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Short Communication

Genetic Diversity and Population Structure of Amphidromous Goby (*Stiphodon semoni*) in Western Part of Southern Java Waters

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Abstract

Fishing activities negatively impact fish populations, potentially causing a decline in fish stocks. Nevertheless, ensuring diversity and connectivity among populations can mitigate these adverse effects. To evaluate the connectivity of river mouths in the western part of Southern Java waters, we sequenced forty Stiphodon semoni individuals from five populations using mitochondrial cytochrome oxidase subunit 1 as molecular markers. The study revealed that S. semoni populations showed high diversity (0.821), with the population in Cimaja displaying the lowest diversity (0.464). Furthermore, the result of the analysis of molecular variance was a Fst value of 0.0630 with a p-value of 0.22. These results, along with the result of the haplotype network, indicated no significant genetic differences among these populations. This implies that the river mouths in the western part of Southern Java waters are interconnected. The distribution of mismatches showed a single peak, indicating that the populations have undergone demographic expansion. This information could be valuable for the conservation and management of S. semoni in the western part of the Southern Java waters.

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1. Introduction

Fishing activities in estuaries are typically small-scale fisheries, such as those carried out by coastal communities around Palabuhanratu Bay. The fishermen catch larvae and juvenile fish at the mouth of the river, which is also known as nyalawean. This type of fishing activity takes place in several areas of the western part of Southern Java, ranging from Lebak to Cianjur Districts. The activity is carried out once a month during the new moon phase, with the peak period being on the 25th of Hijri (Simanjuntak *et al*., 2021). *Nyalawean* fishing mainly catches larvae and juvenile amphidromous fishes, which are called *impun* in the local language. In the western part of the Southern Java waters, *Stiphodon semoni* larvae and juveniles are among the most abundant and widely distributed targets of *nyalawean* fishing in several river mouths. The Gobiidae family dominates the *impun* composition (Baihaqi *et al*., 2022).

Although *nyalawean* is a small-scale fishery, continuous fishing can lead to the depletion of fish stocks and even cause the extinction of some fish species. Amphidromous fish are particularly vulnerable to population decline because they move between freshwater and marine environments during their lifecycle (McDowall, 2007; O'Dwyer *et al*., 2021). Fishing activities on the river mouth can disrupt the recruitment and survival of these fishes (Selbie *et al*., 2011; Walter *et al*., 2012) because they use the river mouth to recruit. Furthermore, fishing activities in the estuaries can directly impact the fish population (Selbie *et al*., 2011).

Effective management and conservation policies are essential to minimize the negative impact of fishing on fish populations. A well-managed protection system is expected to increase biodiversity levels (Zhao *et al*., 2023). Genetic diversity and structure can serve as indicators of effective protection management (Madduppa *et al*., 2021). Additionally, Sabatino *et al*. (2022) have suggested that identifying patterns of genetic structure, diversity, and connectivity is useful for conservation purposes. The genetic structure can also reveal whether populations are distinctive or intermingling, and this information can improve the effectiveness of management processes (Madduppa *et al*., 2021; Kitada, 2022). Genetic information can also be used to detect hybridization. For instance, Kitada (2022) reported that landlocked Ayu (*Plecoglossus altivelis altivelis*) have interbred with native amphidromous and altered population structure. Furthermore, genetic information can help to differentiate between species and locations (Xuan *et al*., 2021; Afiati *et al*., 2022; Hidayani *et al*., 2022).

Fish conservation management should be based on connectivity because species migration, active migrations, and passive dispersal should be considered when determining the geographic scale at which to enact conservation measures to protect fish species (O'Dwyer *et al*., 2021; Berkström *et al*., 2022; Sabatino *et al*., 2022; Zhao *et al*., 2023). Recent studies suggest that knowledge about connectivity can help identify patterns of migration and gene flow that are crucial for population stability and persistence (Sabatino *et al*., 2022), particularly for diadromous species (Liao *et al*., 2021; Lisi *et al*., 2022; Sabatino *et al*., 2022). Previous studies on *S. semoni* have focused on various aspects, such as the composition and abundance of impugn (Baihaqi *et al*., 2022), recruitment patterns (Simanjuntak *et al*., 2021), temporal variations (Prabowo *et al*., 2022), trophic ecology (Amaliah *et al*., 2023).

However, the genetic diversity and population structure of amphidromous have not been studied. Currently, there is a lack of data on the population structure and river mouth connectivity for amphidromous species in the western part of the Southern Java waters. Therefore, this study seeks to fill the data gap by exploring the connectivity of river mouths in the western part of the Southern Java waters based on the genetic diversity and population structure of the amphidromous goby S. semoni. The results of this study are expected to provide scientific data that can be used to determine appropriate management strategies. Data-based management is expected to be more effective, and the results can also serve as the basis for further studies.

