (Yan et al. 2017). Nevertheless, pairwise FST analysis proved that population differentiations were not observed among all populations (Table 3).

Table 1. *Channa striata* of the south region of Central Java, Indonesia population, number of individuals (N), number of haplotypes (nhp), haplotype diversity (h), nucleotide diversity (μ), Tajima's D, Fu's Fs, Sum of Squared deviation (SSD), and Harpending's Raggedness index (HRI)

Population	N	nhp	Genetic diversity		Neutrality test		Mismatch distribution	
			h	π	D	Fs	SSD	HRI
Overall	74	11	0.541±0.065	0.0025±0.0017	-2.053***	-3.808*	0.008^{ns}	0.172^{ns}
Sempor Reservoir	27	4	0.214±0.103	0.0008±0.0008	-2.094***	-1.565*		
Karangbolong	26	3	0.151±0.093	0.0012±0.0011	-2.270***	-0.629^{ns}		
Sumpiuh	4	3	0.833±0.222	0.0037±0.0030	-0.780^{ns}	-0.134^{ns}		
Kedungreja	6	2	0.333±0.215	0.0006±0.0008	-0.933^{ns}	-0.003^{ns}		
Jatijajar	6	2	0.533±0.172	0.0010±0.0010	0.851^{ns}	0.625^{ns}		
Keburuan	5	2	0.400±0.237	0.0021±0.0020	-1.048^{ns}	1.688^{ns}		

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

Moreover, freshwater population structure is also affected by the movement capability of the species among freshwater bodies (Comte and Olden 2018). *C. striata* can live out of the water for 20 h and able to do terrestrial movement (Kuznetsov 2022). Nevertheless, after 2-3 minutes of movements, *C. striata* rested more than moving times (Kuznetsov 2022). The limited dispersal capability of *C. striata* individuals, with the closest distance estimated at 23 km in this study, supports the observation of significant genetic structure among populations in south-central Java. This is attributed to the complete separation of habitats by land masses (Figure 1), which prevents movement of *C. striata* individuals between populations due to long geographic distances.

C. striata is popular Asian fish introduced to the United States of America (Courtenay and Williams 2004; Herborg et al. 2007). This *Channa* species is indigenous or native to Asia, including Indonesia (Coad et al. 2016; Ansyari et al. 2020). No study reported the introduction effort of *C. striata* on Java Island because it is native to the island (Gustiano et al. 2021), even though the introduction of other *Channa micropeltes* has been reported in Sempor Reservoir (Setyaningrum et al. 2020). Therefore, the genetic structure pattern of *C. striata* in south-central Java is considered to be naturally driven and can be attributed to the formation of Java Island. The findings of this study align with previous research, indicating that physical barriers among populations contribute to genetic differences among separated populations (Barasa et al. 2014; Yan et al. 2017).

Small-scale significant genetic structure was rarely observed in closed-distance populations when the analysis used the COI gene, except for animals with low and limited mobility, such as *Aedes aegypti* (Mohammed et al. 2021). However, fine-scale genetic structures were found in some species when the analyses were performed using microsatellites (Gouskov et al. 2016; Abbas et al. 2017; Yan et al. 2017) and D-Loop (Verma et al. 2016). In contrast, other studies proved that no genetic structure was observed, although it was analyzed using microsatellites (Basharat et al. 2016; Cheng et al. 2017). Therefore, it seems that the genetic structure of some fishes are complex phenomena, whatever genetic markers are used (Parmaksiz and Eksi 2017; Achrem et al. 2017; Nuryanto et al. 2019; Parmaksiz 2019).

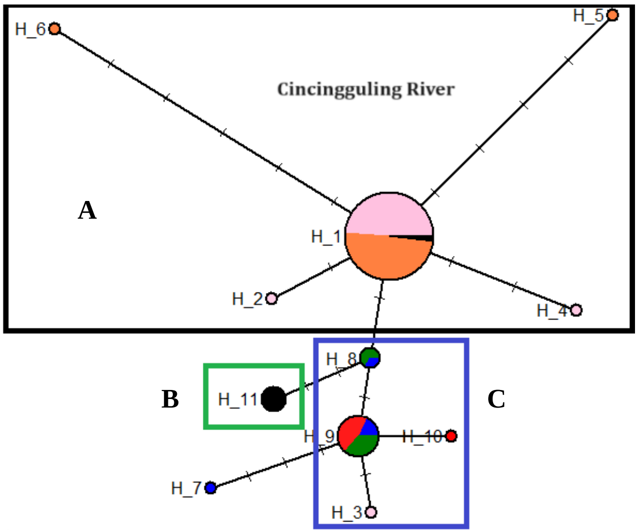

Figure 2. Haplotype networks indicate three clusters of *Channa striata* populations in the southern region of Central Java, Indonesia. A. Black box; B: Green box; C: Blue box

