Characteristics of Meristics, Morphometrics, and Analysis of Cytochrome Oxidase Gen Fragmen Subunit 1 (CO1) Hampala Barb (Hampala Sp) from Jatigede Reservoir, Sumedang, West Java, Indonesia

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Abstract

Hampala barb in Jatigede Reservoir was one of the native fish of the Cimanuk River that needed to be conserved. This fish was included in the IUCN Red List with the category of Least Concern. The purpose of this study was to collect information related to biometric (morphometric and meristic) and molecular characteristics of the platinum fish in the Jatigede Reservoir. Morphometric and meristic analysis results showed that Hampala barb originating from Jatigede Reservoir, both those with spot (HS) and those without (HNS), were both identified as Hammpala macrolepidota Kuhl and Van Hasselt, 1823. Analysis of nucleotides and amino acids from gene fragments Cytochrome Oxidase Subunit 1 (CO1) showed differences in composition between fish samples that had spots (HS) and those that did not have spots (HNS). In the HS sample, the number of amino acids Leucine (Leu) was found the most, while Alanin (Ala) was present predominantly in the HNS sample. Meanwhile, the results of the BLAST analysis showed that the HS sample had a closeness to Hampala dispar (Acc No. MK448077.1) originating from Thailand, with a similarity percentage of 91.07%. Meanwhile, the HNS sample fish found no similarities to the CO1 database in Genbank, however, phylogenic analysis showed a close relationship between HS and HNS sample fish, and both were separate from other Hampala clade species in Genbank.

Keyword: Hampala, Morphometric, Meristic, CO1

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Introduction

Hampala bab was one of the freshwater fish that was included in the IUCN Red List with the category of Least Concern (Herawati et al., 2019; Suryamingsih et al., 2021). The hampala barb that breed in the Jatigede Reservoir came from the inundated Cimanuk River Herawati et al. (2017). In Jatigede Reservoir, hampala barb was a fiscivorous fish, the main food in the form of fish, with a preponderance index of 59%, but in the dry season these fish could utilize plants as a food source Herawati et al. (2019).

Hampala barb were rarely caught in the Citarum River (Kartamihardja, 2006), in Singapore these fish had even become extinct (Hui et al., 2020). The locations for spreading fish included: Malay Peninsula, Sumatra Island, Java Islnad, Borneo Island, and Indochina Region (Ryan et al., 2006). Habitat of hollow fish was in fresh water; wetlands in rural areas, in the form of rivers and including waterfalls, liked the substrate base of muddy sand waters so that it could be found in most bodies of water except streams, torrents, and shallow swamps (Vidthayanon, 2002).

Hampala barb by the local people were usually consumed and also as ornamental fish. As consumed fish, it was sold as fresh and processed products that were usually sold in the market. Meanwhile, as ornamental fish, this fish could be found in ornamental fish shops, with a higher selling price compared to fish sold as consumption.

In Jatigede Reservoir there were species of hampala barb that had black spots located between the dorsal and ventral fins, and other types that did not have these spots. This spot sign was very important because it was used as a key identifier for morphometric and meristic identification of empty fish species (Kottelat et al., 1993; Kottelat et al., 2013). Some researchers considered that species identification of an organism that used morphometric and meristic characteristics could be difficult and often had errors. Therefore, to ensure that vultures that had spots and which did not have spots were the same species or not, identification needed to be done using biometric methods and at the same time using molecular markers (barcoding).

DNA barcoding was used to identify species using DNA sequences from certain areas that had a high level of conservation. The CO1 gene (cytochrome c oxidase subunit 1) was a marker that was often used to identify groups of animals and investigated the biodiversity of their species (Dawnay, 2003; Hebert et al., 2013).

The study related to DNA barcoding for endemic freshwater fish had been conducted by researchers as an effort to preserve germplasm. This study was conducted as an effort to conserve fish in the Jatigede Reservoir, so that it could be used as a material for planning endemic fish conservation activities in the Jatigede Reservoir in West Java, Indonesia.