2. Materials and Methods

2.1 Materials

The materials and equipment used in this study were 40 juveniles of *S. semoni*, 96% ethanol, absolute ethanol, Tissue Genomic DNA Mini Kit (Geneaid), primers, ddH_2O, MyTaq HS Red Mix, 2x (Bioline), agarose (Vivantis PC0701), TAE buffer ultra-pure grade (Vivantis PB0940), nucleic acid staining solution (RedSafe), 6X DNA loading dye (Thermo Fisher Scientific), Sequencing Kit (Applied Biosystems), 1L sample bottles, lift net (*sirib*), stereomicroscope (ZEISS Discovery V8), analytical balance (Adam PW 254), vortex mixer (Corning LSE 6775), high-speed microcentrifuge (Corning LSE 6765-HS), analog magnetic stirrer hot plate, block heater digital (HX Mini), UV transilluminators, GeneExplorer thermal cycler (BIOER), submarine electrophoresis system (Mupid-exU), and ABI 3500xL sequencer (Applied Biosystems).

2.1.1 Ethical approval

This study does not require ethical approval because it does not use experimental animals.

2.2 Methods

2.2.1 Sample collection

Samples were collected using a lift net (sirib) with a mesh size of 0.48 mm (Simanjuntak *et al*., 2021). Samples collection was conducted from September to November 2022 following the spring tide period since the larvae and juveniles of diadromous fishes utilize the tide force for recruiting to the estuary (Maeda and Tachihara, 2005; Simanjuntak *et al*., 2021; Prabowo *et al*., 2022). Sampling sites within Palabuhanratu Bay were selected to study the connectivity of river mouths inside the bay. Additionally, to explore the connectivity of river mouths between the inside and outside of the bay, sites on the bay's west and east sides were included. Five river mouths were sampled; three are located inside the Palabuhanratu Bay, while the others are outside the bay (Figure 1). These sites are anticipated to illustrate the connectivity of the river mouths between the bay's western side, inside the bay, to the eastern side. The samples were preserved in 96% ethanol, and since the size of the samples is small, all parts of the body were used for molecular analysis.

ing the manufacturer's instructions. A 670 bp fragment of the cytochrome oxidase I (COI) mitochondrial gene was amplified using primers TelF1-5'TCGACTAAT-CAYAAAGAYATYGGCAC3' and TelR1-5'ACTTCT-GGGTG-NCCAAARAATCARAA3' (Taillebois *et al*., 2013). In this study, mitochondrial DNA (mtDNA), a widely-used molecular marker, served as a molecular marker, renowned for its sensitivity in detecting the population genetic structures of marine fish species (Wang *et al*., 2008). This is attributed to the ample reference data and better evolutionary dynamics for phylogeographic research (Modeel *et al*., 2023). The mtDNA cytochrome oxidase I (COI) gene, in particular, is extensively utilized as a molecular marker for determining population structure and diversity in genetic studies of various amphidromous taxa (Lord *et al*., 2015; Engman *et. al*., 2019; Liao *et al*., 2021).

Polymerase chain reaction (PCR) was performed in 25 µL total volume, consisting of 4.5 µL