Table 2. Amova result showing genetic difference among *Channa striata* populations in the southern region of Central Java, Indonesia

Source of variation	**Degree of difference d.f.**	**Sum of Square**	**Variance components**	**Percentage of variation**
Among populations	5	10.864	0.191 Va***	59.38
Within population	68	8.879	0.113 Vb	40.62
Total	73	19.743	0.321	
Fixation index (FST)	:	0.594	P= 0.000±0.000	

Note: p>0.05: ns (non-significant), 0.05>p>0.01: significant*, p<0.01: highly significant***, d.f.: degree of freedom

Table 3. Pairwise FST values among populations of *Channa striata* in the southern region of Central Java, Indonesia

Population	**Sempor Reservoir**	**Karangbolong**	**Sumpiuh**	**Kedungreja**	**Jatijajar**	**Kutoarjo**
Sempor Reservoir	0.000					
Karangbolong	-0.015^{ns}	0.000				
Sumpiuh	0.662***	0.722***	0.000			
Kedungreja	0.759***	0.809***	0.043^{ns}	0.000		
Jatijajar	0.709***	0.761***	-0.153^{ns}	0.025^{ns}	0.000	
Kutoarjo	0.690***	0.752***	0.403*	0.636***	0.528***	0.000

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

Furthermore, the present study observed 21 polymorphic sites out of 564 bp (3.54%), resulting in the identification of eleven haplotypes within the *C. striata* populations in the southern region of Central Java, Indonesia. The haplotypes were further classified into three distinct haplogroups, as depicted in Figure 2. Haplogroup A (black box) consisted of five haplotypes (53 individuals) collected from Cincingguling River and Sempor Reservoir, Kebumen District and one individual from Keburuan, Purworejo District. Haplogroup B comprised one haplotype (four individuals) from Keburuan, Purworejo District, as well as one individual from Keburuan, Kebumen District. Haplogroup C was formed by samples from Kedungreja, Cilacap District, Sumpiuh, Banyumas District, Sempor Reservoir and Jatijajar, Kebumen District. Notably, Haplotype 1 was found to be dominant and present in Sempor Reservoir and Cincingguling River, as indicated in Figure 2. This observation further supports the results of the AMOVA analysis, which revealed genetic homogeneity along the Cingcingciguling River, indicating that the presence of the reservoir did not lead to genetic fragmentation within the *C. striata* population (Setyaningrum et al. 2022a).

Conservation

The latest assessment put *C. striata* as the least concern species. The placement was based on the fact that there was no significant threat. However, no information about regional assessment was available in the assessment report (Chaudhry et al. 2019). Therefore, to strengthen the current conservation status of *C. striata*, it is necessary to do further studies of *C. striata* by extending the geographic coverage of study sites. Several studies reported that the local population of *C. striata* in Indonesia had been over-exploited (Cia et al. 2018; Nurdawati et al. 2019). This possibility encourages Ahmadi and Mangkurat (2018) to declare that *C. striata* are threatened species in the Batanghari River. Recently, exploitation has also been reported in the *C. striata* population in Cingcingguling River, Kebumen District, Central Java, Indonesia Setyaningrum et al. (2022b), leading to low genetic diversity (Setyaningrum et al. 2022a).

The natural population of snakeheads in Indonesia is also threatened by habitat loss and fragmentation. According to Pavlova et al. (2017), fragmentation has lowered genetic diversity and decreased population. In addition, fragmentation can prevent gene flow and cause significant genetic structure among populations (Gouskov et al. 2016). The current study observed that the genetic analysis of *C. striata* populations in the southern region of Central Java, Indonesia, revealed their division into three distinct genetic units. This finding highlights the importance of considering these populations as separate conservation units. The identified units are the Cincingguling population, the Keburuan population, and a group consisting of the Kedungreja, Sumpiuh, and Jatijajar populations. Populations that exhibit high genetic similarity hold significant conservation value as gene flow among them can help mitigate the negative effects of small population sizes. Therefore, it is recommended that genetically similar populations be managed as a single conservation unit to ensure their effective conservation and preservation (Vargas et al. 2016).