Material and Methods

Sampling of Hampala barb

Samples of hampala barb that had black spots (HS) and did not have spots (HNS) were taken from the waters of the Jatigede Reservoir (Figure 1). The fish were caught using monofilament gill nets measuring 80 m - 100 m long and 10 m wide, 3 – 5 inch mesh sizes, installing nets for 24 hours from 08.00 to 08.00 the next day.

Fish samples were then sent to the Biology Laboratory, Indonesian Institute of Sciences (LIPI) Cibinong for the morphometric and meristic identification process, using the Kottelatt et al. (1993). Meanwhile, molecular identification with CO1 gene markers was carried out at the Laboratory of Microbiology and Molecular Biotechnology (MICROMOL), Faculty of Fisheries and Marine Sciences, Universitas Padjadjaran.

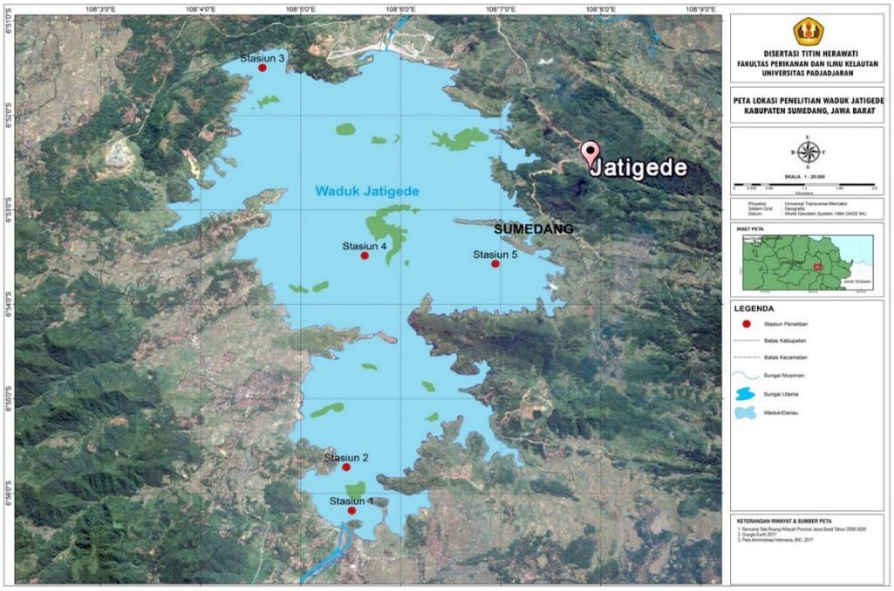


Figure 1: Location Map of Hampala Fish Sampling in Jatigede Reservoir, Sumedang, West Java

Morphometric and Meristic Identification

In the morphometric and meristic identification stages, fish samples used were whole fish, female fish with a total length of 327.17 mm and 372.62 mm. The basic measurements as in Figure 2 and Table 1.

