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Mitochondrial phylogeny and genetic diversity of Hampala barb (*Hampala macrolepidota*) from the Brantas River, Indonesia

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Abstract. *Amalia E, Sufaichusan I, Dailami M, Faqih AR, Widodo MS, Wiadnya DGR, Kusuma WE. 2025. Mitochondrial phylogeny and genetic diversity of Hampala barb* (Hampala macrolepidota) *from the Brantas River, Indonesia. Biodiversitas 26: 5694-5702.* Hampala barb (*Hampala macrolepidota*), a native freshwater fish species in the Brantas River, East Java, is currently under severe threat due to intense fishing and environmental perturbations. The urgency of this situation necessitates the immediate implementation of effective genetic management and conservation programs, which rely on basic scientific information such as genetic characterization. This study aimed to assess the phylogenetic relationships, genetic diversity, population structure, and demographic history of *H. macrolepidota* using a 1,095 bp sequence of the mitochondrial DNA cytochrome b from 23 individuals collected across four sampling locations. Phylogenetic analysis showed that *H. macrolepidota* from the Brantas River clustered together and formed a single group with very shallow genetic divergence. Population structure analyses (both Φst and AMOVA) showed high gene flow and an absence of significant genetic clustering, suggesting the sampled area constitutes a single, interconnected population unit. The combination of high haplotype diversity (hd: 0.714-1.000) but low nucleotide diversity (π: 0.00156-0.00728), along with the negative Tajima's D and Fu's Fs values, suggested that the species has likely experienced a bottleneck effect followed by a rapid expansion in the past. This decline of genetic diversity points to a reduced long-term adaptive potential, causing the species to be highly vulnerable to future environmental changes, despite its current abundance. The results highlight the urgent need for effective genetic management and conservation programs for the species to focus on maintaining genetic connectivity and minimizing anthropogenic pressures to preserve the genetic integrity and long-term viability of the population. Future studies incorporating nuclear or genome-wide markers would provide finer resolution of population structure and complement the mitochondrial data presented here.

Keywords: Conservation, cytochrome b, demographic history, genetic management, population structure

INTRODUCTION

Hampala barb (*Hampala macrolepidota* (Kuhl & Van Hasselt, 1823)) is a native freshwater fish belonging to the family Cyprinidae widely distributed across Indonesia, including Sumatra, Borneo, and Java Islands (Eschmeyer et al. 2016; Froese and Pauly 2025). This fish typically occurs in clear water with moderate or low current, inhabiting small and large rivers, lakes, as well as water canals, but is not found in small creeks, torrents, and shallow swamps (Cottet and Visser 2017). The body identification process is by distinctive body color pattern, a dark bar between the dorsal and pelvic fins, red coloration in the caudal fin with thick black stripes along the edges, and barbels longer than the eye width (Rainboth 1996; Vidthayanon 2002). In Java, *H. macrolepidota* is considered an important species with high economic value, both as an ornamental fish and for human consumption (Makmur et al. 2020; Hidayat et al. 2023).

The Brantas River is the second-longest river on Java after the Solo River and is located in the eastern part, spanning approximately 320 km (Hartini and Dewi 2021). The Brantas River is critically important, serving as a major water source and being widely used for industrial purposes, agricultural irrigation, hydropower, and domestic activities by local communities (Priatna et al. 2016; Waskitho and Wibowo 2024). However, continuous anthropogenic activities, particularly heavy water pollution, habitat degradation and alteration, overfishing, and the introduction of alien invasive species have the potential to cause several negative impacts. These cumulative pressures in the Brantas River significantly contribute to population declines, increased inbreeding risk, disruption of gene flow, and reduced population connectivity in freshwater fish species. Consequently, this situation increases the risk of significant genetic diversity loss, which can lead to localized population extinctions (Dudgeon et al. 2006; Pavlova et al. 2017; Manel et al. 2020).

In East Java, Indonesia, *H. macrolepidota* can be found in lakes, dams, and rivers, particularly in the Brantas River. Currently, there have been no reports of population decline for *H. macrolepidota* in the Brantas River, although several native species, such as *Neolissochilus soro* (Valenciennes, 1842) and *Channa gachua* (Hamilton, 1822), have become increasingly difficult to find (Listyarini et al. 2022; Kusuma et al. 2024). This species is also classified as "Least Concern" by the IUCN Red List of Threatened Species (IUCN 2025). Despite its ecological and economic importance, there is currently a lack of genetic information

on *H. macrolepidota* in the Brantas River i.e., to date, no studies have assessed the genetic diversity, population structure or demographic history of this species in this region. Due to the significant environmental perturbations occurring in the Brantas River and the importance of genetic data in guiding effective conservation and management strategies, monitoring the genetic diversity of *H. macrolepidota* populations is essential for detecting early signs of population fragmentation or loss of genetic diversity, even in the absence of visible population decline.

