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Genetic and morphological analyses of Ngongok fish (*Hampala macrolepidota*) from Ngebel Lake, Ponorogo District, Indonesia

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Abstract. *Amalia E, Paricahya AF, Dailami M, Faqih AR, Kusuma WE. 2026. Genetic and morphological analyses of Ngongok fish* (Hampala macrolepidota) *from Ngebel Lake, Ponorogo District, Indonesia. Biodiversitas 27 (4): d270410. https://doi.org/10.13057/biodiv/d270410.* *Hampala macrolepidota* is a freshwater fish widely distributed in Southeast Asia and plays an important ecological and economic role. However, increasing anthropogenic pressures raise concerns about population structure and genetic integrity. This study aimed to confirm species identity and assess genetic diversity and population differentiation using an integrated morphological and molecular approach. Specimens were collected from Ngebel Lake (Ponorogo, East Java) and compared with populations from East Java, South Sumatra, and Thailand. The morphological identification and inventory profile of *H. macrolepidota*, combined with genetic studies based on Cytochrome b (Cytb) of mitochondrial DNA (mtDNA) at the population level in Ngebel Lake, have not been explored before. Ngongok fish in Ngebel Lake has been confirmed as *H. macrolepidota* based on morphological and genetic studies. *H. macrolepidota* sequences from Ngebel Lake represent the Insular Asia region separated from the Mainland Asia region population based on haplotype network, phylogenetics, and genetic p-distance measurements. The *H. macrolepidota* population in Ngebel Lake has historically experienced rapid growth, as indicated by high genetic diversity (hd = 1.0000, $\pi = 0.0086$) and a favorable demographic history (D = 0.6479, Fs = -0.4208). The genetic closeness of *H. macrolepidota* and *Cyprinus carpio* presents potential for hybridization in the future. Despite morphological similarity across regions, genetic divergence highlights the importance of molecular data in resolving population-level differences. These findings suggest that *H. macrolepidota* populations are shaped by geographic and biogeographic barriers, with potential implications for conservation and management. The observed genetic patterns also suggest a potential hybridization risk under increasing environmental pressure, although further investigation using nuclear markers is required.

Keywords: Cytochrome b, demographic history, haplotype, Ngongok fish, phylogenetic

INTRODUCTION

High human activity in Ngebel Lake may impose anthropogenic pressure on aquatic organisms, particularly indigenous species. Ngebel Lake is a volcanic lake at the foot of Mount Wilis (Luthfi 2017; Sumarsono et al. 2020) and supports native fauna, including Ngongok fish (*Hampala macrolepidota* (Kuhl & Van Hasselt, 1823)) and Java barb (*Barbonymus gonionotus* (Bleeker, 1849)) (Haraningtias et al. 2018). Fish mass mortality has been reported annually, especially in May and August (Yudiantoro et al. 2020). Such events can be associated with algal blooms, oxygen depletion (Njiru et al. 2015), disease, pollutants (Bedassa 2019), pathogen infection (Raja et al. 2019), climate change (Reddythota and Timetowes 2022), upwelling and sediment elevation (Wiradana et al. 2022), and they are often linked to anthropogenic impacts (Baladia et al. 2025). In Ngebel Lake, these pressures are intensified by fish farming (Prasetyowati et al. 2014), tourism, and community activities (Saddhono et al. 2019), and a hydroelectric power plant (Yudiantoro et al. 2020). Introduced fishes such as common carp (*Cyprinus carpio* (Linnaeus, 1758)) (Haraningtias et al. 2018) and Nile tilapia (*Oreochromis niloticus* (Linnaeus, 1758)) (Nugroho et al. 2022) further increase the risk of predation, competition, disease transmission, habitat change, and hybridization (Gozlan et al. 2010). These conditions justify studies in Ngebel Lake using both morphological and genetic approaches.

H. macrolepidota is widely distributed in Indonesia and the Greater Sundaland region, and its ecological role deserves documentation in multiple localities. In Indonesia, this species is also known as *Sasau* (Risdawati 2011), *Kemencut*, *Arongan*, *Sebarau* (Makmur et al. 2014), and *Palung* (Melisa et al. 2021; Angkasawan et al. 2022). It is a carnivorous predator and a top predator in Ranau Lake (Makmur et al. 2018; Permana and Delami 2022), suggesting ecological importance in Ngebel Lake as well. However, morphological identification within *Hampala* remains challenging because species in this genus show ambiguous morphological profiles (Doi and Taki 1994; Taki and Kawamoto 1997), and environmental pressure may also promote adaptive variation (Kristensen et al. 2020). Morphological measurements of *H. macrolepidota* have been reported from Thailand (Doi and Taki 1994), Ranau Lake, South Sumatra (Makmur et al. 2018), and from Kalimantan, Sumatra, and Java (Restuningsih et al. 2023). Nevertheless, the report from Java does not provide a clear locality, and no specific publication has documented the morphological measurements of *H. macrolepidota* from Ngebel Lake, Ponorogo, Indonesia, is not available.

Genetic analysis can be combined with morphological analysis for both identification and population health assessment purposes. Species confirmation through genetic analysis using nuclear DNA (nDNA) (Wang et al. 2007; Moghaddam et al. 2013) and mitochondrial DNA (mtDNA) (Bingpeng et al. 2018; Zeng et al. 2018) is a widely applied method. Advanced analysis at the population level of freshwater fish can be applied using the mtDNA region, including for population health purposes (Lamadi et al. 2023), or to observe distribution (Paricahya et al. 2024). In freshwater fish, mitochondrial variation can be applied with Cytochrome C Oxidase I (COI) (Baisvar et al. 2019; Asiah et al. 2020), NADH dehydrogenase subunit 5 (ND5) (Nabilsyafiq et al. 2019), Cytochrome C Oxidase II (COII) (Zhao et al. 2020), Cytb (Alam et al. 2021; Kuang et al. 2021; Kim et al. 2023), Displacement loop (D-loop) (Pan et al. 2021; Wang et al. 2021; Nishida et al. 2025), and NADH Dehydrogenase subunit 1 (ND1) (Das and Chakraborty 2025). Genetic analysis in *H. macrolepidota* using Cytb has been previously studied in Malaysia (Ryan and Esa 2006); however, there have been no publications from the Java populations. Furthermore, genetic research on *H. macrolepidota* in another region, specifically the COI, has already been conducted in Ranau Lake, South Sumatra (Makmur et al. 2014).