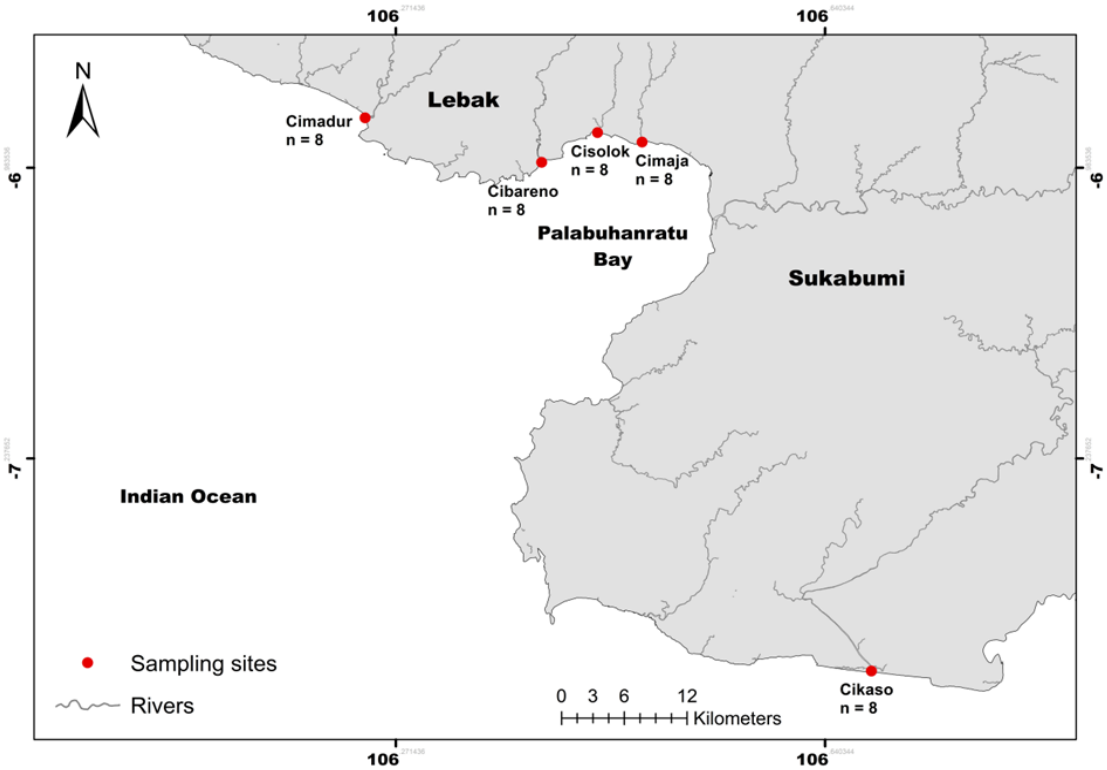

Figure 1. Sampling locations and number of specimens used at each location (n).

2.2.2 Molecular analysis

Samples were first identified morphologically under a stereomicroscope (ZEISS Discovery V8) using available literature (Leis and Carson-Ewart, 2004; Sahami *et al*., 2020) prior to molecular analysis. A total of 40 specimens were used in the molecular analysis, with eight specimens at each sampling site. DNA was extracted in a Tissue Genomic DNA Mini Kit (Geneaid) by following the manufacturer's instructions. A 670 bp fragment of the cytochrome oxidase I (COI) mitochondrial gene was amplified using primers TelF1-5'TCGACTAAT-CAYAAAGAYATYGGCAC3' and TelR1-5'ACTTCT-GGGTG-NCCAAARAATCARAA3' (Taillebois *et al*., 2013). In this study, mitochondrial DNA (mtDNA), a widely-used molecular marker, served as a molecular marker, renowned for its sensitivity in detecting the population genetic structures of marine fish species (Wang *et al*., 2008). This is attributed to the ample reference data and better evolutionary dynamics for phylogeographic research (Modeel *et al*., 2023). The mtDNA cytochrome oxidase I (COI) gene, in particular, is extensively utilized as a molecular marker for determining population structure and diversity in genetic studies of various amphidromous taxa (Lord *et al*., 2015; Engman *et. al*., 2019; Liao *et al*., 2021).

Polymerase chain reaction (PCR) was performed in 25 µL total volume, consisting of 4.5 µL ddH_2O, 1.5 µL each primer, 12.5 µL MyTaq HS Red Mix, 2x (Bioline), and five µL template DNA. The PCR amplifications were carried out in a GeneExplorer thermal cycler (BIOER) by a pre-denaturation step of 5 min at 94 °C followed by 35 cycles of denaturation at 94 °C for 45 s, annealing at 54 °C for 60 s, extension at 72 °C for 60 s with the terminal elongation at 72 °C for 5 min. A 2.5 µL of each PCR product was used for 1.2% agarose gel electrophoresis with 5 µL nucleic acid stain-

ing solution (RedSafe) for verifying the amplified fragment. PCR products were purified using a PCR Product Pre-Sequencing Kit (Applied Biosystems). These purified products were the template DNA for cycle sequencing reactions that run on an ABI 3500xL sequencer (Applied Biosystems). The forward TelF1 and reverse TelR1 primers were used for the direct cycle sequencing reaction.

2.2.3 Analysis data

The sequences were checked and corrected manually using BioEdit v7.7.1 (Hall, 1999). They were aligned using the MUSCLE method with MEGA v11 (Tamura *et al*., 2021) and blasted in the database on NCBI ((National Center for Biotechnology Information). All sequences used in this study were submitted to the GenBank online database (accession numbers PP379809–PP379813, PP379918–PP379922, PP381862–PP381869, PP383968–PP383978, and PP390191–PP390201).

Genetic diversity indices such as the number of haplotypes (H), haplotype diversity (Hd), Jukes-Cantor corrected nucleotide diversity (π; Jukes and Cantor, 1969), number of pairwise differences (K), and number of segregating sites (S) were calculated using the software DNAsp v6 (Rozas *et al*., 2017). Subsequently, the fixation index (Fst; Hudson *et al*., 1992) is used to assess the genetic structure among sampling locations and was estimated by DNAsp v6 with 1000 permutations to evaluate statistical significance. The result of Fst will disclose whether sites are genetically different or pan-mixing. Analysis of molecular variance (AMOVA) (Excoffier *et al*., 1992) was used to further examine genetic differentiation among and within populations.