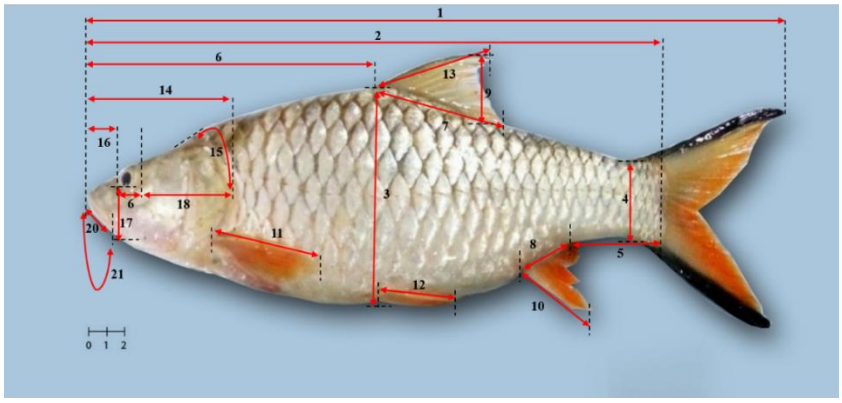


Figure 2: Basis of Morphometric Measurement of Hampala Fish

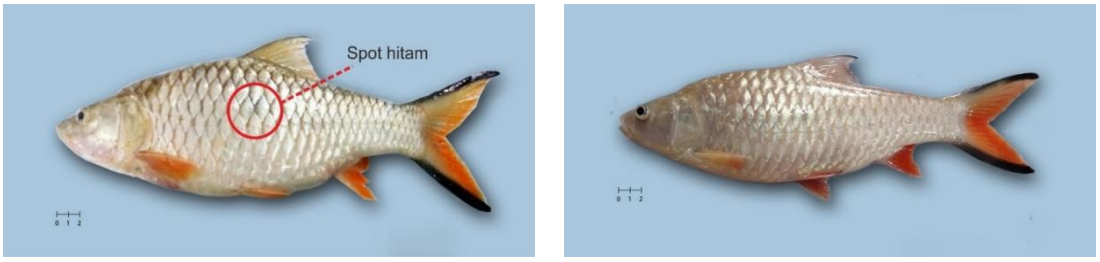
Table 1: Morphometric Characteristic Information (Figure 1b) and Meristic (Figure 1b)

No.	Characteristic	Code
1.	Total Length (mm)	TL
2.	Standard Length (mm)	SL
3.	Body Depth (mm)	BD
4.	Caudal Peduncle Depth (mm)	CPD
5.	Caudal Peduncle Length (mm)	CPL
6.	Pre-dorsal Length (mm)	PL
7.	Length of Dorsal Base (mm)	LDB
8.	Length of Anal Base (mm)	LAB
9.	Height of Dorsal Fin (mm)	HDF
10.	Height of Anal Fin (mm)	HAF
11.	Length of Pectoral Fins	LPF
12.	Length of Pelvic Fins (mm)	LPVF
13.	Length of Longest Dorsal Spine (mm)	LLDS
14.	Head Length (mm)	HL
15.	Head Width (mm)	HW
16.	Snout Length (mm)	SNL
17.	Suborbital Width (mm)	SW
18.	Orbit to Preopercle Angle (mm)	OPA

No.	Characteristic	Code
19.	Eye Diameter (mm)	ED
20.	Upper Jaw Length (mm)	UJL
21.	Gape Width (mm)	GW
22.	Dorsal Fin Spines	DFS
23.	Dorsal Soft Ray	DSR
24.	Anal Spines	AS
25.	Anal Soft Rays	ASR
26.	Total Pectoral Rays	TPR
27.	Scales Along LL	SALL
28.	Scales Above LL	SABL
29.	Scales Below LL	SBLL
30.	Scales Before Dorsal Fin	SBDF
31.	Scales Around Caudal Peduncle	SACP

Molecular Identification

Meanwhile, fish samples used for molecular identification were 2 (two) female Hampala fish. Fish that had black spots (HS) weighed 1,130 g, total length was 420 mm (Figure 3a), and hampala fish that had no black spots (HNS) weighed 788 g, total length was 343 mm (Figure 3b).



(a) (b)

Figure 3: Hampala Fish from Jatigede Reservoir (a) Have Spot (HS), (b) Have No Spot (HNS)

For molecular identification purposes, tail fins were taken from each fish 50 mm long using sterile scissors, stored in a preservation solution (EtOH), and stored in a -200C freezer until ready for use.

Isolation of Genomic DNA and Amplification of CO1 Gene Fragments

The DNA of the Hampala fish genome was isolated using the gSYNC DNA Extraction Kit (GeneaidTM). The genomic DNA was then visualized by electrophoresis in 0.8% agarose gel. Amplification of the CO1 gene fragment was carried out using Polymerase Chain Reaction (PCR) with the help of the primary pair Fish-F1 (5'-TCA-ACC-AAC-CAC-AAA-GAC-ATT-GGC-AC-3 ') and Fish-R1 (Fish-R1) 5'-TAG-ACT-TCT-GGG-TGG-CCA-AAG-AAT-CA-3 ') with a target of 707 bp amplicon. The thermocycling amplifier conditions were as follows: pre-denaturation (94°C for 5 minutes), 35 denaturation cycles (94°C for 30 seconds), anneling (52°C for 30 seconds) and extension (72°C for 30 seconds) , followed by an extension (72°C for 5 minutes) and termination (4°C for 5 minutes) (Fahmi, 2017). The amplicon was then visualized using 1% agarose gel and the positive ampsicon was then sent to the 1st BASE Sequencing Service (http://www.base-asia.com) for disequensing.

