



## RESEARCH PAPER

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## Morphological, meristic characteristics and mtDNA analysis of *Hampala* Fish (*Hampala macrolepidota* Kuhl & Van Hasselt 1823) from ranau lake, Indonesia

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### Abstract

*Hampala* (*Hampala macrolepidota*) in Ranau Lake is locally known by three size-based names: small size as *Kemencut*, medium as *Arongan* and large as *Sebarau*. To know whether these local names are the same or different species, morphometric measurements, meristic counts and mtDNA analysis were carried out. Five individuals of each size, 157–172 mm total length for *Kemencut*, 264–295 mm for *Arongan* and 374–445 mm for *Sebarau* were taken. Morphometric measurements showed significant correlation ( $p > 0.01$ ). The strongest correlation between body part and percent of total length was predorsal length (PL) with  $r = 0.980$ . Meristic character counts fit to the identification of Weber and Beaufort (1916). Number of scales along the lateral line and total number of pectoral fin were 27-28 and 15-16, respectively. Nucleotide base composition of *H. macrolepidota* did not show any variation in nucleotide situs. Intraspecific *COI* (Cytochrome Oxidase Subunit I) gene nucleotide-based phylogram of *Hampala* created a genetic relationship supported by bootstrap value of 100%. No haplotypic variation was formed. All *Hampala* samples were included in *H. macrolepidota* (Kuhl & Van Hasselt 1823).

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### Introduction

Ranau Lake located in South Ogan Komering Ulu (South OKU) Regency, South Sumatera Province and

West Lampung Regency, Lampung Province. It possesses water surface of  $\pm 12,590$  km<sup>2</sup> and maximum depth of  $\pm 229$  m. It is located on 540 m

above sea level with water volume  $\pm 21,950 \times 10^6 \text{ m}^3$  (Sulastri *et al.*, 1999). In Ranau Lake waters, *Hampala* (*Hampala* macrolepidota, Kuhl & Van Hasselt 1823) is recognized with three different local names, *Kemencut* (small size), *Arongan* (medium) and *Sebarau* (large). This name difference is based on the fish size and the fishing gear used. *Kemencut* is caught using *kebang* (gill net) of 1 inch, 1½ inch and maximum 1¾ inch mesh size. *Arongan* is caught using 2½ inch-gill-nets, while *Sebarau* is caught using lance or speargun.

The presence of three different local names of hampala in Ranau Lake often results in mistakes in species identification or species determination since the identification has been merely done by comparing the photographs from various literatures. A comprehensive identification is firstly carried out by looking at the morphological characteristics and mtDNA for determining whether the three local names of hampala belong to the same species.

Main characteristic of adult *Hampala* is having line-like black spots between dorsal fins and ventral fins which then become vague in larger fish, but the color patterns of the adults and the juveniles are distinct in different rivers (Kottelat *et al.*, 1993). Genus *Hampala* of Family Cyprinidae is widely distributed almost all regions of Southeast Asia, such as Thailand, Malaysia, Vietnam, Philippine and Indonesia. It has five species, *H. macrolepidota* including *H. m. Sabana* as sub-species, *H. ampalong*, *H. bimaculata*, *H. lopezi* and *H. dispar*. Disimilarity of these five species is shown in the external morphological characteristics, especially in color patterns. The last new species of *Hampala* that successfully identified by Japanese scientist was *Hampala salweenensis* found in Mae Surin River, Mae Pae Valley Salween Tribury, Thailand (Doi and Taki, 1994). *Hampala* (*H. macrolepidota*) distribution in Indonesia includes

river, lake, swamp and reservoir in Sumatera, Kalimantan and Java. Other species, *H. ampalong* are only recorded in Sumatera and Kalimantan, and even *H. bimaculata* is only found in Kalimantan.

Species identification can be done by looking at morphometric and meristic characteristics or DNA analysis. Morphometric characteristic is the character illustrating the body shape, while meristic character counted number, series or structure. Both morphometric and meristic characters are mostly used to identify variety of fish species (Turan *et al.*, 2006). Morphometric and meristic studies are strong tools to measure the discreteness of the same species (Gharaei, 2012). Moreover, mtDNA (Mitochondrial DNA) analysis uses the DNA found in the mitochondria. The output of mtDNA analysis in this study will be registered new barcode or registered in the *GenBank*. Therefore, these identification methods were used to identify three groups of *Hampala* (*Kemencut*, *Arongan* and *Sebarau*) in Ranau Lake, Indonesia. This study determined whether the three groups of *Hampala* are distinct species or not.