The median-joining network (Bandelt *et al*., 1999) was constructed in PopART v1.7 (Leigh and Bryant, 2015) to deduce the relationship among all haplotypes. Additionally, the maximum likelihood (ML) tree was reconstructed using MEGA v.11 based on the Jukes-Cantor with a combination discrete gamma distribution model (JC+G) and evaluated with 1000 bootstrap replicates. Model selection was based on the value of AICc (Akaike Information Criterion, corrected); the lowest-value model (JC+G) was selected. Sequences of *S. palawensis* (accession numbers: MK496968) were used as an outgroup. Neutrality (equilibrium) was assessed by calculating the R_2 statistic (Ramos-Onsins and Rozas, 2006) in DNAsp v6; R2 is recommended for small-size samples (Ramírez-Soriano *et al*., 2008).

3. Results and Discussion

3.1 Results

3.1.1 Genetic diversity

A total of 40 mtDNA COI sequences (451 bp) were obtained from five populations of *S. semoni*. of the 451 nucleotide positions, 38 polymorphic sites were observed, including 28 singleton variable sites (6.21 % of 451 bp) and 10 parsimony informative sites. Haplotype and nucleotide diversities ranged from 0.464 ± 0.200 to 1.000 ± 0.063 and 0.0033 ± 0.0008 to 0.0116 ± 0.0034, respectively (Table 1).

The haplotype diversity was considered high, except for the Cimaja population, while the nucleotide diversity of all populations was pretty low. The haplotype diversity of *S. semoni* as metapopulations was 0.821 ± 0.062, while the nucleotide diversity was 0.0071 ± 0.0014 (Table 1). As metapopulations, *S. semoni* in the western part of Southern Java waters showed high genetic diversity.

Considering sample sizes is essential when estimating genetic diversity in populations. Sample size influences the variance and relative error of the estimate, as well as the probability of accepting a false null hypothesis (Bashalkhanov *et al*., 2009; Gorbachev, 2012). The minimum sample size is affected by the marker, species, and the nature of the population, whether it is endemic or widely dispersed (Gorbachev, 2012; Sánchez-Montes *et al*., 2017). Sánchez-Montes *et al*. (2017) suggested that for reliable estimates, the minimum sample size should be around 20 per population. Nonetheless, other studies indicate that sampling a greater number of populations with smaller samples can yield more accurate estimations and reduce biases, as opposed to sampling a smaller number of populations with larger sample sizes (Bashalkhanov *et al*., 2009; Gorbachev, 2012). Gorbachev (2012) noted that sample size alone does not ensure its representativeness. Consequently, owing to budget constraints, our study utilizes eight individuals from five distinct populations rather than twenty individuals from two populations to assess the genetic diversity index of *S. semoni* populations within and outside Palabuhanratu Bay.

Low genetic diversity in the Cimaja population may be due to the high fishing pressure or modification of the habitat. Local communities utilize the Cimaja River by collecting the rocks and using them as construction materials. Cisolok River has same situation as Cimaja where the local community collects the rocks from the river, more than that in Cisolok River there is a sand mine. Even though Cisolok still has a high genetic diversity, the phenomenon of collecting rocks and sand mining can affect the populations in the long term. Many amphidromous fishes, including *S. semoni*, occupy rocky habitats, and they spawn in rocky rivers (Pinacho-Pinacho *et al*., 2023). The Cibareno River that have the highest genetic diversity is more pristine compared to others.

Table 1. Genetic diversity indices of Stiphodon semoni: number of samples (n); number of haplotypes (H); haplotype diversity (Hd), Jukes-Cantor corrected nucleotide diversity (π); number of pairwise differences (K); number of segregating sites (S)

Region	Locations	n	H	Hd	π	K	S
Inside Bay	Cibareno	8	8	1.000 ± 0.063	0.0061 ± 0.0010	2.75	8
	Cisolok	8	5	0.857 ± 0.108	0.0033 ± 0.0008	1.5	4
	Cimaja	8	3	0.464 ± 0.200	0.0050 ± 0.0032	2.25	9
Outside Bay	Cimadur	8	5	0.857 ± 0.108	0.0075 ± 0.0022	3.357	12
	Cikaso	8	5	0.786 ± 0.151	0.0116 ± 0.0034	5.179	16
	Total	40	21	0.821 ± 0.062	0.0071 ± 0.0014	3.173	39