Analysis of Sequencing and Phylogenetic Tree Construction Results

The results of sequencing in the form of fasta were processed using BioEdit software alignment and matched with the COI sequence database in GenBank carried out through the BLAST (Basic Local Alignment Search Tool) program. Alignment analysis of nucleotide sequences was also carried out using Clustal 2.0, amino acid translation and Open Reading Frame (ORF) searches were performed with the help of the Expasy translate tool and phylogenetic tree

construction was carried out with the help of MEGA 7.0.

Result

Morphometric and Meristic Identification Results

Two Hampal fish that had different characteristics, in the form of the presence or absence of black spots between the dorsal and ventral fins, were taken from the waters of the Jatigede Reservoir, Sumedang, West Java. Then identified based on the meristic and morphometric characteristics, the results of observing meristic characteristics and morphometric measurements could be described as in Table 2 and Table 3.

Table 2: Description of Hampala Fish from Jatigede Reservoir Based on Meristic Characteristics

Hampala Had black spot between the dorsal fin and the ventral fin (HS)	Hampala Had No black spot between the dorsal fin and the ventral fin (HNS)
1. The body of relatively large and elongated, with a standard length was 327.17 mm.	1. The body of relatively large and elongated, with a standard length was 372.62 mm.
2. Mouth wide, and equipped with a pair of tentacles.	2. Mouth wide, and equipped with a pair of tentacles.
3. At the end of the lower snout was black.	3. At the end of the lower snout was black.
4. The last part of the dorsal fin was hardened and jagged.	4. The last part of the dorsal fin was hardened and jagged.
5. Grayish body color on the upper side, while on the bottom brown.	5. Grayish body color on the upper side, while on the bottom was brown.
6. Between the dorsal and dorsal fins were black patches.	6. There were no black patches between the dorsal and abdomen fins.
7. The hardened dorsal fin was blackish.	7. The top and bottom caudal fins were blackish, and the middle was orange.
8. Chest fins, pelvic fins and anal fins were brownish in the center, orange in color.	8. Number of hard fingers of dorsal fin II and 8 soft fingers.
9. The top and bottom tail fins were blackish, and the middle was orange.	9. The number of hard fingers of the pectoral fin I and 16 soft fingers.
10. Number of hard fingers of dorsal fin II and 9 soft fingers.	10. The number of hard fingers of the pelvic fin I and 9 soft fingers.
11. Number of hard fingers of the pectoral fin I and 16 soft fingers.	11. Number of hard fingers anal fin II and 6 soft fingers.
12. The number of hard fingers of the abdominal fin I and 9 soft fingers.	12. lateral line with 29 scales.
13. Number of hard fingers anal fin II and 6 soft fingers.	
14. Lateral line with 30 scales.	

Table 3: Morphometric Measurement Results of Hampala Fish

No.	Characteristic	Size (mm)	
		Hampal which has Black Spot (HS)	Hampal that has no Black Spots (HNS)
1	Total Length (TL) mm	327.17	372.62
2	Standard Length (SL) mm	264.50	289.55
3	Head Length (HL) mm	85.67	89.21
4	Head Width (HW) mm	43.35	44.46
5	Head Height mm	52.95	53.65
6	Snout Length (SNL) mm	27.67	29.92
7	Suborbital Width (SW) mm	12.60	14.09
8	Orbit to Preopercle Angle (OPA) mm	24.88	24.96
9	Length Before the Dorsal Fin (starting from the back of the head to the beginning of the dorsal fin)	91.56	100.47
10	Length Before the Dorsal Fin (starting from the tip of the mouth to the beginning of the dorsal fin) mm	144.68	156.80
11	Length Before the pelvic Fin mm	138.13	141.56
12	Length Before anal Fin (mm)	209.24	209.99
13	Height (mm)	79.57	79.92
14	Body Width (mm)	47.77	47.96
15	Tail Base Height (mm)	37.53	37.56
16	Tail Base Length (mm)	35.53	35.87
17	Base length of Dorsal Fin (mm)	41.34	41.64
18	Dorsal Fin Height (mm)	60.57	62.65