Genetic diversity is a fundamental component of a species' long-term evolutionary potential, enhancing the ability to survive and adapt in response to environmental changes. It also plays a significant role as the primary basis for selective breeding in genetic improvement programs in aquaculture (Milla et al. 2021; Zhang et al. 2022). In this context, mitochondrial DNA (mtDNA) markers have been widely used and proven effective for studying genetic diversity, phylogenetic relationships, and population structure in various fish species. mtDNA is maternally inherited, showing high mutation rate, generally lacks recombination, and is devoid of introns (Li et al. 2019; Mar'ie and Allam 2019). Among the 13 protein-coding genes within the mitochondrial genome, cytochrome b (Cyt*b*) is commonly used for intraspecific genetic characterization in fish. The Cyt*b* gene has been applied in phylogenetic reconstruction and genetic diversity analysis in several cyprinid fish species, including *Hampala* spp. (Ryan and Esa 2006), *Rasbora lateristriata* (Bleeker, 1854) (Kusuma et al. 2016), *Puntius* complex (Ren et al. 2020), and *Barbus* sp. (Rossi et al. 2021).

In this study, field sampling was performed to collect *H. macrolepidota* from the Brantas River. Subsequently, the mtDNA Cyt*b* gene was sequenced to assess genetic diversity, intraspecific phylogenetic relationships, population structure, and historical population dynamics. The results were expected to provide a valuable foundation for future genetic management and effective conservation strategies of *H. macrolepidota*, particularly for populations inhabiting the Brantas River, East Java, Indonesia.

MATERIALS AND METHODS

Specimen collection and identification

Field sampling was performed to collect *Hampala macrolepidota* from the Brantas River, East Java, Indonesia, between February 2023 and April 2024 (Figure 1). Twenty-three individuals of *H. macrolepidota* were collected from four localities, with the geographic coordinates of each sampling site provided in Table 1. Although the sample size per locality was relatively small (4-7 individuals), this was due to low capture success, despite our repeated sampling efforts. The four sampling locations were selected to represent the spatial and ecological variation of the Brantas River, including upstream and downstream sections of the river. All specimens were obtained using fishing rods and morphologically identified following the diagnostic criteria described by Robert (1986). A small portion of the right pectoral fin was excised from each species, preserved in 99% ethanol in a 1.5 mL microcentrifuge tube, and stored at -20°C for DNA extraction. Whole-body specimens were fixed in 10% formalin and transferred to 70% ethanol for long-term preservation. Furthermore, preserved specimens were deposited at the specimen depository, Depositorium Ichthyologicum Brawijayae, Faculty of Fisheries and Marine Science, Universitas Brawijaya, Indonesia, with voucher numbers listed in Table 1.