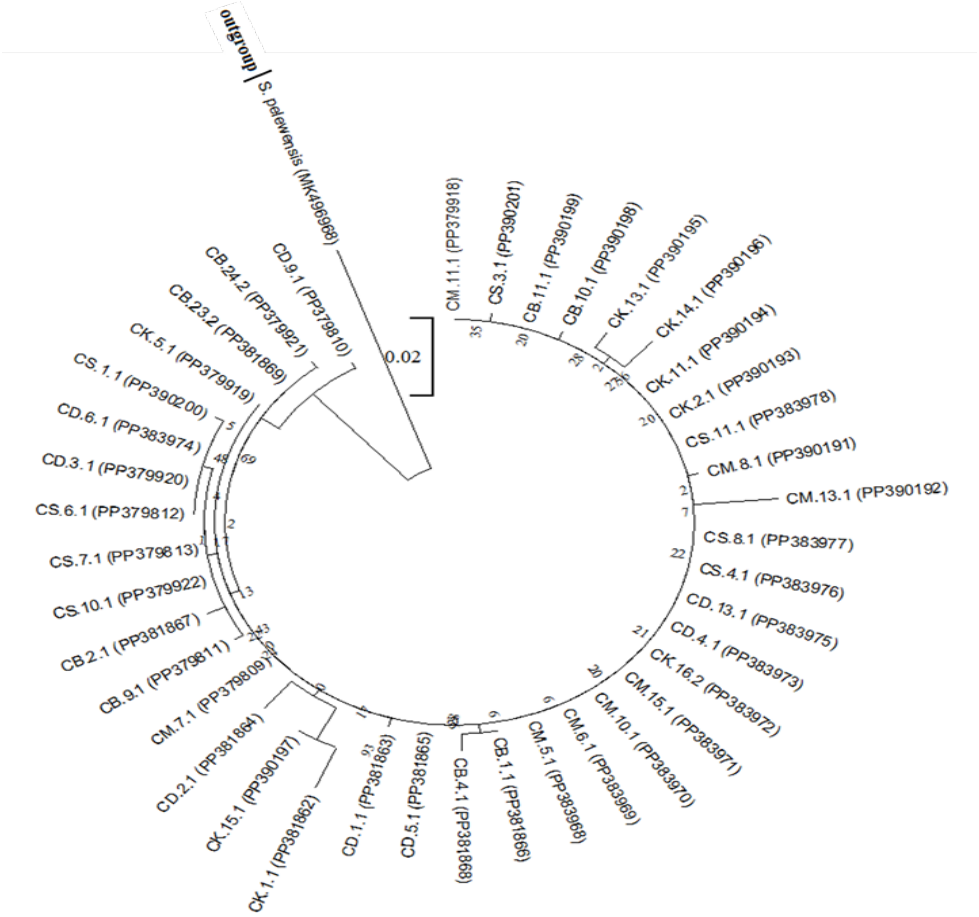

Figure 2. Phylogenetic tree of Stiphodon semoni based on maximum likelihood method. Jukes-Cantor with combination discrete gamma distribution model (JC+G) was used with 1000 bootstrap replicates. Abbreviation represent the sampling locations: CB (Cibareno); CM (Cimaja); CS (Cisolok); CD (Cimadur); CK (Cisolok).

Table 2. AMOVA result of Stiphodon semoni per population, Fst values are under the diagonal and p-values are above the diagonal

	Cibareno	Cisolok	Cimaja	Cimadur	Cikaso
Cibareno		0.29	0.18	0.29	0.31
Cisolok	0.042		0.24	0.4	0.33
Cimaja	0.091	0.118		0.24	0.38
Cimadur	0.013	-0.036	0.025		0.33
Cikaso	0.094	0.124	0.057	0.065	

Populations of *S. semoni* in the western part of Southern Java waters have high haplotype and low nucleotide diversities. The same pattern, high haplotype and low nucleotide diversities are similar to *S. percnopterygionus* (Lord *et al*., 2015), amphidromous gobies of a different genus (Taillebois *et al*., 2013; Liao *et al*., 2021; Abdulmalik-Labe *et al*., 2023), and other non-finfish species (Madduppa *et al*., 2021; Zhao *et al*., 2023). High haplotype diversity but low nucleotide diversity implies a bottleneck, a significant reduction in population size (Liao *et al*., 2021). The ability to adapt to environmental stressors may be reduced if populations have experienced population bottlenecks (Perrier *et al*., 2017; Klerks *et al*., 2019). However, the limited sample size in this study may lead to false positive results regarding the bottleneck (Peery *et al*., 2012; Sabatino *et al*., 2022). Therefore, further evaluation using a larger and more robust dataset is necessary, along with power estimates to detect bottlenecks across various possible demographic histories.