No.	Characteristic	Size (mm) Hampal which has Black Spot (HS)	Hampal that has no Black Spots (HNS)
19	Basic Length of anal Fin (mm)	26.83	26.96
20	Anal Fin Height (mm)	49.04	49.44
21	Base Length of Abdominal Fin (mm)	14.57	16.78
22	Abdominal Fin Height (mm)	43.06	45.46
23	Basic Length of Pectoral Fin (mm)	14.13	14.29
24	Tinggi sirip dada (mm)	49.48	49.81
25	Length of Caudal Fin (mm)	81.74	82.72
26	Length of Snout (mm)	15.20	15.46

Based on meristic and morphometric characteristics as presented in Tables 2 and 3 and the results of matching with Kottelat et al., (1993), the two fish samples were identified as Hampala macrolepidota Kuhl, Van & Hasselt 1823 or often referred to by other names Capoeta macrolepidota Valenciennes 1842, with the following classification:

Kingdom : Animalia
Phylum : Chordata
Ordo : Cypriniformes
Familia : Cyprinidae
Genus : Hampala
Species : *Hampala macrolepidota*

This species was well distributed in the Asian region; especially in the Mekong River Basin (China), Chao Phraya (Thailand), Peninsular Malaysia, and Indonesia.

Molecular Identification Results

Results of Amplification of Cytochrome Oxidase Subunit 1 (CO1) Gene Fragments

The CO1 gene was part of the mitochondrial genome, which played an important role in the process of cellular respiration. This gene area was often used as a barcode in the identification of animals, including fish, because in this region there were sequences with mutations that were fast enough so that they could distinguish clearly between species that areree relatively close, but on the other hand also had a fairly sustainable area among species in one genus (Hebert et al., 2003). With annealing temperature of 520 ^{o}C, the primary pair of fish-f1 and fish-r1 in this study succeeded in amplifying the CO1 gene fragment at a size of ± 700 bp (Figure 4).

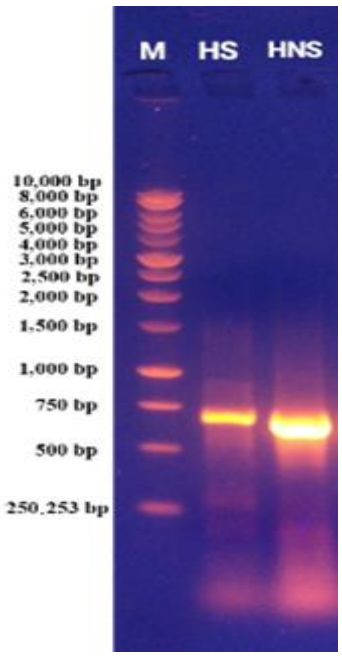


Figure 4: Electropherogram Results of Amplification of CO1 Fragments from the DNA of Hampala Fish Genomes Originating from Jatigede Reservoir, M: Marker/DNA Ladder 1 kb; HS: Samples of Hampal with Spot; HNS: Hampala Fish Samples Without Spot

In the complete genome structure of Hampala macrolepidota, CO1 gene fragments were in the area between the 5000th to 7000 base sequences (Figure 5).