This study aims to inventory the species conditions and population scope of *H. macrolepidota*, using a morphological profile approach and genetic analysis based on the Cytb gene, in Ngebel Lake, Ponorogo. We hypothesize that morphological characters are sufficient to confirm the species identity of *H. macrolepidota* across sampling sites, while mitochondrial Cytb sequences will reveal significant population-level genetic differentiation among Ngebel Lake, East Java, and Mainland Asia populations. Specifically, we expect Indonesian (Insular Southeast Asia) populations to be more genetically similar to each other than to the Mainland Asia population, reflecting geographic isolation and restricted gene flow.

MATERIALS AND METHODS

Sampling site

Specimens in this study were collected at Ngebel Lake, Ngebel, Ponorogo District, East Java, Indonesia, at coordinates 7°47'23.57"S and 111°38'01.47"E. Sampling was performed using fishing rods in May and June 2024. The geographical conditions of the sampling area are visualized in Figure 1.

Morphological identification

Species in the genus *Hampala* are widely distributed in Asia, and four of its seven species are reported to be found in Indonesia. The *Hampala* species in Indonesia, based on scientific publications, is *Hampala bimaculata* (Popta, 1905) (Makmur et al. 2014; Soetignya et al. 2016), *Hampala ampalong* (Bleeker, 1852) (Makmur et al. 2014; Afriansyah et al. 2023), *Hampala macrolepidota* (Suryaningsih et al. 2018; Panitvong and Hui 2025), and *Hampala lopezi* (Herre, 1924) (Iskandar et al. 2020). The genus *Hampala* in Indonesia is distributed only in Greater Sundaland, and the distribution of *Hampala* on Java Island includes *H. macrolepidota* (Makmur et al. 2014).

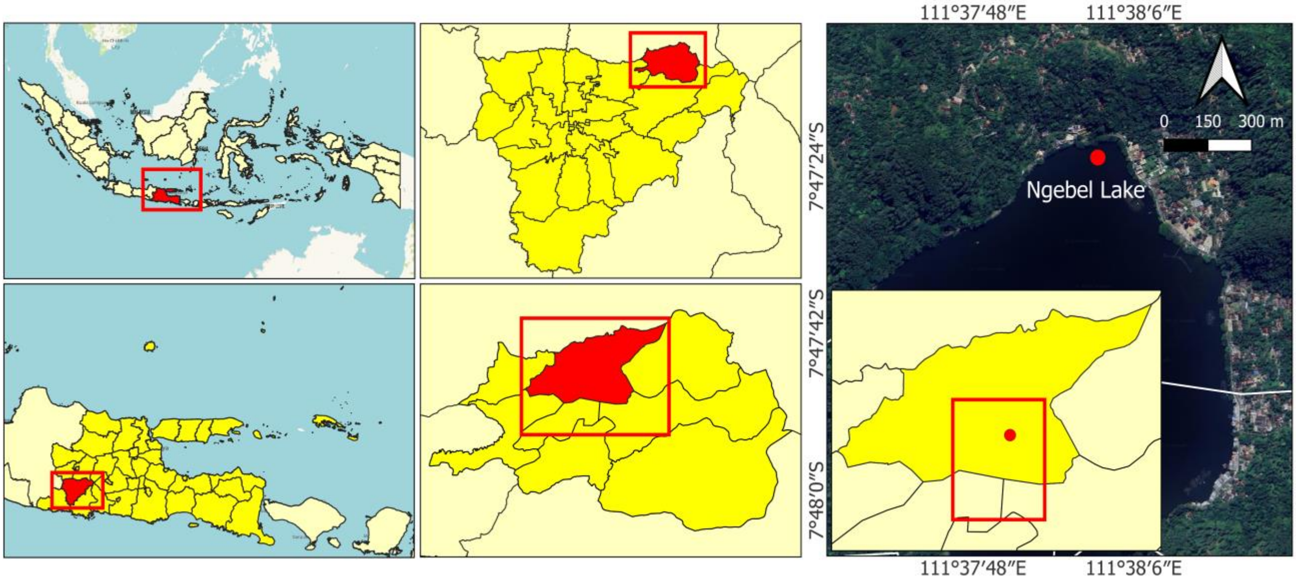

Figure 1. Location of *Hampala macrolepidota* sample collection at Ngebel Lake, Ngebel, Ponorogo District, East Java, Indonesia

Confirmation of the genus *Hampala* from Ngebel Lake, Ponorogo, East Java, can be based on the illustration (Figure 2) and the literature (Inger and Kong 1962). Species in the genus *Hampala* differ from other genera in the Cyprinidae family based the ventral edge not keeled (G1); no symphyseal knob on the mandible (G2); lateral line mostly curves slightly downwards (G3); deep groove from snout and upper lip (G4, towards the subfamily Cyprininae); lateral line ending below middle of the caudal base (G5); the last simple dorsal rays denticulate on posterior border (G6); proportional head structure without ridges and folds (G7); and only one pair of barberls (G8, towards the genus *Hampala*). Species identifiers can follow the identification key below:

1. a. Generally, no black margin in the upper and lower lobes of the caudal fin→ 2
   b. Black margin in the upper and lower lobes of the caudal fin (S1) → 3
2. Two blotches on the body, aligned below the dorsal fin and above the anal fin; maximum size around 15.5 cm TL → *Hampala ampalong*
3. a. Longitudinal black stripes along the body; maximum size around 8.5 cm → *Hampala lopezi*
   b. No longitudinal black stripes along the body (S2) → 4
4. a. Two vertical blotches in adult stages, the blotch sometimes becomes circular when they mature; maximum size around 50 cm → *Hampala bimaculata*
   b. Only one vertical blotch in the adult stage, blotch on the posterior body is generally only found during the juvenile stage (S3); maximum size around 70 cm → *Hampala macrolepidota*

A total of 11 Ngongok fish specimens from Ngebel Lake, Ponorogo, East Java, were identified, and morphological measurements (Figure 3) confirmed that they were *H. macrolepidota*. Five individuals were tagged, and then 20 mg of muscle tissue was taken for DNA extraction. Morphological measurement results were then compared with those of other specimens from 3 different locations, in East Java, totaling 14 individuals (4 individuals from Malang, 4 individuals from Jombang, and 6 individuals from Banyuwangi), the other locations, South Sumatra (5 individuals), and Thailand (15 individuals).