The findings of this study demonstrate that the *C. striata* populations inhabiting the river and swampy areas in south-central Java, Indonesia, exhibit a distinct genetic structure, with three genetic units (A, B, and C) identified. The observed fragmentation of these populations has resulted in reduced genetic diversity within each subpopulation. Consequently, it is crucial to recognize and manage the *C. striata* populations in south-central Java as three separate genetic conservation units.

The recognition of these genetic conservation units is of utmost importance for effective conservation and management strategies. By acknowledging the genetic distinctiveness of each subpopulation, conservation efforts can be tailored to address the specific needs and challenges faced by these populations. This approach will help ensure the preservation of the striped snakehead's genetic diversity, which is vital for the long-term viability and resilience of the species in the region. Furthermore, the identification of low genetic diversity within the genetic units highlights the urgency of conservation actions. Efforts should be focused on mitigating the factors that contribute to population fragmentation and promoting connectivity between genetic units where possible. Implementing measures to safeguard habitat connectivity, such as maintaining and restoring corridors between the river and swampy areas, can facilitate gene flow and promote genetic exchange among genetic units. Finally, the genetic structure observed in the *C. striata*populations in south-central Java, along with the low genetic diversity within each genetic unit, underscores the necessity of treating these populations as distinct genetic conservation units. By prioritizing the conservation and management of these units, we can enhance the long-term viability and resilience of *C. striata* in the region, ensuring its continued existence for future generations.

In conclusion, *C. striata* populations in south-central Java showed fine-scale genetic structure and can be divided into three different genetic populations. This population structuring implies that the populations must be managed as three different conservation units.

ACKNOWLEDGEMENTS

We thank the Directorate of Research and Community Services, Directorate General of Higher Education, Ministry of Education, Culture, Research, and Technology, and the Republic of Indonesia for the funding (contract: T/1029/UN23.18/PT.01.03/2022). The authors also thank the Research and Community Service Institute of Universitas Jenderal Soedirman, Banyumas, Indonesia for administrative support and the Faculty of Biology, Universitas Jenderal Soedirman for facilities support. We highly appreciate the fishermen for their help during the field trips.

REFERENCES

Abbas K, Xiaoyun Z, Weimin W. 2017. Microsatellite markers reveal genetic differentiation of Chinese dojo loach *Misgurnus anguillicaudatus* in the Yangtze River basin. Turk J FishAquat Sci 17: 1167-1177. DOI: 10.4194/1303-2712-v17_6_10.

Achrem M, Skuza L, Kirczuk L, Domagala J, Pilecka-Rapacz M, Czerniawski R. 2017. Assessment of genetic variability in common whitefish from the catchment area of the Oder River using microsatellite markers. Acta Biol 24: 5-13. DOI: 10.18276/ab.2017.24-02.

Ahmadi A, Mangkurat UL. 2018. The length weight relationship and condition factor of the threatened snakehead (*Channa striata*) from Sungai Batang Hari River, Indonesia. Pol J Nat Sci 33 (4): 607-623.

Amilhat E, Lorenzen K. 2005. Habitat use, migration pattern and population dynamics of chevron snakehead Channa striata in a rainfed rice farming landscape. J Fish Biol 67 (Supplement B): 23-34. DOI: 10.1111/j.1095-8649.2005.00927.x

Ansyari P, Ahmadi S. 2020. Food habits and biolimnology of snakehead larvae and fingerlings from different habitats. AACL Bioflux 13 (6): 3520-3531.

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. DOI: 10.1080/24701394.2018.1532416.

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*. Proc Nat Acad Sci India Sect B Biol Sci 89: 1455-1463. DOI: 10.1007/s40011-018-01070-w.

Bandelt HJ, Foster P, Rohl A. 1999. Median-joining networks for inferring intraspecific phylogenies. Mol Biol Evol 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. Afr J Aquat Sci 39 (3): 287-293. DOI: 10.2989/16085914.2014.933734.