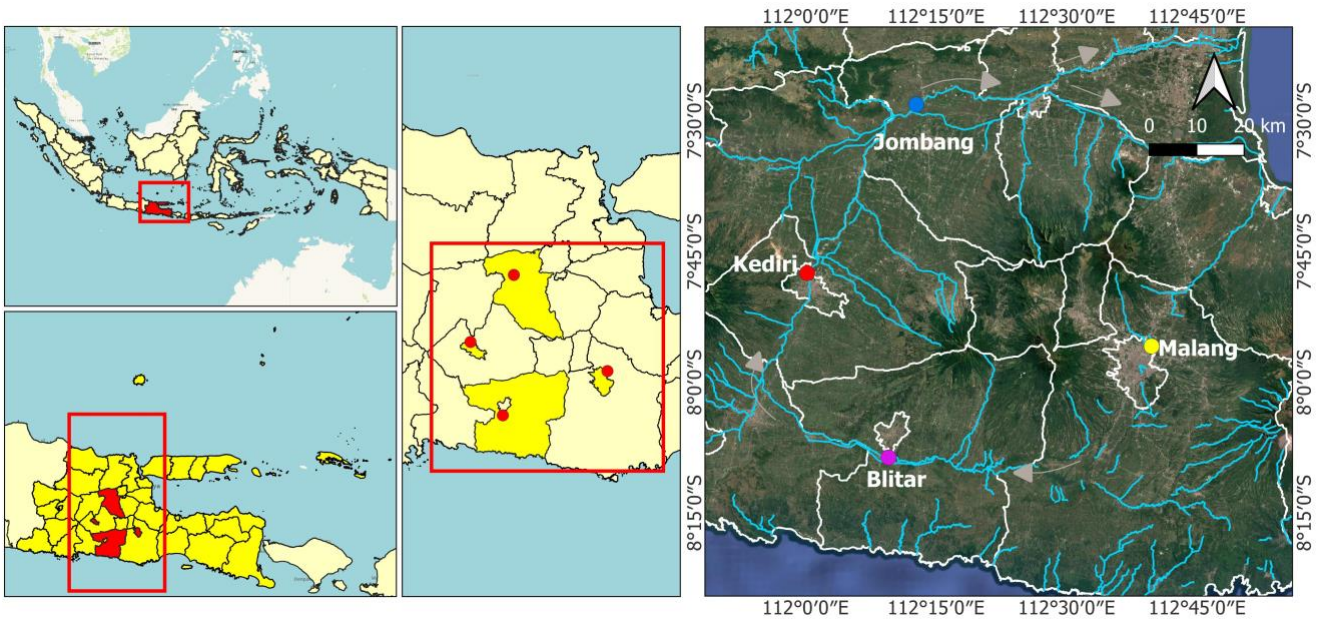

Figure 1. Sampling locations of *Hampala macrolepidota* in the Brantas River, East Java, Indonesia. The grey arrows indicate the direction of river flow

Table 1. Sampling locations of *Hampala macrolepidota* in the Brantas River, East Java, Indonesia

Locations detail	Sample size	Geography coordinates		Voucher number	GenBank accession number
		S	E		
Arjosari, Blimbing, Malang	7	7°55'49.9"	112°39'46.0"	UB.1722.1-7	PV537214-20
Karangsari, Sukorejo, Blitar	6	8°06'41.7"	112°09'42.8"	UB.1723.1-6	PV537225-30
Ngampel, Mojoroto, Kediri	4	7°47'26.7"	112°00'12.1"	UB.1724.1-4	PV537221-24
Tanggungkramat, Ploso, Jombang	6	7°28'05.9"	112°12'43.6"	UB.1725.1-6	PV537231-36

DNA extraction, PCR amplification, and sequencing

Genomic DNA was extracted from preserved fin tissue using the Wizard® Genomic DNA Purification Kit (Promega, Madison, Wisconsin, USA) following the manufacturer's protocol. A fragment of the mitochondrial cytochrome b (Cyt*b*) gene was amplified through Polymerase Chain Reaction (PCR) using the primer pair LA-Cyp (5′-ATGGCAAGCCTACGAAAAAC-3′) and HA-Cyp (5′-TCGGATTACAAGACCGATGCTT-3′). This primer has been frequently used to elucidate phylogenetic relationships of cyprinid fishes (e.g., Tang et al. 2010). PCR amplification was performed using a miniPCR® thermal cycler (miniPCR bio™, Cambridge, Massachusetts, USA). Each 30 µL PCR reaction mixture contained 15 µL of GoTaq® Green Master Mix (Promega), 2 µL of genomic DNA, 1 µL of each primer (forward and reverse), and 11 µL of nuclease-free water. A negative control (using nuclease-free water in place of template DNA) was included in all PCR runs to monitor for potential contamination, and no amplification was observed in these controls. The thermal cycling conditions were as follows: initial denaturation at 94°C for 2 minutes, 40 cycles of denaturation at 94°C for 45 seconds, annealing at 58°C for 45 seconds, and extension at 72°C for 60 seconds, followed by a final extension at 72°C for 7 minutes (Kenthao and Jearranaiprepame 2020). The quality of PCR amplification was assessed by electrophoresis on a 1.5% agarose gel in 1× Tris-Borate-EDTA buffer, stained with GelGreen® Nucleic Acid Gel Stain (Biotium, Fremont, California, USA). Subsequently, electrophoresis was conducted using the GELATO miniPCR biosystem at 100 volts for 30 minutes. The PCR products were submitted to Apical Scientific Sdn. Bhd. (Selangor, Malaysia) for both directions Sanger sequencing.