Genetic analysis

The DNA of *H. macrolepidota* from Ngebel Lake, Ponorogo, East Java, was isolated using the Wizard® Genomic DNA Purification (Promega, Madison, Wisconsin, USA) protocol. After homogenizing the muscle tissue, EDTA and nucleic lysis solution were added. The Cytb sequence was amplified using the Polymerase Chain Reaction (PCR) method, with 15 µL GoTaq Green (Promega Madison, Wisconsin, USA), 11 µL Nucleic Acid-Free Water, 2 µL isolate, and each 1 µL from primer LA-Cyp: 5'-`ATGGCAAGCCTACGAAAAAC`-'3 and HA-Cyp: 5'-`TCGGATTACAAGACCGATGCTT`-'3 (Tang et al. 2010). The PCR reaction process was carried out using the protocol: pre-denaturation at 94°C for 2 min, followed by 40 cycles of denaturation at 94°C for 45 s, annealing at 58°C for 45 s, and extension at 72°C for 60 s, then ended with a final extension at 72°C for 7 min (Kenthao et al. 2018). The amplicons were subjected to electrophoresis on a 1.5% agarose gel in 1X Tris-Borate-EDTA and GelGreen Nucleic Acid Stain (Biotium, Fremont, San Francisco, USA). The electrophoresis process lasted for 30 min at 100 volts. The amplicon samples were then sequenced using the Sanger method by Apical Scientific Sdn Bhd (Selangor, Malaysia).

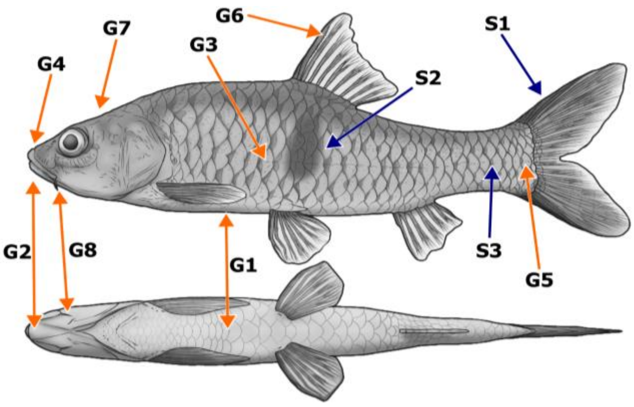

Figure 2. Illustration of a Ngongok fish specimen (*Hampala macrolepidota*) with morphological characters. Codes G1 to G8 are key identifiers leading to the genus *Hampala*, while codes S1 to S3 are key species-level diagnostic identifiers leading to *Hampala macrolepidota*. All codes can be matched with the explanation of morphological identification in the text

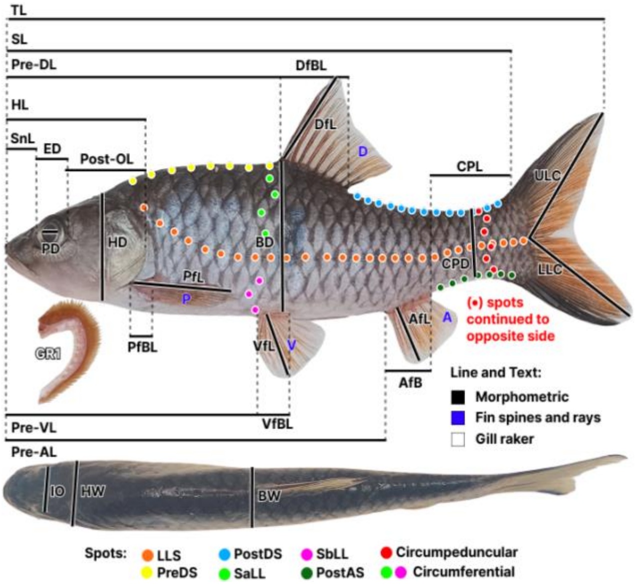

Figure 3. Morphometric and meristic measurements of *Hampala macrolepidota* in this study. TL: Total Length, SL: Standar Length, Pre-DL: Pre-Dorsal Length, Pre-VL: Pre-ventral Length, Pre-AL: Pre-anal Length, HL: Head Length, SnL: Snout Length, ED: Eye Diameter, PD: Pupil Diameter, HD: Head Depth, PfBL: Pectoral fin Base Length, PfL: Pectoral fin Length, VfBL: Ventral fin Base Length, VfL: Ventral fin Length, DfBL: Dorsal fin Base Length, DfL: Dorsal fin Length, AfBL: Anal fin Base Length, AFL: Anal Fin Length, CPD: Caudal Peduncle Depth, CPL: Caudal Peduncle Length, ULC: Upper Lobe Caudal, LLC: Lower Lobe Caudal, IO: Inter-Orbital width, HW: Head Width, BW: Body Width, LLS: Lateral Line Scale, Pre-DS: Pre-Dorsal Scale, Post-DS: Post-Dorsal Scale, SaLL: Scale above Lateral Line, SbLL: Scale below Lateral Line, Post-AL: Post-Anal Scale, Circumpeduncular, and Circumferential

Data analysis

The length of the assembled electrophoregram sequences data was around 1,095 bp, analyzed and edited using Chromas v.2.6.6 (Technelysium Pty Ltd, South Brisbane, Australia), then the forward and reverse sequences consensused using Unipro UGENE v.1.31.1. All sequences in this study are aligned using Mesquite v.3.5.1. Analyses of genetic p-distance and phylogenetic reconstruction were conducted using MEGA v.11. Phylogenetic reconstruction was analyzed using Maximum Likelihood estimation (ML), with Hasegawa-Kishino-Yano substitution model (HKY), using the (G+I) Gamma distribution and Invariant sites, followed by 1,000 replications. Genetic data were also analyzed for genetic diversity and demographic history through neutrality tests and goodness-of-fit analyses using DnaSP v.6.0 and Arlequin v.3.5.2. The haplotype network was reconstructed using the Network software (Fluxus Technology Ltd, UK), with Median Joining estimation.

RESULTS AND DISCUSSION

Morphology

The morphometric and meristic data for *Hampala macrolepidota* from Ngebel Lake in this study were compared with those of *H. macrolepidota* from several populations in East Java (Malang, Jombang, and Banyuwangi), also with *H. macrolepidota* from Ranau Lake, South Sumatra (Makmur et al. 2014), and Thailand (Doi and Taki 1994), in Tables 1 and 2, respectively.