The population of *S. semoni* in the western part of Southern Java waters as metapopulation, however, is considered to have high genetic diversity. Wide-range distribution of amphidromous may cause high genetic diversity, active migration, and passive dispersal, leading to the transfer of genetic material between different populations (Madduppa *et al*., 2021). A high number of unique and diverse haplotypes also impact the genetic diversity of a population (Garcia *et al*., 2021). Moreover, Sabatino *et al*. (2022) stated that the result of genetic diversity is also influenced by markers used in the study. The choice of genetic markers can significantly influence the perceived genetic diversity within species. For instance, the genetic diversity of *Alosa alosa* was reported to be higher than that of *A. fallax* when analyzed using microsatellite markers (Sabatino *et al*., 2022). However, this finding is at odds with studies employing mtDNA and allozymes (Alexandrino *et al*., 2006; Faria *et al*., 2012). The discrepancy arises because microsatellites exhibit greater variability than mtDNA and allozymes, with the chosen markers being subject to mutation rates, genetic drift, and selection (Galtier *et al*., 2009; Sabatino *et al*., 2022). Moreover, certain markers can identify the preliminary phases of speciation, while others cannot (Cheng *et al*., 2019; Xuan *et al*., 2021; Orlova *et al*., 2024). The number of samples can also influence the estimation of genetic diversity, impacting more on the dispersion of values than the precision of the measurement (Gorbachev, 2012), as well as the result of statistical significance due to sampling variation (Xuan *et al*., 2021). Therefore, additional testing with more markers throughout the genome and an increased sample size is necessary to obtain comprehensive information.

Genetic diversity has a potential effect, directly or indirectly, on the population and might follow the impact on the community and ecosystem levels (Hughes *et al*., 2008). High genetic diversity will increase the population's ability to respond to changes in environmental conditions (Madduppa *et al*., 2021). Thus, the loss of genetic diversity will reduce the population's ability to adapt. The low genetic diversity in the Cimaja population may be due to overfishing or habitat modification. Madduppa *et al*. (2021) reported that the most exploited population has lower genetic diversity than others. Populations with low genetic diversity are vulnerable to rapid decline or extinction, although they may still exist in nature. The population of *S. semoni* within Palabuhanratu Bay exhibits greater genetic diversity, with a diversity index of 0.833, compared to the 0.817 index of those outside the bay. This disparity is attributed to gene flow, the transfer of genetic material across different populations, and gene flow, which is known to enhance genetic diversity significantly (Madduppa *et al*., 2021). The absence of geographical barriers inside the bay facilitates higher gene flow, allowing for more population mixing.

3.1.2 Genetic structure and distribution

The maximum likelihood phylogenetic tree showed clades with no significant differentiation of *S. semoni* based on location, and there was no significant split of clades among populations (Figure 2). The degree of pairwise population Fst showed that the genetic differences among populations ranged from -0.0362 to 0.1241. The highest genetic difference was between Cikaso and Cisolok (0.1241), while the closest populations were Cisolok and Cimadur (Table 2). The genetic difference between the Cikaso population and other populations was considered moderate, even though all the pairwise Fst values were not statistically significant in all populations ($p > 0.05$; Table 2). Moreover, the result of the AMOVA test as metapopulation of *S. semoni* was a Fst value of 0.0630 with a p-value of 0.22, indicating that Fst was insignificant.

A total of 21 haplotypes were found: 2 shared haplotypes (hap1 and hap4) and 19 private haplotypes. Hap1 was the only haplotype shared by five populations, while hap4 was shared only by two populations (Figure 3). The frequency of hap1 was the highest with a value of 42.5%, while the remaining haplotypes were under 10%, less frequent. Shared haplotype in all five populations suggested no evidence of genetic structure within these populations. The result of the median-joining haplotype network is supported by the values of pairwise Fst, which are very low and insignificant among all locations (Table 2).