Phylogenetic and haplotype network analyses

The resulting electropherogram data for each sequence were manually inspected, corrected, and trimmed using Chromas v.2.6.6 (Technelysium Pty Ltd, South Brisbane, Australia) to ensure high-quality peaks and to correct any ambiguous base calls. Consensus sequences were generated from forward and reverse reads with Unipro UGENE v.1.31.1 (Okonechnikov et al. 2012). The sequences obtained in this study, along with outgroup sequences downloaded from GenBank, were then manually arranged and aligned using Mesquite v.3.5.1 (Maddison and Maddison 2018). The final alignment matrix was unambiguously aligned, confirmed to maintain the 1,095 bp length of the Cyt*b* fragment. The outgroup sequences used for phylogenetic analysis included the Cyt*b* gene of *Hampala dispar* (Smith, 1934) (GenBank accession number: AP011245) and *Hampala salweenensis* (Doi & Taki, 1994) (MW548258). These species were selected as they were the only congeneric species with comparable Cyt*b* data of sufficient length available in GenBank. Phylogenetic analysis was conducted using the Maximum Likelihood (ML) method. The phylogenetic tree was reconstructed using RAxML (Stamatakis 2014) implemented in raxmlGUI v.2.0 (Edler et al. 2021) with a random starting tree. We employed 1,000 bootstrap replicates to assess node support and the other parameters were set to default. The General Time Reversible model with Gamma distribution and Invariant sites (GTR+G+I) was selected as the best-fit substitution model based on the Bayesian Information Criterion, as implemented in jModelTest v.2.1.3 (Posada 2008). The topology with the highest likelihood score was retained. Additionally, a haplotype network was constructed using Network v.10.2.0.0 (Fluxus Technology Ltd., UK) through the Median-Joining Method (Bandelt et al. 1999).

Genetic diversity, population structure, and demographic history analyses

Genetic diversity indices, including the number of haplotypes (nh), haplotype diversity (hd), and nucleotide diversity (π), were calculated using DnaSP v.6.0 (Rozas et al. 2017). The assessment of genetic differentiation between *H. macrolepidota* locations in the Brantas River was based on pairwise Φst values. At the same time, population structure was evaluated using Analysis of Molecular Variance (AMOVA) implemented in Arlequin v.3.5.2 (Excoffier and Lischer 2010). Demographic history was inferred using Tajima's D (Tajima 1989) and Fu's Fs (Fu 1996) neutrality tests, which was designed to evaluate departure from neutrality under the assumption of constant population size and mutation rate, which was also performed in DnaSP v.6.0. Statistical significance for neutrality indices was evaluated using 10,000 coalescent simulations.

RESULTS AND DISCUSSION

Sequence characteristics

Specimens of *Hampala macrolepidota* were successfully collected from four different locations along the river, namely Malang, Blitar, Kediri, and Jombang. Partial mtDNA Cyt*b* sequences of 1,095 bp were successfully obtained from 23 individuals and submitted to the GenBank database under accession numbers PV537214 to PV537236 (Table 1). A total of 17 haplotypes were identified among these sequences, with H1 and H9 shared by individuals

from different locations (Table 2). Overall, 26 segregating sites were detected, where 10 were parsimony-informative and 16 were singletons. While our sample size per locality was relatively modest, previous studies have shown that limited sample size (≥5) can still provide reliable insight into patterns of genetic diversity and population structure, particularly when care is taken in interpretation (Goodal-Copestake et al. 2012; Phillips et al. 2019). Nevertheless, we recognize that increasing sample sizes in future work would further enhance confidence in these estimates.

Phylogenetic and haplotype network

Phylogenetic analysis is used to investigate inter- and intraspecific relationships and the evolutionary processes shaping organisms (Dornburg and Near 2021; Cano-Barbacil et al. 2022). Similarly, haplotype network reconstruction provides insights into mutation patterns, relationships among species, and microevolutionary dynamics (Paradis 2018; Selle et al. 2021). In this study, phylogenetic analysis of *H. macrolepidota* based on mtDNA Cyt*b* sequences showed no distinct genetic clustering among the 17 haplotypes identified across four locations in the Brantas River (Malang, Blitar, Kediri, and Jombang) (Figure 2). The lack of clear phylogenetic structure, with haplotypes intermingled among locations, suggested a high degree of genetic exchanges among populations throughout this river system. Furthermore, the haplotype network analysis corroborated the phylogenetic reconstruction results, showing no distinct genetic clustering among the haplotypes. This suggested that the population was not strongly genetically differentiated across the sampled range (Figure 3).