Basharat H, Gafhoor A, Chavhan A, Zafar MM, Abbas K, Tabasum J, Parveen R. 2016. Microsatellite markers revealed poor genetic structure of *Wallago attu* in Punjab, Pakistan. Intl J Life Sci 4: 385-393.

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. J Biol Sci 16: 37-43. DOI: 10.3923/jbs.2016.37.43.

Candek K, Kuntner M. 2015. DNA barcoding gap: Reliable species identification over morphological and geographical scales. Mol Ecol Resour 15 (2): 268-277. DOI: 10.1111/1755-0998.12304.

Chan B, Ngor PB, So N, Lek S. 2017. Spatial and temporal changes in fish yields and fish communities in the largest tropical floodplain lake in Asia. Intl J Limnol 53: 485-493. DOI: 10.1051/limn/2017027.

Chan B, Brosse S, Hogan ZS, Peng BNPB, Lek,S. 2020. Influence of local habitat and climatic factors on the distribution of fish species in the Tonle Sap Lake. Water 2 (3): 786. DOI: 10.3390/w12030786.

Chaudhry S, de Alwis Goonatilake S, Fernando M, Kotagama O. 2019. *Channa striata*. The IUCN Red List of Threatened Species 2019: e.T166563A60591113.

Cheng F, Zhao S, Schmidt BV, Ye L, Hallerman EM, Xie S. 2017. Morphological but no genetic differentiation among fragmented populations of *Hemiculter leucisculus* (Actinopterygii, Cyprinidae) from a lake complex in the middle Yangtze, China. Hydrobiologia 809: 185-200. DOI: 10.1007/s10750-017-3464-0.

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. [Indonesian]

Coad BW. 2016. Contribution to the knowledge of the snakeheads of Iran (Family Channidae). Iran J Ichthyol 3 (1): 65-72. DOI: 10.7508/iji.2016.01.007.

Coleman RA, Gauffre B, Pavlova A, Beheregaray LB, Kearns J, Lyon J, Sasaki M, Leblois R, Sgro C, Sunnucks P. 2018. Artificial barriers prevent genetic recovery of small isolated populations of a low-mobility freshwater fish. Heredity 120: 515-532. DOI: 10.1038/s41437-017-0008-3.

Comte L, Olden JD. 2018. Fish dispersal in flowing waters: A synthesis of movement- and genetic- based studies. Fish Fisheries 19: 1063-1077. DOI: 10.1111/faf.12312.

Courtenay WR, Williams JD. 2004. Snakeheads (Pisces, Channidae): A Biological Synopsis and Risk Assessment. Roston, VA: US Geological Survey.

Djumanto, Setyobudi E, Simanjuntak CPH, Rahardjo MF. 2020. Estimating the spawning and growth of striped snakehead *Channa striata* Bloch, 1793 in Lake Rawa Pening Indonesia. Sci Rep 10: 19830. DOI: 10.1038/s41598-020-76825-5.

Dohna TA, Timm J, Hamid L, Kochzius M. 2015. Limited connectivity and a phylogeographic break characterize populations of the pink anemonefish, *Amphiprion perideraion*, in the Indo-Malay Archipelago: Inferences from a mitochondrial and microsatellite loci. Ecol Evol 5 (8): 1717-1733. DOI: 10.1002/ece3.1455.

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. Genet Sel Evol 51: 52. DOI: 10.1186/s12711-019-0495-1.

Excoffier L, Lischer HEL. 2015. Arlequin suite Ver 3.5: An integrated software package for population genetics data analysis. https://cmpg.unibe.ch/software/arlequin35/man/Arlequin35.pdf.

Fernandez-Alias A, Razinkovas-Baziukas A, Morkune R, Ibanez-Martinez H, Bacevicius E, Munoz I, Marcos C, Perez-Ruzafa A. 2022. Recolonization origin and reproductive locations, but not isolation from the sea, lead to genetic structure in migratory lagoonal fishes. Mar Environ Res 181: 105732. DOI: 10.1016/j.marenvres.2022.105732.

Froese R, Pauly D. 2024. FishBase. World Wide Web electronic publication. www.fishbase.org.

Fu FX. 1997. Statistical test of neutrality of mutations against population growth, hitchhiking and background selection. Genetics 147: 915-925.