#### Materials and method

Sample used in this study was *Hampala* caught by fishermen in 2013 in Banding Agung waters, Ranau Lake, Indonesia (Fig. 1). Fishing gears used were 1½ inch and 1¾ inch mesh-sized gill net for *Kemencut*, 2½ inch mesh-sized gill net for *Arongan* and lance or spear for *Sebarau*. Fifteen fish samples were grouped based on total length into three groups, each consists of five individuals with the following length ranges: 157–172 mm for *Kemencut* (A), 264–295 mm for *Arongan* (B), and 374–445 mm for *Sebarau* (C). The samples were preserved in 10% formaldehyde, dorsal fins were tagged and separated with size group (for morphological observations). The mtDNA analysis used the caudal fin stored in 95% ethanol-containing labelled vial tube and kept in room temperature.

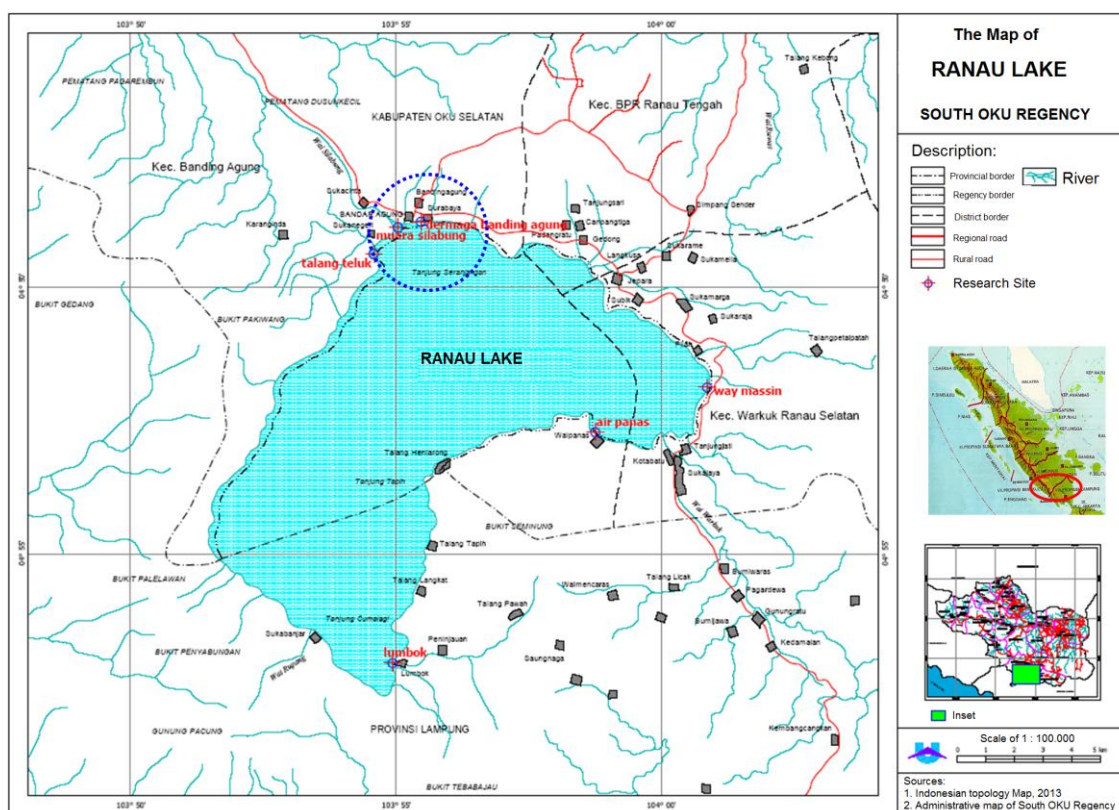


Fig. 1. Sampling site of *Hampala* in Ranau Lake.