Table 2. Distribution of Mitochondrial DNA Cyt*b* haplotypes among *Hampala macrolepidota* from four locations in the Brantas River, East Java, Indonesia

Haplotype no.	Locations			
	Malang	Blitar	Kediri	Jombang
H1	4	1	1	
H2	1			
H3	1			
H4	1			
H5			1	
H6			1	
H7			1	
H8		1		
H9		1		1
H10		1		
H11		1		
H12		1		
H13				1
H14				1
H15				1
H16				1
H17				1

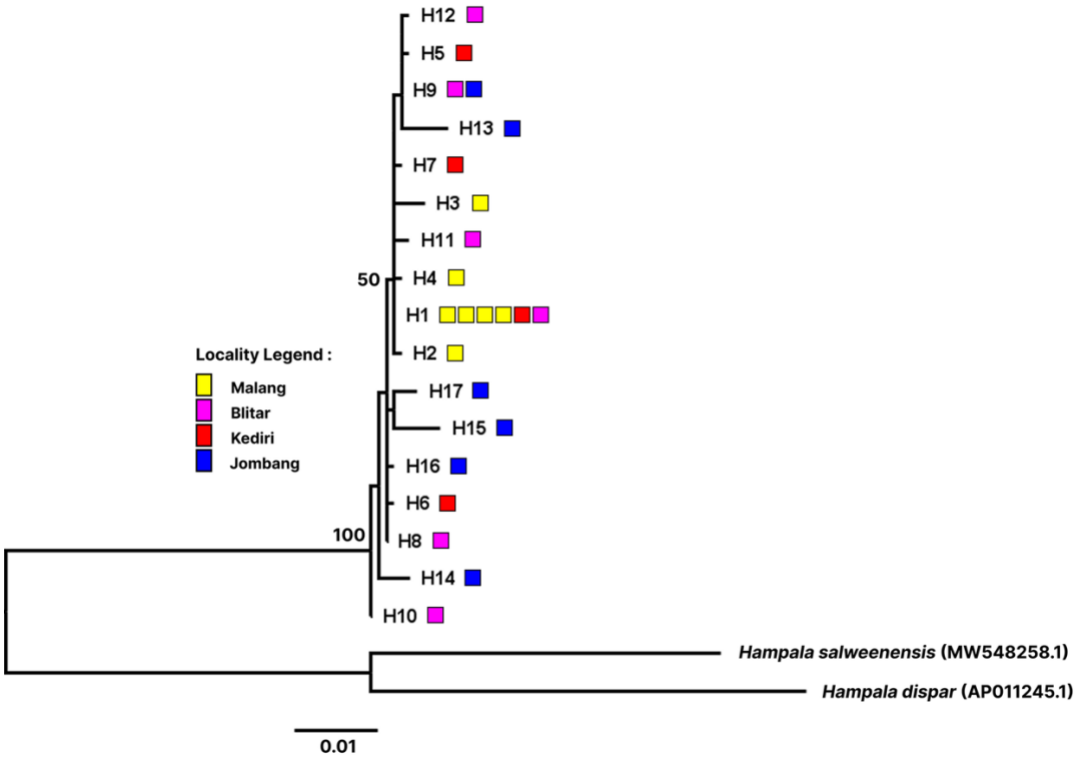

Figure 2. Maximum likelihood tree of *Hampala macrolepidota* based on partial mitochondrial DNA Cyt*b* haplotype. The number of squares represents the number of individuals (refer to Table 2 for the members of each haplotype). Different colors represent different sampling locations associated with each haplotype as depicted in the figure legend. Bootstrap values were shown only with values ≥50%. Bootstrap analysis was performed using 1,000 replicates