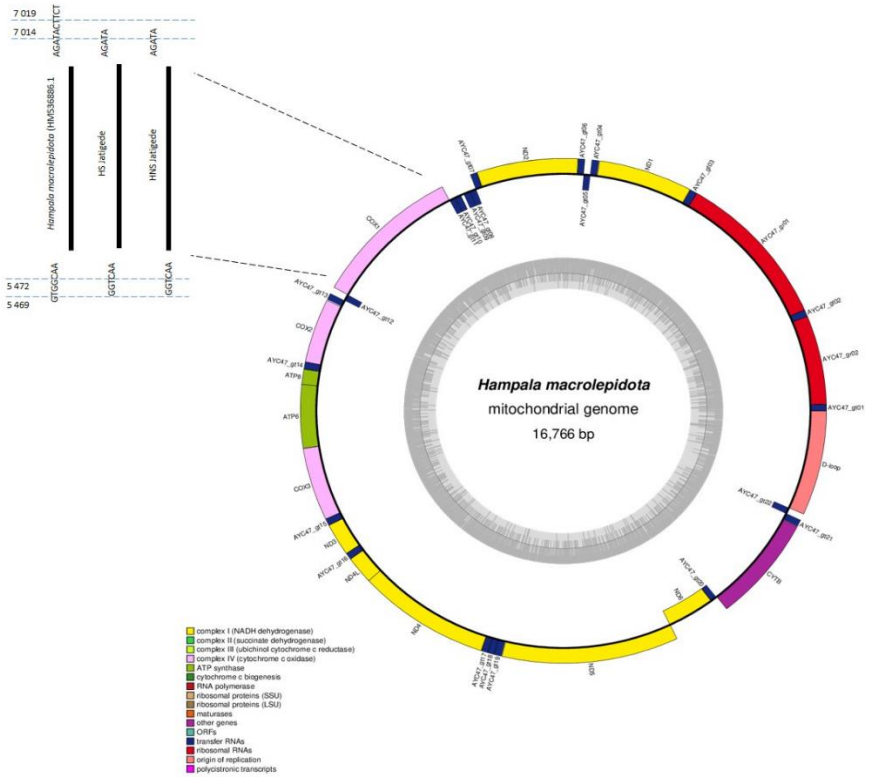


Figure 5: Position of the CO1 gene in the mitochondrial genome of the Hampala macrolepidota

Analysis of Nucleotide Fragment of CO1 Gene in Hampala Fish

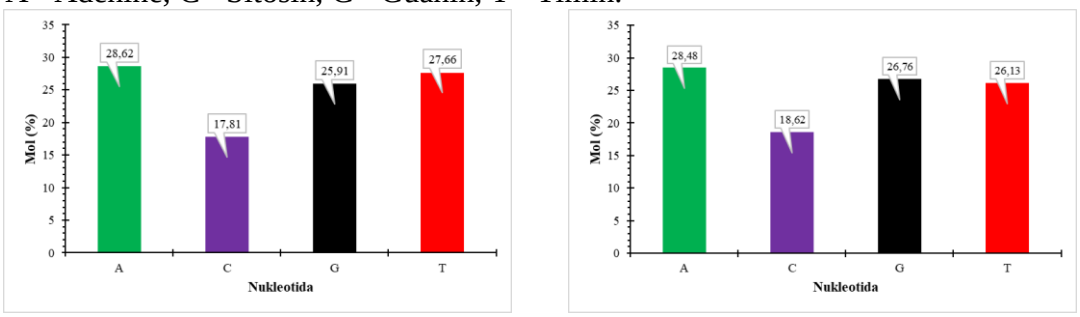
The results of sequencing of CO1 gene amplicons from the Hampala fish genome originating from the Jatigede Reservoir provided information that CO1 fragments from HS samples had a length of 629 bp, while HNS was 639 bp long. The characteristics of the CO 1 fragment nucleotides from the two samples and their comparison with CO 1 fragments from several other Hampala species were presented in Table 4 and Figure 6.