Galib SM, Rashid MA, Chaki N, Mohsin A, Joadder MAR. 2016. Seasonal variation and community structure of fishes in the Mahananda River with special reference to conservation issues. J Fish 4 (1): 325-334. DOI: 10.17017/jfish.v4i1.2016.139.

Gouskov A, Reyes M, Wirthner-Bitterlin L, Vorburger C. 2016. Fish population genetic structure shaped by hydroelectric power plants in the upper Rhine catchment. Evol Appl 9: 394-408. DOI: 10.1111/eva.12339.

Gumiri S, Ardianor, Syahrinudin, Anshari GZ, Komai Y, Taki K, Tachibana H. 2018. Seasonal yield and composition of an inland artisanal fishery in a humic floodplain ecosystem of Central Kalimantan, Indonesia. Biodiversitas 19 (4): 1181-1185. DOI: 10.13057/biodiv/d190401.

Hall T. 2017. BioEdit: Biological Sequence Alignment Editor for Wina95/98/NT/2K/XP. https://thalljiscience.github.io/.

Henriques R, Von Der Heyden, S., Lipinski, MR, Du Toit N, Kainge P, Bloomer P, Matthee CA. 2016. Spatio-temporal genetic structure and the effects of longterm fishing in two partially sympatric offshore demersal fishes. Mol Ecol 25: 5843-5861. DOI: 10.1111/mec.13890.

Herborg L-M, Mandrak NE, Cudmore BC, MacIsaac HJ. 2007. Comparative distribution and invasion risk of snakehead (Channidae) and Asian carp (Cyprinidae) species in North America. Can J Fish Aquat Sci 64: 1723-1735. DOI: 10.1139/F07-130.

Irmawati, Tresnati J, Nadiarti, Fachruddin L, ArmaNR, Haerul A. 2017. Identification of wild stock and the first generation (F1) of domesticated snakehead fish, *Channa* spp. (Scopoli 1777) using partial cytochrome c oxidase subunit I (COI) gene. Jurnal Iktiologi Indonesia 17 (2): 165-173. DOI: 10.32491/jii.v17i2.356. [Indonesian]

Karanovic I. 2015. Barcoding of ancient lake Ostracods (Crustacea) reveals cryptic speciation with extremely low distances. PLoS One 10 (3): e0121133. DOI: 10.1371/journal.pone.0121133.

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. DOI: 10.13057/biodiv/d211054.

Kuznetsov AN. 2022. How big can a walking fish be? A theoretical inference based on observations on four land-dwelling fish genera of South Vietnam. Integr Zool 17: 849-878. DOI: 10.1111/1749-4877.12599.

Leyton KKS, Dempson B, Snelgrove PVR, Duffy SJ, Messmer AM, Peterson IG, Jeffrey NW, Kess T, Horne JB, Salisbury SJ, Ruzzante DE, Bentzen P, Cote D, Nugent CM, Ferguson MM, Leong JS, Koop BF, Bradbury IR. 2020. Resolving fine-scale population structure and fishery exploitation using sequenced microsatellites in a northern fish. Evol Appl 13: 1055-1068. DOI: 10.1111/eva.12922.

Liu B, Zhang K, Zhu K, Shafi M, Gong L, Jiang L, Liu L, Muhammad F, Lü Z. 2020. Population genetics of *Konosirus punctatus* in Chinese coastal waters inferred from two mtDNA genes (COI and Cytb). Front Mar Sci 7: 534. DOI: 10.3389/fmars.2020.00534.

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. DOI: 10.13057/biodiv/d220219.

Muslimin B, Rustadi, Hardaningsih, Retnoaji B. 2020. Morphometric variation of Cork fish (*Channa striata* Bloch, 1793) from nine populations in Sumatra Island, Indonesia. Iran J Ichthyol 7: 209-221.