The morphometric measurement results, specifically SL in each population as follows: Ngebel Lake, Ponorogo (11 individuals) ranged from 116.0-207.0 mm, East Java (14 individuals) ranged from 109.0-132.0 mm, South Sumatra (5 individuals) ranged from 124.0-132.0 mm, and Thailand (15 individuals) ranged from 71.8-244.6 mm; based on morphological measurements that were compared with other populations of *H. macrolepidota* from Thailand (Doi and Taki 1994) and South Sumatra (Makmur et al. 2014), the Ngongok fish from Ngebel Lake is *H. macrolepidota*. Several parameters that were not measured in the comparative data were still measured in this study, such as the morphometric data BW, HD, HW, Pre-AL, Pre-VL, VfBL, PfBL, ULC, LLC (Table 1), and meristic data V, Pre-DS, Post-DS, Post-AS, CFS, GR (Table 2). The similarity of meristic data in 4 populations for characters D, V, A, and CPS was observed. Still, an anomaly was found in the Pre-DS from the East Java population, as comparative data in this study, with a minimum of 9 scales. In contrast, other *H. macrolepidota* populations were consistent with 10 scales. The morphometric characters of *H. macrolepidota* in this study reveal differences in Pre-AL, Pre-VL, and PfBL; however, some measurements show overlapping results among the populations, including Pre-DL, LLD, DfBL, VfBL, ULC, LLC, and LJL.

Table 1. Morphometric measurements of *Hampala macrolepidota* from Ngebel Lake, with several sets of comparative data. The comparative data are from East Java (research data of specimens from Malang, Jombang, and Banyuwangi), Thailand (Doi and Taki 1994), and South Sumatra (Makmur et al. 2014). All measurements are in millimeters (mm)

	Ngebel Lake, Ponorogo (n = 11)			**East Java (n = 14)**			**South Sumatra (n = 5)**			**Thailand (n = 15)**		
	Min	**Max**	**Mean**	**Min**	**Max**	**Mean**	**Min**	**Max**	**Mean**	**Min**	**Max**	**Mean**
TL	194.0	245.0	223.0	133.0	160.0	146.5	157.0	172.0	165.6	-	-	-
SL	116.0	207.0	161.5	109.0	132.0	130.2	124.0	132.0	127.8	71.8	244.6	-
BD	46.2	53.7	49.9	28.2	32.2	30.2	36.2	40.2	38.0	29.6	34.4	31.7
BW	18.9	24.4	21.6	12.1	13.1	12.6	-	-	-	14.3	17.7	15.7
HL	51.0	65.0	58.0	35.0	41.0	38.0	32.9	40.8	37.3	30.3	34.3	32.1
HD	29.8	35.9	32.8	17.6	22.8	20.5	-	-	-	18.5	22.3	20.5
HW	23.4	27.9	25.7	14.6	18.1	16.4	16.4	19.2	17.2	-	-	-
CPD	19.8	23.2	21.5	13.1	15.0	14.0	15.7	17.8	16.5	13.0	14.3	13.5
CPL	32.4	36.2	34.3	20.8	27.4	24.1	20.1	21.7	20.8	16.7	19.1	17.9
Pre-DL	94.0	115.0	104.5	61.0	73.0	67.0	62.8	67.6	66.9	53.4	55.8	54.7
Pre-AL	122.0	153.0	137.5	78.0	98.0	88.0	-	-	-	74.2	77.7	75.8
Pre-VL	82.0	106.0	94.0	55.0	72.0	63.5	-	-	-	51.2	55.3	53.2
LLD	29.0	41.0	35.0	22.0	29.0	25.5	25.8	31.1	29.4	20.9	23.8	22.2
DfBL	22.0	29.0	25.5	14.0	27.0	20.5	16.7	19.4	18.4	14.7	17.6	16.6
LLA	21.0	30.0	25.5	14.0	19.0	16.5	15.8	19.9	18.3	14.4	17.0	15.6
AfBL	14.0	19.0	16.5	9.0	11.0	10.0	10.7	11.8	11.4	9.2	10.7	10.5
LLV	25.0	30.0	27.5	16.0	21.0	18.5	20.4	23.1	21.6	16.2	18.1	17.0
VfBL	8.0	10.0	9.0	5.0	9.0	7.0	-	-	-	-	-	-
LLP	30.0	39.0	34.5	21.0	26.0	23.5	22.9	26.2	24.7	17.7	20.7	18.7
PfBL	9.0	11.0	10.0	5.4	7.2	6.3	-	-	-	-	-	-
ULC	46.0	61.0	53.5	34.0	38.0	36.0	-	-	-	31.2	34.2	32.3
LLC	38.0	52.0	45.0	29.0	45.0	35.0	-	-	-	26.8	32.0	30.1
SnL	13.0	15.0	14.0	8.0	9.9	9.0	11.2	12.8	12.0	32.0	36.9	34.9
ED	9.0	11.0	10.0	7.0	8.0	7.5	8.3	9.8	9.2	-	-	-
PD	5.0	6.0	5.5	3.0	4.0	3.5	-	-	-	5.6	13.7	-
MxBL	14.1	17.9	16.0	8.9	11.4	10.1	-	-	-	70.2	100.0	89.4
IO	7.0	9.0	8.0	4.0	5.0	4.5	5.1	6.6	5.7	28.5	35.5	32.8
UJL	13.0	19.0	16.0	10.0	11.0	10.5	12.9	15.4	14.4	-	-	-
LJL	11.0	17.0	14.0	8.0	12.0	9.5	-	-	-	-	-	-

Note: The symbol (-) in the table represents the absence of data measured

Table 2. Meristic measurements of *Hampala macrolepidota* from Ngebel Lake, with several sets of comparative data. The comparative data are from East Java (research data of specimens from Malang, Jombang, and Banyuwangi), Thailand (Doi and Taki 1994), and South Sumatra (Makmur et al. 2014)

	Ngebel Lake, Ponorogo (n = 11)			**East Java (n = 14)**			**South Sumatra (n = 5)**			**Thailand (n = 15)**		
	Min	**Max**	**Mean**	**Min**	**Max**	**Mean**	**Min**	**Max**	**Mean**	**Min**	**Max**	**Mean**
D	IV.8	IV.8	IV.8	IV.8	IV.8	IV.8	IV.8	IV.8	IV.8	IV.8	IV.8	IV.8
P	14	15	14.5	12	14	13	15	16	-	14	16	15
V	9	9	9	9	9	9	-	-	-	9	9	9
A	III.5	III.5	III.5	III.5	III.5	III.5	III.5	III.5	III.5	III.5	III.5	III.5
LLS	27	28	27.5	24	28	26	28	28	28	25	27	25.9
SaLL	5	5	5	5	5	5	5	5	5	4.5	4.5	4.5
SbLL	3	3	3	3	3	3	3	3	3	2.5	2.5	2.5
Pre-DS	10	10	10	9	10	9.5	-	-	-	10	10	10
Post-DS	11	14	12.5	12	14	13	-	-	-	-	-	-
Post-AS	8	8	8	7	7	7	-	-	-	-	-	-
CPS	12	12	12	12	12	12	12	12	12	12	12	12
CFS	8	9	8.5	8	9	8.5	-	-	-	-	-	-
GR	1+6	2+9	1.8+7.2	2+6	2+8	2+6.9	-	-	-	1 + 8	2 + 9	1.4 + 8.4