Table 4: Characteristics and Comparison of Nucleotide Fragments of CO1 Gene in Hampala Fish

Species	Length (base pairs/bp)	Double-stranded weight (Dalton)	Nucleotides	Amount		Mol (%)	Nucleotide distribution pattern
HS Jatigede	629	381,709	G+C=43.72%	A	180	28.62	A>T>G>C
			A+T=56.27%	C	112	17.81	
				G	163	25.91	
				T	174	27.66	
HNS Jatigede	639	387,983	G+C=45.38%	A	182	28.48	A>G>T>C
			A+T=54.62%	C	119	18.62	
				G	171	26.76	
				T	167	26.13	
Hampala macrolepidota Ranau (KM213072.1)	617	374,523	G+C=44.41%	A	165	26.74	T>C>A>G
			A+T=55.59%	C	172	27.88	
				G	102	16.53	
				T	178	28.85	
Hampala dispar Thailand (MK448077.	630	382,384	G+C=44.13%	A	178	28.25	A>T>C>G
			A+T=55.87%	C	169	26.83	
				G	109	17.30	
				T	174	27.62	

1) *Hampala macrolepidota* (HM536886.1)	625	379,438	G+C=44.96% A+T=55.04%	A C G T	163 175 106 181	26.08 28.00 16.96 28.96	T>C>A>G
Hampala macrolepidota Voucher *(>KF410690.1)*	630	382,537	G+C=45.56% A+T=54.44%	A C G T	169 185 102 174	26.83 29.37 16.19 27.62	C>T>A>G

Explanation: A= Adenine, C= Sitosin, G= Guanin, T= Timin.



(a) H (b) HNS

Figure 6: Comparison of Nucleotide Base Composition of Hampala Fish Originating from Jatigede Reservoir, (a) HS: Hampala Fish with Spot, (b) HNS: Hampala Fish without Spot

The results of the nucleotide analysis of CO1 gene fragments from hampala fish originating from the Jatigede Reservoir showed that: in the HS sample the distribution of nitrogen bases on the fragment strand was adenine (A) = 28.62%; thymine (T) = 27.81%; cytosine (C) = 17.11% and guanine (G) = 25.91%. From Table 4 it was also found that in the CO1 fragment the percentage of Purine bases (A + T) was greater than that of the Pyrimidine bases (G + C) in all the compared hampala fish.

The results of the alignment analysis of nucleotide sequences using Clustal 2.0 showed 80.72% similarity between nucleotide fish that had spots (HS) and those that had no spots (HNS), with 519 same base sequences and 124 different base sequences.

Analysis of Amino Acid Fragments of CO1 Gene in Hampala Fish

The results of the nucleotide translation in the form of amino acids from CO1 gene fragments of Hampala fish samples originating from the Jatigede Reservoir, between Hampala fish that had spots (HS) and those that had no spots (HNS), showed a difference between the two. The HS sample had the number of amino acids Leucine (Leu) that dominated the Open Reading Frame (ORF) of the fragment, while the amino acid Alanin (Ala) was seen most commonly found on the ORF fragment CO1 from the HNS sample (Figure 7).

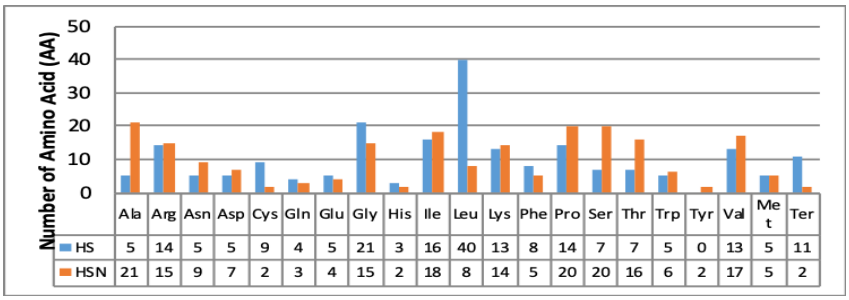


Figure 7: Amino Acid Composition in ORF Fragment CO1 from Hampala Fish Originating from Jatigede Reservoir, HS: Hampala Fish with Spot, HNS: Hampala Fish without Spot

The difference in amino acid composition in ORF fragments of CO1 could be an indication of the possibility of differences in protein synthesis from the two fish samples (HS and HNS), including structural proteins, could can affect differences in both phenotypes, including the possibility of presence or absence of spots.