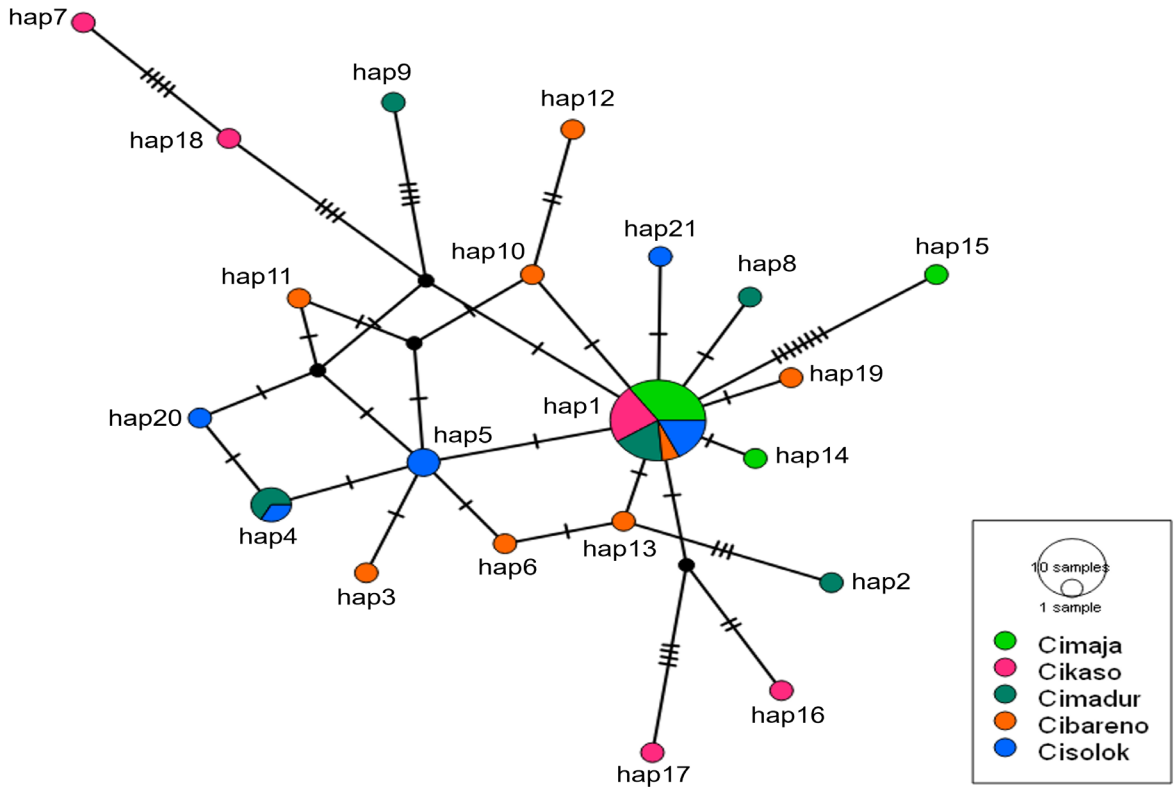

Figure 3. Median-joining network of *Stiphodon semoni*. Colors represent different sampling locations; the size of the pie chart is proportional to the number of individuals; the dash represents a single mutation step, and each black dot represents haplotypes not collected in this study.

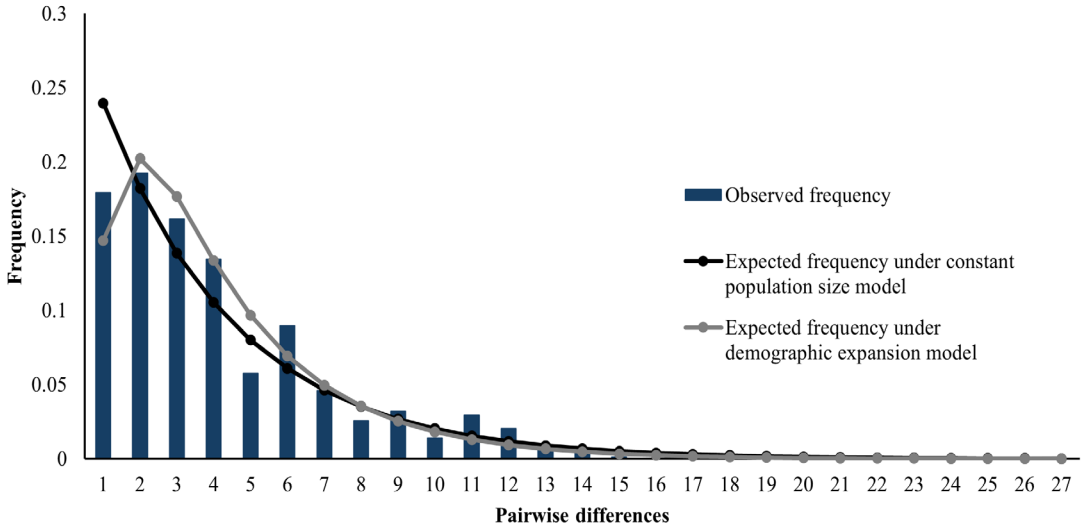

Figure 4. Mismatch distribution of *Stiphodon semoni* populations in western part of Southern Java waters

The result of equilibrium analysis showed that the R_2 value was significant (p-value = 0.044), indicating more unique nucleotide site variants than expected under a neutral evolution model. It can be the result of population expansion. The observed value closely matches the expected frequency under the demographic expansion model, and it appears that the mismatch distribution appeared unimodal (Figure 4). The significant value of R_2 and unimodal mismatch distribution indicated that the *S. semoni* in the western part of Southern Java waters has undergone population expansion recently. Furthermore, this is supported by the haplotype network.

The reconstruction of the phylogenetic trees indicates that no distinct clades are formed based on location for *S. semoni* populations. This suggests that

the populations are either closely related genetically or derived from a common ancestor. Furthermore, the haplotype network shows that the most common haplotype was hap1, and about 2 of 5 samples had hap1. Shared haplotypes existed between distant populations (Cimadur and Cikaso), indicating that the dispersal of this species may occur over long distances (Zhao *et al*., 2023). This also suggests that shared haplotypes are related to high migration rates and large population sizes (Eastwood *et al*., 2016; Zhao *et al*., 2023).