Note: The symbol (-) in the table represents the absence of data measured

Table 3. Nucleotide variation per site from *Hampala macrolepidota* Ngebel Lake, Ponorogo

Sequence	**Accession number**	**57**	**75**	**90**	**162**	**270**	**351**	**405**	**444**	**540**	**709**	**729**	**741**	**762**	**849**	**855**	**912**	**978**	**1083**
PNG001	PV765901	C	A	C	C	C	A	C	C	T	C	T	C	A	T	T	C	A	C
PNG002	PV765902	T	G	T	T	T	.	T	.	.	A	.	T	T	C	C	T	G	T
PNG003	PV765903	.	.	.	.	.	G	.	G	T	.	.	.	T	.	.	.	.	.
PNG004	PV765904	T	G	T	T	T	.	.	.	.	.	.	T	T	C	C	.	G	T
PNG005	PV765905	T	G	T	T	T	.	.	.	.	.	C	T	T	C	C	.	G	T

Sequence characteristics

In this study, a total of 5 samples of *H. macrolepidota* from Ngebel Lake, Ponorogo, were analyzed using a genetic approach based on partial Cytb sequences, each with a length of 1,095 bp. Sequences can be accessed at https://www.ncbi.nlm.nih.gov/ with the (Accession Number: PV765901-PV765905). A total of 5 sequences used in this study are sufficient to provide intrapopulation genetic information of *H. macrolepidota* from Lake Ngebel, Ponorogo, East Java. Five sequences were obtained (Table 3), representing 5 haplotypes, indicating that each *H. macrolepidota* sequence has different mutations. The *H. macrolepidota* Ngebel Lake, Ponorogo sequence has 18 segregation sites, of which 11 are parsimony-informative, and 7 are singletons.

Phylogenetic and haplotype network

The haplotype network and phylogenetic tree analyses reveal the relationships of *H. macrolepidota* from Ngebel Lake, Ponorogo, spanning both intraspecific and interfamily levels (Figure 4), which are exploratory and not aimed at resolving deep phylogenetic relationships. The haplotype network and phylogenetic tree show that the *H. macrolepidota* population from Ngebel Lake represents the Insular Asia region, with a distance of at least about 20 bp from the populations of Laos and China, representing the Mainland Asia region, and a phylogenetic bootstrap value of 100%. This is most likely influenced by the geographical isolation of the two regions, which is further strengthened by the presence of the Laos and China populations in a single haplotype node. The interspecies distance of *Hampala*, based on two results, shows a higher closeness between the species *Hampala dispar* (Smith, 1934) and *Hampala salweenensis* (Doi & Taki, 1994), with a bootstrap value of 99%, compared to both species with *H. macrolepidota*, whether from the Mainland Asia region or the Insular Asia region, with a bootstrap value of 99%. All *Hampala* sequences are clearly distinct from those of other Cyprinidae genera, particularly as indicated by the haplotype network and phylogenetic tree. The intergenus distance of Cyprinidae, based on phylogenetics, shows a closer relationship between *C. carpio* and *Tor tambra* (Valenciennes, 1842) compared to *B. gonionotus*, with a bootstrap value of 95%. However, the haplotype network results are slightly different. The *C. carpio* clade has a distance of at least about 114 bp to the *T. tambra* clade, while at least only about 95 bp towards *B. gonionotus*. Based on the phylogenetic tree, the *Puntius tetrazona* (Bleeker, 1855) clade is the most distant clade within the Cyprinidae family, but it is barely noticeable on the haplotype network. Furthermore, based on the haplotype network, the ingroup clade Cyprinidae is closest to the outgroup, *Nemacheilus spiniferus* (Kottelat, 1984) from the family Nemacheilidae, with *H. macrolepidota* at a distance of at least 157 bp. The distance of shorter compared to the distance from the clade *H. macrolepidota* to the clade *C. carpio,* which is at least 162 bp, and at least 255 bp to the clade *B. gonionotus*.

Genetic p-distance analysis results based on Cytb (Table 4) tend to show clear distinctions between intrapopulation, interpopulation-intraspecies, interspecies-intragenus, intergenus-intrafamily, and interfamily-intraorder distances. The range of genetic p-distances within intrapopulation (gray column) in the dataset, between 0.0% and 5.9%, while the interpopulation-intraspecies (blue column) ranges from 0.1% to 3.1%. Results for *H. macrolepidota* Mainland Asia (China and Laos) have a distance from the population in Greater Sundaland, specifically the Ponorogo samples. The genetic p-distances between interspecies and intragenus *Hampala* (green column) range from 9.2% to 12.7%, which is significantly different from the interspecies distance of *H. macrolepidota*.

Table 4. Genetic p-distance of *Hampala macrolepidota* from Ngebel Lake, with various comparative data from interspecies to interfamily. The gray column indicates intrapopulation distance, the blue column interpopulation-intraspecies, the green column interspecies-intragenus, the yellow column intergenus-intrafamily, and the red column interfamily-intraorder (OG: Outgroup)

		1	2	3	4	5	6	7	8	9	10
1	HMA Ngebel	0.009									
2	HMA China	**0.031**	0.001								
3	HMA Laos	0.029	**0.001**	0.001							
4	HDI	**0.127**	0.123	0.116	**0.000**						
5	HSA	0.121	0.118	0.120	**0.092**	**0.000**					
6	PTE	0.210	0.200	0.204	0.210	0.206	0.046				
7	BGO	0.174	0.164	0.169	0.192	0.195	0.229	0.029			
8	CCA	0.160	0.153	0.161	0.181	0.182	0.209	**0.136**	0.009		
9	TTA	0.203	0.191	0.200	0.194	0.194	**0.233**	0.174	0.159	0.001	
10	OG: NSP	0.238	**0.230**	**0.231**	0.277	0.264	**0.281**	0.249	0.241	0.277	**0.059**