BLAST and Genetic Kinship Analysis

The alignment results of CO1 fragment sequences from hampala fish originating from the Jatigede Reservoir with CO1 databases in Genbank (NCBI) through the BLAST (Basic Local Alignment Search Tool) program provided information that the HS sample had closeness to Hampala dispar (Acc No. MK448077.1) originating from Thailand, with 91.07% similarity. Meanwhile, HNS fish samples were not found similar to the CO1 data base in Genbank. Nevertheless, phylogenetic analysis showed a close relationship between HS and HNS fish samples (Figure 8). Phylogenetic analysis was used to be able to describe the exact kinship relationship between organisms (Li and Graur 1991).

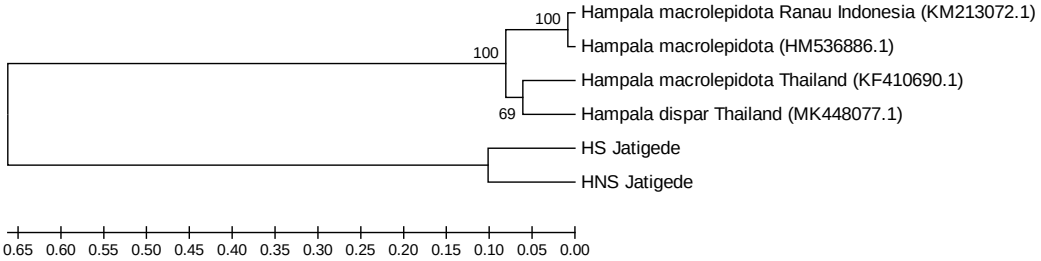


Figure 8: Phylogenetic Tree Relationship Between Samples of Hampala Fish Originating from the Jatigede Reservoir with CO1 Database of Other Hampala Fishes (UPGMA, 1000x boostrap)

The results of the phylogenetic analysis also showed that the Jatigede clade (HS and HNS) was separate from the other Hampala clades. This could indicate that hampala fish originating from Jatigede had genetic differences from those in other places. Evaluation of genetic distance was presented in Table 5. It could be seen that HS and HNS had the closest genetic distance to *Hampala dispar* Thailand (Acc No. MK448077.1), each at 1.25.

Table 5: Genetic Distance of Hampala from Jatigede Reservoir Compared to Other Types of Hampala Based on CO1 Fragment Analysis

No.	Spesies	1	2	3	4	5	6
1	HS Jatigede						
2	HNS Jatigede	0.19					
3	*Hampala macrolepidota* Ranau (KM213072.1)	1.37	1.30				
4	*Hampala_macrolepidota* (HM536886.1)	1.35	1.30	0.02			
5	*Hampala dispar* Thailand (MK448077.1)	1.25	1.25	0.18	0.18		
6	*Hampala_macrolepidota* Thailand (KF410690.1)	1.36	1.34	0.15	0.15	0.14	

From Table 5 it could also be seen that Thailand *Hampala dispar* had the closest genetic distance to Thailand *Hampara macrolepidota* (Acc No. KF410690.1) with a genetic distance of only 0.14.

Conclusion

The conclusions of this study are:

1. Based on morphometric and meristic analysis, it was known that hampala fish originating from the Jatigede Reservoir, both those who had spot/HS or those who did not have spot/HNS were identified as *Hampala macrolepidota* Kuhl and Van Hasselt, 1823.

2. The analysis of nucleotide fragments of CO1 gene in vultures showed information that there were differences in the composition of the nucleotides of the two samples, but the percentage of Purine (A/T) and Primidin (G/S) bases of all samples was always the same, with a higher Purin percentage,
3. Analysis of the amino acid fragments of CO1 gene in hampala fish also provided information that there were differences in composition between the two samples, in the HS sample, the number of amino acids Leucine (Leu) was found the most, while Alanin (Ala) was present predominantly in the HNS sample.
4. The results of the BLAST analysis showed that the HS sample had closeness to *Hampala dispar* (Acc No. MK448077.1) originating from Thailand, with a similarity percentage of 91,07%. Meanwhile, HNS fish samples were not found similar to CO1 databases in Gen bank, however, phylogenetic analysis showed a close relationship between HS fish samples and HNS, and the two were separated from other clade species in Gen bank.

Acknowledgements

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