A low value of Fst indicated high gene flow between populations (Madduppa *et al*., 2021). Freshwater species with an amphidromous life cycle exhibit higher dispersal ability and potentially a lower degree of population structure (Lord *et al*., 2012; Lord *et al*., 2015). Despite, some diadromous species migrating to locations where they originate from, natal homing (McDowall, 2001; Lohmann and Lohmann, 2019), other individuals sometimes migrate to distinct locations from where they are born and then they reproduce in their non-natal locations which leads to the high gene flow (Salmenkova, 2017).

The results of mismatch distribution and neutrality showed that populations of *S. semoni* in the western part of Southern Java waters have undergone a recent demographic expansion. Populations that expanse spatially will have lower Fst value and higher gene flow than constant populations (Liao *et al*., 2021). This phenomenon leads to no genetic structure among populations of *S. semoni* in the western part of Southern Java waters. The same result was also reported for other amphidromous fishes (Watanabe *et al*., 2006; Lord *et al*., 2015; Liao *et al*., 2021). The inter-population genetic homogeneity and intra-population genetic variability may result from frequent gene flow through passive dispersal pelagic larval duration (PLD) in the ocean (Watanabe *et al*., 2006; Lord *et al*., 2015; Liao *et al*., 2021). Generally, the longer the PLD, the wider the range of dispersal (Hoareau *et al*., 2007; McDowall, 2007; Hall *et al*., 2019). The short PLD of amphidromous species may constrain the larval dispersal and account for endemism (Lord *et al*., 2010; Liao *et al*., 2021). Therefore, we hypothesize that larvae of *S. semoni* have long PLD and high dispersal in the western part of Southern Java waters.

The ocean current around the region and tidal current may play a key role in dispersing to neighboring rivers in Palabuhanratu Bay and rivers outside the bay, even though passive transportation of larval fish by oceanic currents is complicated. Hohenlohe (2004) reported that spawning season, length of PLD, characteristics of planktonic environments, seasonal variation of currents, and other biological and physical factors may influence dispersal. Furthermore, Sabatino *et al*. (2022) stated that while straying among populations within the regions is common, individuals regularly return to their natal river if possible.

The study indicates that the populations of *S. semoni* in the western part of Southern Java waters have high gene flow and low subdivision, and thus should be treated as pan-mixing populations. Therefore, a common conservation and management plan should be developed to cover all regions. Cibareno River, which has high genetic diversity, could be a potential protected area. The protected areas with high genetic diversity also had a higher management effectiveness (Liu *et al*., 2021; Zhao *et al*., 2023). Cibareno River has the potential to serve as a reservoir and a source of migrants for nearby rivers. This study, however, has several limitations, including a small sample size, limited geographical scope, and the use of a restricted number of molecular markers. To comprehensively understand the connectivity of river mouths in the western part of Southern Java waters, it is necessary to conduct further studies on the population genetic structure using additional markers a larger sample size, and by including different species of amphidromous fish. Moreover, expanding the study area to include river mouths located on the east side of the Cimadur River and on the west side of the Cikaso River would provide broader geographical coverage in subsequent studies. Additional crucial studies include the ecological interactions, comparative phylogeography, and the early life history of *S. semoni*, encompassing pelagic larval duration, growth rates, and patterns of larval dispersal.

4. Conclusion

The Haplotype diversity of *Stiphodon semoni* is high in most populations, except for the Cimaja population, while nucleotide diversity is low in all populations. However, when considered as a metapopulation, the genetic diversity of *S. semoni* is high. The genetic difference among populations is insignificant, and it indicates that there is no genetic structure within these populations. This result is supported by populations undergoing demographic expansion. Cibareno River has the potential to be a protected area for conservation efforts. Nevertheless, further studies are necessary to fully understand the connectivity of river mouths in the western part of Southern Java waters. This includes studies on genetic diversity and population structure using other markers and larger samples, as well as considering other species of amphidromous fish and expanding the study locations, further east from the Cimadur River and further west from the Cikaso River. In addition, studies on ecological interactions, comparative phylogeography, and early life history of *S. semoni*, including pelagic larval duration, growth rate, and larval dispersal patterns

are also crucial to conduct.

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Authors' Contributions

All authors have contributed to the final manuscript. Each author's contribution is as follows: AR collected the data, analyzed the data, drafted the manuscript, and designed the figures and tables. CPHS, S, and AS devised the main conceptual ideas, evaluated the final data, and critically revised the article. All authors discussed the results and contributed to the final manuscript.

Conflict of Interest

All the authors declare that they have no competing interests upon the publication of this paper.

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