Note: The bold black numbers represent the lowest distance, and the bold red numbers represent the highest distance within each category

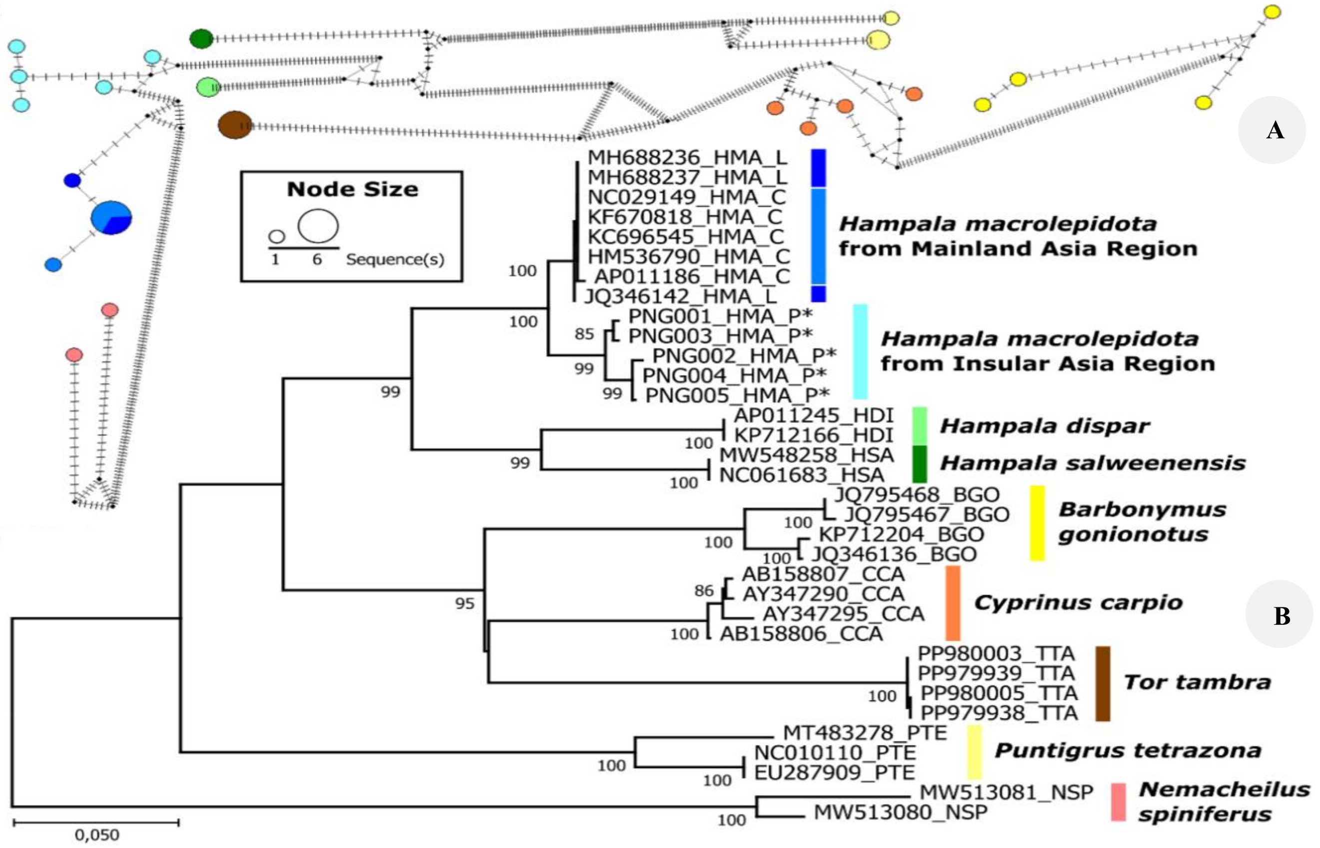

Figure 4. Mitochondrial Cytb, A. Haplotype network, and B. Phylogenetic tree on the *Hampala macrolepidota* data set from Ngebel Lake, Ponorogo. Comparative data are used to compare the results of estimating interpopulation, interspecies, intergenus, and interfamily relationships. The color codes on nodes in the haplotype network and branches in the phylogenetic tree are the same. The numbers at the nodes are bootstrap support values. The hash marks in the haplotype network indicate inferred mutations that were not observed in the dataset

Table 5. Results of genetic diversity and demographic history of *Hampala macrolepidota* from Ngebel Lake, in this study

Genetic diversity		**Neutrality test**				**Goodness-of-fit**	
hd	π	Fs	p-value	D	p-value	SSD	Hri
1.0000	0.0086	-0.4208	0.2370	0.6479	0.7120	0.0918	0.1200

Note: hd: Haplotype diversity, π: Nucleotide diversity, Fs: Fu's Fs value, D: Tajima's D value, SSD: Sum Square Deviation, Hri: Harpending's raggedness index

The results confirm the genetic proximity between the Ponorogo population and the Mainland Asia population. Furthermore, the genetic p-distances results of intergenus-intrafamily Cyprinidae (yellow column) in the dataset range between 13.6% and 23.3%, and specifically, the distances between intergenus-intrafamily within the genus *Hampala* and other genera range between 15.3% and 21.0%. Intersecting range on genetic p-distance value found in intergenus-intrafamily distances, with the highest value 23.3%, with interfamily-intraorder distances (red column, ingroup-outgroup) that is Cyprinidae-Nemacheilidae with the lowest value of 23.0%.