Nei M, Jin L. 1989. Variances of the average numbers of nucleotide substitutions within and between populations. Mol Biol Evol 6: 290-300

Nurdawati S, Fahmi Z, Supriyadi F. 2019. Parameter populasi ikan betook (*Anabas testudineus* Bloch, 1792) di ekosistem paparan banjir Sungai Musi, Lubuk Lapam. Berita Biologi Jurnal Ilmu-Ilmu Hayati 18 (1): 25-35. DOI: 10.14203/beritabiologi.v18i1.3107. [Indonesian]

Nuryanto A, Bhagawati D, Abulias MA, Indarmawan. 2015. Fauna ikan di Sungai Cikawung Kabupaten Cilacap Jawa Tengah. Jurnal Iktiologi Indonesia 15 (1): 25-37. DOI: 10.32491/jii.v15i1.73. [Indonesian]

Nuryanto A, Komalawati N, Sugiharto. 2019. Genetic diversity assessment of *Hemibagrus nemurus* from rivers in Java Island, Indonesia using COI gene. Biodiversitas 20: 2707-2717. DOI: 10.13057/biodiv/d200936.

Nuryanto A, Baghawati D, Sastranegara MH, Rachmawati FN. 2020. Molecular characterization of *Anguilla* from Cibereum and Sapuregel Rivers Segara Anakan watersheds Cilacap, Central Java. Biogenesis 8 (2): 145-156. DOI: 10.24252/bio.v8i2.15532.

Nuryanto A, Riani S, Bhagawati D, Winarni ET, Rachmawatai FN. 2022. Molecular characterization of *Anguilla* sp. with special notes on its population genetic in the rivers of Cilacap Central Java, Indonesia. Biosaintifika 14: 103-116. DOI: 10.15294/biosaintifika.v14i1.35421.

Padmavathi P, Srinu G. 2019. Genetic divergence and phylogenetic analysis of fish fauna from Lake Kolleru based on COI sequences. Curr Trends Biotechnol Pharm 13 (2): 173-189.

Parmaksiz A, Eksi E. 2017. Genetic diversity of the cyprinid fish *Capoeta trutta* (Heckel, 1843) populations from Euphrates and Tigris rivers in Turkey based on mtDNA COI sequences. Indian J Fish 64 (1): 18-22. DOI: 10.21077/ijf.2017.64.1.62396-03.

Parmaksiz A. 2019. Population genetic diversity of yellow barbell (*Carasobarbus luteus*) from Kueik, Euphrates, and Tigris Rivers based on mitochondrial DNA D-loop sequences. Turk J Fish Aquat Sci 20 (1): 79-86. DOI: 10.4194/1303-2712-v20_1_08.

Pavlova A, Beheregaray LB, Coleman R, Gilligan D, Harrisson KA, Ingram BA, Kearns J, Lamb AM, Lintermans M, Lyon J, Nguyen TTT, Sasaki 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. Evol Appl 10: 531-550. DOI: 10.1111/eva.12484.

Petit-Marty N, Liu M, Tan IZ, Chung A, Terrasa B, Guijarro B, Ordines F, Ramı´rez-Amaro S, Massutı´ E, Schunter C. 2022. Declining population sizes and loss of genetic diversity in commercial fishes: A simple method for a first diagnostic. Front Mar Sci 9: 872537. DOI: 10.3389/fmars.2022.872537.

Righi A, Splendiani A, Fioravanti T, Casoni E, Gioacchini G, Carnevali O, Barucchi V. C. 2020. Loss of mitochondrial genetic diversity in overexploited Mediterranean Swordfish (*Xiphias gladius*, 1759) population. Diversity 12: 170. DOI: 10.3390/d12050170.

Rogers AR. 1995. Genetic evidence for Pleistocene population expansion. Evolution 49: 608-615.

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.

Setijadji LD, Kajino S, Imai A, Watanabe K.2006. Cenozoic island arc magmatism in Java Island (Sunda Arc, Indonesia): Clues on relationships between geodynamics of volcanic centers and ore mineralization. Resour Geol 56: 267-292. DOI: 10.1111/j.1751-3928.2006.tb00284.x.

Setyaningrum N, Sugiharto, Susatyo P. 2020. Species richness and status guilds of fish in Sempor Reservoir Central Java. DEPIK 9 (3): 411-420. DOI: 10.13170/depik.9.3.15094.

Setyaningrum N, Nuryanto A, Lestari W, Krismono. 2021. Spatial distribution and abundance of *Channa striata* Bloch, 1793 in Sempor Reservoir, Kebumen Central Java. E3S Web Conf 322: 01029. DOI: 10.1051/e3sconf/202132201029.