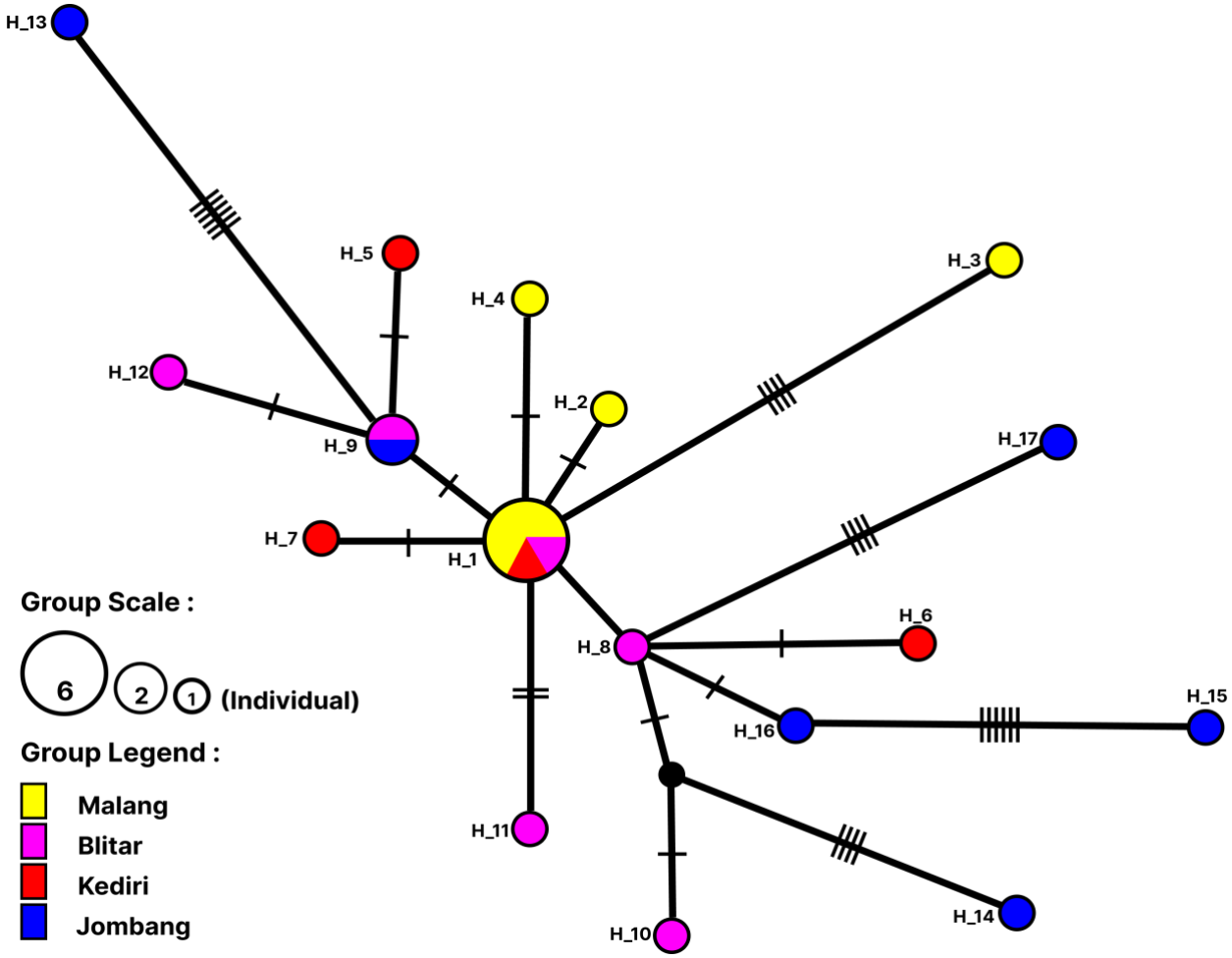

Figure 3. Median-joining network showing relationships among mtDNA Cyt*b* haplotypes of *Hampala macrolepidota* in the Brantas River. The size of each circle is proportional to its relative haplotype frequency with different colors indicating different locations as indicated in the figure legend. The number of dashed lines represents the number of mutations that separate two haplotypes. A black circle indicates the median vector or missing haplotype

The presence of shared haplotype (H1 and H9) in *H. macrolepidota* from two or more different locations further supports the hypothesis of an interconnected population unit or recent divergence with ongoing gene flow. This pattern of shared haplotype and genetic homogeneity can show ongoing gene flow facilitated by the continuous and connected nature of the Brantas River system, allowing for dispersal and interbreeding among populations (Martin et al. 2018; Gonzalez et al. 2019). Based on observation, this pattern was also found from previous results in freshwater fish species with similar ecological and life history traits, where a continuous river system without major geographic barriers facilitated genetic exchange in *Astyanax mexicanus* (De Filippi, 1853) (Pérez-Rodríguez et al. 2021), *Prochilodus lineatus* (Valenciennes, 1837) (Perini et al. 2021), and *Labeo gonius* (Hamilton, 1822) (Roy et al. 2024) species.