Genetic diversity and demographic history

Genetic diversity and demographic history play a crucial role in determining a population's adaptability to environmental changes, its health, and effective fishery management (Martinez et al. 2018; Kumar 2023). Analysis of genetic diversity using haplotype diversity and nucleotide diversity is a common approach to understanding genetic variation. Genetic analysis can also apply neutrality tests as a statistical index for demographic history analysis (Ouassal et al. 2024), to identify neutral evolution, such as the neutrality tests Tajima's D and Fu's Fs. Haplotype diversity (hd) refers to the accumulation of unique mutations in each nucleotide sequence (Chan et al. 2016). Unique mutations that differ in each nucleotide sequence (Garcia et al. 2021) reveal distinct haplotype groups. At the same time, the nucleotide diversity (π) is the average nucleotide difference estimate among sequences within a group or population (Fan et al. 2024). Moreover, the demographic history can also be analyzed using goodness-of-fit tests (Meng et al. 2015), such as the Sum of Squared Deviation (SSD) and Harpending's raggedness index (Hri). Genetic diversity, according to Grant and Bowen (1998), can be identified as relatively high, if hd>0.5 and π>0.005, which indicates that the *H. macrolepidota* in this study have high haplotype diversity with hd = 1.0000 and slightly high nucleotide diversity value, with π = 0.0086 (Table 5). The results are consistent with the neutrality test and goodness of fit for *H. macrolepidota* from Ngebel Lake, which show positive values for D = 0.6479 and negative values for Fs = -0.4208. Even though Tajima's D shows a positive value, the goodness-of-fit statistics (SSD = 0.0918 and Hri = 0.1200, Table 5) support the hypothesis that *H. macrolepidota* from Ngebel Lake exhibits unimodal shapes in its mismatch distribution, indicating a historical population expansion (Ouassal et al. 2024). Furthermore, when comparing the China population genetic diversity results: hd = 0.4000 and π = 0.0008, neutrality test: D = -0.9726 and Fs = 1.0404, with goodness-of-fit: SSD = 0.3200, and Hri = 0.6800, while the Laos population genetic diversity results: hd = 0.6667 and π = 0.0006, neutrality test: D = 0.0000, and Fs = 0.2007, goodness-of-fit: SSD = 0.0898 and Hri = 0.5556, *H. macrolepidota* from Ngebel Lake, still shows higher diversity.

Discussion

Analysis of morphological measurements of *Hampala macrolepidota* from Ngebel Lake confirms the identification, but some comparisons require special consideration. Morphological measurements and genetic results support the interpretation that Ngongok fish are *H. macrolepidota*, and have a wide distribution in Asia, from the Insular Asia region to the Mainland Asia region; however, no distribution records have been found in West Indoburma, either morphological or genetic. Species from the genus *Hampala* in this study exhibit low intrapopulation genetic distances, ranging from 0.000 to 0.009. Low genetic distances often indicate a high signal of homogeneity, one of the effects of gene flow, suggesting the possibility of reproductive connectivity within a population. The close relationship of the *Hampala* clade to other clades within Cyprinidae, compared to the *P. tetrazona* clade, differs from the phylogenetic results based on the combined analysis of Cytb and *Rag1* from nDNA (Sudasinghe et al. 2021). Those differences suggest considering nDNA information in the future classification of Cyprinidae.

The haplotype network and p-distance results also validate the phylogenetic results in this study, although not identical. The closest clade to *P. tetrazona* is the clade *B. gonionotus*, with a genetic p-distance value of 15.6%, roughly equivalent to the distance between the clade *H. macrolepidota* and the clade *C. carpio*. The adoption of general standards for genetic p-distance, such as in Teleost fish, using COI (Bañón et al. 2022), is not absolute because the mutation rate of each taxon varies significantly (Paricahya et al. 2024); therefore, it is essential to understand the taxon-specific trends being discussed.

The genetic diversity value from Ngebel Lake, Ponorogo, is: hd = 1.0000 and π = 0.0086, hd = 1 value, shows that there are unique mutations that differ in each sequence (Table 3), haplotype diversity tends to rapidly increase compared to nucleotide diversity, because it is measured based on unique mutations in each sequence (Ferreira et al. 2017). A high value of hd and low values of π indicate the possibility of population growth occurring in a relatively short time. Those results are also supported by a goodness of fit: SSD = 0.0918 and Hri = 0.1200, where the analysis supports the hypothesis that *H. macrolepidota* from Ngebel Lake is indicated under unimodal shapes of the mismatch distribution, which suggests experienced historical population expansion. Despite the neutrality test having contradictory results between the positive value D = 0.6479 and the negative value Fs = -0.4208, the majority of the analysis results point to the possibility of population growth and the historical population expansion.

Compared with the China population genetic diversity results: hd = 0.4000 and π = 0.0008, supported by a neutrality test: D = -0.9726 and Fs = 1.0404, with the goodness-of-fit: SSD = 0.3200, and Hri = 0.6800, while the Laos population genetic diversity results: hd = 0.6667 and π = 0.0006, with a neutrality test: D = 0.0000, and Fs = 0.2007, goodness-of-fit: SSD = 0.0898 and Hri = 0.5556. The China population exhibits a genetic bottleneck, characterized by low hd and π values, a hypothesis supported by the observation that the China population is under multimodal shapes of the mismatch distribution, which rejects the notion of historical population expansion. While the Laos population has slightly higher hd, indicating the possibility of an early stage of historical population growth, this hypothesis is supported by the Laos population being under weak unimodal shapes of the mismatch distribution. The differences in conditions between *H. macrolepidota* from the Ngebel Lake population and those from China and Laos suggest that the historical population expansion occurred regionally. The high diversity of *H. macrolepidota* in Ngebel Lake, possibly due to the wider mobility of species and its connection to another population (Lamadi et al. 2023), ensures that species mobility and environmental pressures have no immediate impact on the fluctuations in genetic diversity of *H. macrolepidota* from Ngebel Lake, Ponorogo.

The high genetic differentiation of *H. macrolepidota* from the Insular Asia region compared with the Mainland Asia region in this study suggests that *H. macrolepidota* may have been part of a metapopulation in the past during the Pleistocene. The separation of the Insular Asia region from the Mainland Asia region occurred at the close of the Pleistocene. The land separation formed due to a combination of sea-level changes caused by glacial-interglacial and tectonic activities that influenced changes in the ocean basins. The land separation in the past influenced differences in species conditions within it, as shown by genetic analysis, which indicated differences between the *H. macrolepidota* population from the Insular Asia region and the Mainland Asia region population.