Setyaningrum N, Lestari W, Krismono, Nuryanto A. 2022a. Genetically continuous populations of striped snakehead (*Channa striata*) in the Cingcingguling River fragmented by Sempor Reservoir, Central Java, Indonesia. Biodiversitas 23: 222-230. DOI: 10.13057/biodiv/d230128.

Setyaningrum N, Lestari W, Krismono, Nuryanto A. 2022b. Exploitation of striped snakehead (*Channa striata*) in Sempor Reservoir, Central Java, Indonesia: A proposed conservation strategy. Biodiversitas 23 (7): 3584-3592. DOI: 10.13057/biodiv/d230735.

Supmee V, Songrak A, Suppapan J, Sangthong P. 2021. Population genetic structure of ornate threadfin bream (*Nemipterus hexodon*) in Thailand. Trop Life Sci Res 32: 63-82. DOI: 10.21315/tlsr2021.32.1.4.

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. Biochem Syst Ecol 60: 99-105. DOI: 10.1016/j.bse.2015.04.006.

Tim J, Kochzius M, Madduppa HH, Neuhaus AI, Dohna T. 2017. Small scale genetic population structure of coral reef organisms in Spermonde Archipelago, Indonesia. Front Mar Sci 4: 294. DOI: 10.3389/fmars.2017.00294.

Tisthammer KH, Forsman ZH, Toonen RJ, Richmond RH. 2020. Genetic structure is stronger across human-impacted habitats than among islands in the coral *Porites lobata*. PeerJ 8: e8550. DOI: 10.7717/peerj.8550.

Underwood ZE, Mandeville EG, Walters AW. 2015. Population connectivity and genetic structure of burbot (*Lota lota*) populations in the Wind River Basin, Wyoming. Hydrobiologia 757: 329-342. DOI 10.1007/s10750-015-2422-y.

Vargas SM, Jensen MP, Ho SYW, Mobaraki A, Broderick D, Mortimer JA, Whiting SD, Miller J, Prince RIT, Bell IP, Hoenner X, Limpus CJ, Santos FR, Fitz-Simmons NN. 2016. Phylogeography, genetic diversity, and management units of hawksbill turtles in the Indo-Pacific. J Hered 107 (3): 199-213. DOI: 10.1093/jhered/esv091.

Verma R, Singh M, Kumar S. 2016. Unraveling the limits of mitochondrial control region to estimate the fine scale population genetic differentiation in anadromous fish *Tenualosa Ilisha*. Scientifica 2016: 2035240. DOI: 10.1155/2016/2035240.

Viret A, Tsaparis D, Tsigenopoulos CS, Berrebi P, Sabatini A, Arculeo M, Fassatoui C, Magoulas A, Marengo M, Morales-Nin B, Caill-Milly N, Durieux EDH. 2018. Absence of spatial genetic structure in common dentex (*Dentex dentex* Linnaeus, 1758) in the Mediterranean Sea as evidenced by nuclear and mitochondrial molecular markers. PLoS One 13 (9): e0203866. DOI: 10.1371/journal.pone.0203866.

Ward RD, Zemlak TS, Innes BH, Last PR, Hebert PDN. 2005. DNA barcoding Australia's fish species. Phil Trans R Soc B 360: 1847-1857. DOI:10.1098/rstb.2005.1716.

Winarni ET, Simanjuntak SBI, Nuryanto A. 2023. DNA barcoding of crustacean larvae with two new records of *Caridina gracilipes* and *Ptychognathus altimanus* in the western region of Segara Anakan lagoon in Indonesia. Biodiversitas 24 (1): 341-348. DOI: 10.13057/biodiv/d240141.

Yan R-J, Zhang G-R, Guo X-Z, Ji W, Chen K-C, Zou G-W, Wei K-J, Gardner JPA. 2017. Genetic diversity and population structure of the northern snakehead (*Channa argus* Channidae: Teleostei) in central China: Implications for conservation and management. Conserv Genet 19: 467-480. DOI: 10.1007/s10592-017-1023-x.

Yudha IGNAP, Arya IW, Suryani SAMP. 2018. Study of species diversity identification, feeding habits and food habits of demersal fish in the lower reaches of the Yeh Sungi River, Tabanan, Bali. Gema Agro 23 (2): 114-123. DOI: 10.22225/ga.23.2.883.114-123. [Indonesian]

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. Genet Sel Evol 48: 24. DOI: 10.1186/s12711-016-0203-3.