Genetic diversity

Genetic diversity is an essential aspect for species as a fundamental source of the adaptive ability of organisms in response to environmental perturbations (He et al. 2025). According to the criteria proposed by Grant and Bowen (1998), haplotype diversity can be considered high when the value hd>0.5, while the value π>0.005 is for nucleotide diversity. In this study, genetic diversity analyses for *H. macrolepidota* from four different locations in the Brantas River indicated high haplotype diversity values in all populations (hd: 0.714-1.000). Meanwhile, nucleotide diversity measured was found to be low in Malang, Blitar and Kediri (π: <0.005). However, the locality of Jombang is a noteworthy exception, displaying a high nucleotide diversity value (π: 0.00728) that surpasses the threshold for low diversity, unlike the upstream sites (Table 3). While we cannot rule out the possibility that these results are influenced by the modest sample size per locality in this study, one plausible explanation is that individuals of *H. macrolepidota* tend to drift or disperse more easily downstream. This causes downstream populations tend to receive more individual source than upstream and, thus, accumulate more intraspecific genetic diversity (higher genetic diversity) compared to the upstream ones (Paz-Vinas et al. 2015; Washburn et al. 2020). Similar patterns i.e., high haplotype diversity but low nucleotide diversity were reported from previous studies on several other cyprinids species, such as *Anaecypris hispanica* (Steindachner, 1866) (Alves et al. 2001), *Chondrostoma*

lusitanicum (Collares-Pereira, 1980) (Mesquita et al. 2001), and *Barbodes semifasciolatus* (Günther, 1868) (Wang et al. 2023). Genetic diversity of *H. macrolepidota* from the Brantas River had higher hd but lower π compared to two species of *H. macrolepidota* and *H. bimaculata* (Popta, 1905) from Malaysian populations. *Hampala macrolepidota* from this region had hd = 0.686 and π = 0.0093, while *H. bimaculata* had hd = 0.617 and π = 0.0105 (Ryan and Esa 2006). The mtDNA Cyt*b* is particularly useful for assessing genetic diversity at the population level due to its relatively high mutation rate, which allows the detection of recent evolutionary events (Li et al. 2019; Zhai et al. 2019a).

Generally, the genetic diversity pattern observed in *H. macrolepidota* in this study was characterized by high haplotype diversity coupled with low nucleotide diversity. The high value of haplotype diversity occurred from a rapid accumulation of mutations, while the development of nucleotide polymorphisms typically required a longer period to accumulate (Bowen et al. 2001; Souza-Shibatta et al. 2018; Zhang et al. 2020). The combination of high haplotype diversity and low nucleotide diversity was often indicative of historical demographic events, suggesting that the population of *H. macrolepidota* in the Brantas River could experience a sudden reduction in size due to a bottleneck or founder event, followed by rapid population growth (Grant and Bowen 1998). Furthermore, contemporary anthropogenic pressures, such as water pollution, habitat degradation and alteration, overexploitation, as well as the introduction of non-native species, could contribute to a reduction in effective population size (Pavlova et al. 2017; Martinez et al. 2018; Blanton et al. 2025; Cavalcante et al. 2025). In particular, industrial and organic pollution might act as a strong selective pressure, rapidly eliminating sensitive genotypes and thus contributing directly to the low nucleotide diversity observed across upstream populations. This pattern of depressed genetic diversity has been documented in other river systems in Java. For example, analysis of the COI gene in the related cyprinid, *Barbodes binotatus* (Valenciennes, 1842), showed the absence of both haplotype and nucleotide diversity (Astuti et al. 2020). Such findings underscore the severity of genetic erosion affecting freshwater fish populations across the region.

Population genetic structure

Based on the measurement of the pairwise Φst index, the results showed that the highest level of differentiation was between Jombang and Malang (p-value>0.001) at 0.17593 while the remaining comparisons provided values between 0.00090 to 0.05828 (Table 4). The value of Φst<0.05 was categorized as low genetic differentiation, while high and significant differentiation was indicated by Φst>0.15 (Wright 1978; Hall 2022). The genetic differentiation among populations of *H. macrolepidota* in the Brantas River falls into the low category. Only Φst between Malang and Blitar is classified as having moderate genetic differentiation. These results indicated that *H. macrolepidota* in the Brantas River had high gene flow and genetic exchange. Meanwhile, AMOVA analysis showed a high variation within populations (94.33%) as presented in Table 5. AMOVA analysis also revealed no statistically significant genetic differentiation among sampling localities (p value = 0.055). Based on the results, genetic variation occurred within rather than between populations, indicating that no statistically supported population genetic structure was detected among populations of *H. macrolepidota* in the Brantas River.

Both Φst and AMOVA analyses showed low genetic differentiation and a lack of population genetic structure among *H. macrolepidota* in the Brantas River. These results suggest that *H. macrolepidota* in this river system can be considered a single, near-panmictic population. The absence of genetic structuring was due to ongoing gene flow among populations (Zhai et al. 2019b; Pereira et al. 2023). This flow can be facilitated by migration connectivity patterns that are not constrained by physical or geographic barriers (LaCava et al. 2021), allowing for unrestricted movement and genetic exchange in the river system (Ferreira et al. 2017). While genetic connectivity is currently high, future alterations to the river system, such as the construction of dams, habitat fragmentation, or changes in tributary connectivity, could disrupt these natural dispersal pathways and potentially reduce gene flow among populations. Therefore, preserving continuous hydrological connections should be a key consideration in future conservation and river management efforts.