The *H. macrolepidota* distribution is reported to be greater compared to that of 7 other *Hampala* species. In comparison, three other species are claimed to be distributed in Indonesia, have a limited distribution, and are typically endemic. Ngongok fish are claimed as an endemic species from Ngebel Lake (Primiani et al. 2020), but in this study, it was confirmed based on morphology and genetics to be *H. macrolepidota*, whose wide distribution in Asia indicates that this claim is incorrect. The distribution of the genus *Hampala* in Southeast Asia is extensive, encompassing Thailand, the Philippines, and Indonesia (Makmur et al. 2014), whereas *H. macrolepidota* was previously reported to be distributed from Sundaland to Burma (Taki and Kawamoto 1977). *H. macrolepidota* distribution is currently believed to be still wide-ranging, covering all rivers in the Mainland Asia region and Greater Sundaland, except Salween and Tenasserim (Panitvong and Hui 2025). Based on the phylogenetic results in this study, it is confirmed that at least one species of *H. macrolepidota* is distributed from Indonesia to Laos and China. *H. ampalong* is typically endemic in the Greater Sunda, especially Sumatra and West Borneo (Taki and Kawamoto 1977), while *H. bimaculata* distribution is typically endemic in Borneo Island (Soetignya et al. 2016); however, *H. bimaculata* is divided into at least two groups based on genetics (Ryan and Esa 2006). The uncommon distribution of *H. lopezi* in Indonesia was reported in a scientific publication in 2020 (Iskandar et al. 2020), without accompanying documentation or adequate evidence. The distribution needs to be confirmed again, as *H. lopezi* thus far has been reported to be distributed only on Busuanga Island, Philippines (Doi and Taki 1994).

Hampala is a genus of Cyprinidae that has a high potential for misidentification, not only due to high morphological similarity but also due to the potential for interspecies hybridization within it. Morphological similarity between interspecies *Hampala* is very high, with the absence of specific characters that can truly differentiate between species, except when combined with body coloration patterns (Doi and Taki 1994). The color pattern of the genus *Hampala* is determined by ontogeny (Taki and Kawamoto 1977). Discussion about morphological similarity among interspecific species of *Hampala*, previously limited to seven species, with the first report of *H. salweenensis* in 1994 (Doi and Taki 1994). Around 30 years later, *Hampala siamensis*, *Hampala lupar*, and *Hampala katibas* were identified in 2025, with the biggest morphological differences being observed using a body coloration pattern approach (Hui and Grinang 2025; Panitvong and Hui 2025). High morphological similarity within *Hampala* also raises the possibility of interspecies hybridization in the wild, such as between *H. macrolepidota* and *H. dispar* in the Mekong River (Taki and Kawamoto 1977).

The potential issues of identification and the possibility of hybridization are also evident from the discrepancies in morphological identification through body patterns, compared to the genetic results of *H. bimaculata* from Malaysia. The phylogenetic analysis results using Cytb revealed that the specimens identified as *H. bimaculata* with two blotches on the body were divided into at least two groups, and *H. bimaculata* type B has a monophyletic relationship with *Hampala sabana* (Inger & Chin, 1962), which has only one blotch on the body. Identification using blotches on the body has been combined with side stripe observations, leading to more accurate morphological identification; however, the potential for hybridization between the two species remains unavoidable (Ryan and Esa 2006). The complexity of *H. sabana* was not only found to be connected with *H. bimaculata,* but *H. sabana* was previously classified as a subspecies named *H. macrolepidota sabana*. There has also been suspicion of interspecies hybridization in the genus *Hampala* (Inger and Kong 1962).

Interspecies hybridization as a mechanism to maintain populations in the genus *Hampala* has unknown impacts on the ecosystem; however, hybridization of indigenous or endemic species with introduced species can have significant consequences. An introduced Cyprinid species in Ngebel Lake is common carp (*C. carpio*) (Haraningtias et al. 2018). The introduced species have various impact on ecology from the common impact, such as predation on local fish parents or their juvenile stages, competition for living space and food, habitat use, including habitat condition changes due to the introduction species behavior, hybridization with other introduced species or local species, and disease transmission, either from mobility in the new environment or diseases from the origin of the introduced species (Gozlan et al. 2010). The closeness of the relationship between introduced species and local species can amplify the impact of the introduction due to the high taxonomic similarity. The similarity in size and mobility between invasive species and indigenous species is known to increase negative interactions in nature, even driving the extinction of indigenous species that fail to coexist with the invasive species (Mills et al. 2004). The genetic similarity of species increases the potential for natural hybridization as an indicator of environmental pressures (Skovrind et al. 2019). This is particularly relevant when the impact of introduction is increasing due to the formation of new cryptic species variations, especially in two taxa that are not completely isolated sexually (Pante et al. 2014). Natural intergeneric hybridization in Cyprinidae has been reported, specifically between *C. carpio* and *T. douronensis* (Valenciennes, 1842) (Budi et al. 2025). Changes in genetic or morphological profiles in the future could serve as early indicators of adaptation in the *H. macrolepidota* population at Ngebel Lake, Ponorogo, potentially due to environmental degradation or natural hybridization. Considering genetic information through nDNA serves to confirm the potential hybridization in the future (Skovrind et al. 2019), and with complementary markers, including microsatellites and SNPs, to enrich the analysis and provide more comprehensive information.

The genus *Hampala* is prone to misidentification due to morphological similarities and the potential for interspecies hybridization. Furthermore, the existing data are insufficient to investigate environmental degradation factors and test suspicions of complex hybridization schemes. The population condition of *H. macrolepidota* from Lake Ngebel, Ponorogo, based on Cytb, indicates historical population growth, presumably due to the connectivity of population mobility with other populations; thus, environmental fluctuations do not have a significant impact. Genetic analysis based on mtDNA is sufficient to indicate population conditions due to environmental fluctuations; however, suspicion of future hybridization schemes requires a combination of morphological approaches and more specific genetic analysis based on nDNA. Furthermore, considering stock monitoring and conservation planning for the species in East Java, additional research, including DNA barcoding and ecological studies on *H. macrolepidota*, is needed.

In conclusion, this study confirms that all examined specimens from Ngebel Lake and comparative populations belong to *H. macrolepidota*, based on consistent morphometric and meristic characters. Genetic analysis revealed high haplotype diversity in the Ngebel population (hd ≈ 1.0000) and moderate nucleotide diversity (π ≈ 0.008-0.009), indicating substantial genetic variation. In contrast, lower diversity was observed in the Mainland Asia population, reflecting regional differences. Genetic distance (p-distance ≈ 0.029-0.031) and phylogenetic reconstruction demonstrated clear separation between Indonesian (Insular Southeast Asia) and Mainland Asia populations. Despite morphological similarity across regions, genetic evidence highlights population-level divergence shaped by geographic isolation. However, this study is limited by the use of a single mitochondrial marker (Cytb) and relatively small genetic sample sizes, which may not fully capture fine-scale population structure. Future research should incorporate nuclear DNA markers (e.g., SNPs or microsatellites) and larger sample sizes to validate population connectivity and test potential hybridization. Integrating ecological and environmental data will further clarify the drivers of genetic differentiation and support more effective conservation and management strategies for *H. macrolepidota*.

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