Table 4. Results of pairwise genetic differentiation (Φst) of four locations of *Hampala macrolepidota* in Brantas River, Indonesia

Location	Malang	Kediri	Blitar	Jombang
Malang	0.00000			
Kediri	0.03441	0.00000		
Blitar	0.05828	-0.08698	0.00000	
Jombang	0.17593*	0.00090	0.00907	0.00000

Note: The Φst number with (*) represents a significant difference with p-value<0.01

Table 3. Results of genetic diversity indices and demographic history parameters of *Hampala macrolepidota* based on Cyt*b* gene sequences

Locations	n	nh	hd (±SD)	π (±SD)	*S*	*D*	*Fs*
Malang	7	4	0.714±0.181	0.00156±0.00070	6	-1.524120	-0.428120
Blitar	6	6	1.000±0.096	0.00249±0.00054	7	-0.630950	-3.513880
Kediri	4	4	1.000±0.177	0.00227±0.00059	5	-0.796840	-1.513810
Jombang	6	6	1.000±0.096	0.00728±0.00129	20	-0.541450	-1.311880

Note: n: Number of samples, nh: Haplotype number, hd: Haplotype diversity, π: Nucleotide diversity, S: Number of segregating sites, *D*: Tajima's D value, *Fs*: Fu's Fs value, SD: Standard Deviation

Table 5. Results of Analysis of Molecular Variance (AMOVA)

Source of variation	d.f.	Sum of squares	Variance components	Percentage of variation
Among populations	3	7.248	0.10825 Va	5.67
Within populations	19	34.218	180094 Vb	94.33
Fixation indices (FST) 0.05670 (p-value = 0.055)				

Demographic history

Tajima's D (Tajima 1989) and Fu's Fs (Fu 1996) are commonly used neutrality tests that can provide insights into historical fluctuations in effective population size, indicating whether the population has experienced expansion or contraction (Rougemont et al. 2020; Loog 2021). In this study, both Tajima's D and Fu's Fs values were negative across all *H. macrolepidota* populations in the Brantas River, suggesting recent population expansion. Tajima's D values ranged from -1.524120 to -0.541450, while Fu's Fs values also showed consistent negativity across all populations (-3.513880 to -0.428120). The neutrality tests for both Tajima's D and Fu's Fs were statistically non-significant in all populations. This result is likely due to the small sample size used in this study.

A value of Tajima's D = 0 suggests neutral evolution and a stable population size. In comparison, negative D values show an excess of low-frequency polymorphisms, typically interpreted as a signature of population expansion or purifying selection (Baselga et al. 2015; Zhang et al. 2015; Kim et al. 2017). The consistently negative Tajima's D values observed in the four populations of *H. macrolepidota* in the Brantas River suggest the presence of many rare alleles, which is indicative of recent population bottlenecks or genetic drift followed by rapid population expansion. Genetic drift, particularly when associated with a reduction in effective population size, is often followed by a recovery phase characterized by population growth (Zhai et al. 2019b). This interpretation is further supported by the negative Fu's Fs values across all populations, as Fs<0 is typically associated with recent demographic expansion (Kitada et al. 2017; Kim et al. 2022).

In this study, *H. macrolepidota* from four locations along the Brantas River showed a pattern of high haplotype diversity but low genetic differentiation and nucleotide diversity, high gene flow, absence of population structure, and signals of recent population contraction followed by expansion. The results showed that *H. macrolepidota* in the Brantas River currently exists as a single or near-panmictic population with substantial genetic connectivity among sampling locations. However, given that this study is based on a single gene of mitochondrial DNA, future research incorporating nuclear or genomic markers would improve the resolution of fine-scale population structure. For future genetic management and conservation, the results suggested the importance of maintaining river connectivity to support natural gene flow and prevent population fragmentation. Therefore, conservation strategies should prioritize habitat preservation, pollution control, and mitigation of anthropogenic disturbances that could reduce effective population size or disrupt existing genetic exchange. Regular genetic monitoring should be conducted to identify early signs of genetic erosion or changes in population structure, allowing for proactive management (Schwartz et al. 2007; Andersson et al. 2022). Because of their ecological and economic importance, these measures are crucial to ensure the long-term sustainability of *H. macrolepidota* in the Brantas River ecosystem.

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