Morphometric and meristic observations were carried out in Fisheries Biology Laboratory, Freshwater Fisheries Research Office (BP3U), Palembang. The genetic diversity of *Hampala macrolepidota* was analyzed based on COI (Cytochrome Oxidase Subunit I) gene in Ecology Molecular Laboratory, BP3U Palembang and First Base DNA Sequencing Service, Singapura (<http://www.base-asia.com>).

*Morphometric and Meristic Characteristics*

Morphometric measurements of the specimen were done using a digital caliper with 0.1 mm accuracy, while meristic characteristics were conducted by manual counts assisted with an enlargement glass, with parts measured and counted shown in Fig. 2, 3 and Table 1. The morphometric and meristic measurements of *Hampala* were done for 32 morphological characters, on left side of the fish body (Cailet *et al.*, 1986). The morphological characters were compared with percent total length and the eye diameter was compared with the head length. Some

major morphometric characters analysed for regression and correlation significance (Zafar *et al.*, 2002; Turan *et al.*, 2006; Hossain *et al.*, 2009; Krishan and Tarana, 2010; Hazarika *et al.*, 2011 and Abbaspour *et al.*, 2013). Various morphological characters (morphometric and meristic) were also compared with those of identification book of Weber and Beaufort (1916) and Kottelat *et al.* (1993).

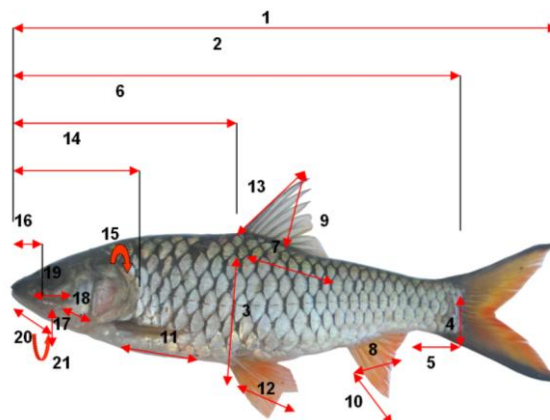


Fig. 2. Morphometric character (A) of *Hampala*.

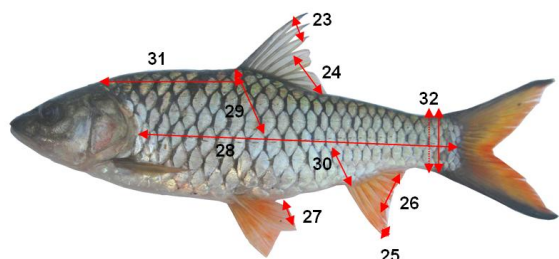


Fig. 3. Meristic character of *Hampala*.

Table 1. Information on Fig. 2 and 3, Morphological and meristic characters.

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

Source: Caillet *et al.*, (1986).

*mtDNA*

DNA analysis includes isolation, extraction and purification steps. DNA extraction used Genomic DNA mini kit for blood (Geneaid) modified

(Muladno, 2006). The amplification of mtDNA *COI* fragments used a universal primer. PCR reaction used *ABI* Applied Biosystem machine. The PCR product was tested using PAGE 6% in buffer 1x TBE (10 Mm Tris-HCL, 1 M boric acid, and EDTA 0.1 Mm) run at 200 Mv condition for 50 min. The DNA was then stained with silver sensitive coloration (Tegelstrom, 1986).

Genetic diversity data analysis was carried out through homologous side observation of the nucleotide base trace or amino acid trace of *COI* gene. The mtDNA of *Hampala* obtained was then paralleled (multiple alignment) compared with that *COI* gene from the partial *GenBank*. The chromatogram sequence was shown and manually edited using *BIOEDIT* (Applied Biosystems, Foster City, CA, USA). The initial multiple alignment used *MAFFT* online version (<http://mafft.cbrc.jp/alignment/server/>) (Katoh *et al.*, 2002). The multiple alignment was redone using *MUSCLE* (Dereeper *et al.*, 2008) (<http://phylogeny.lirmm.fr/phylo.cgi/alacarte.cgi>), the alligment curation used *GBLOCK* (Castresana *et al.*, 2000) and the phylogenetic construction used *PhyML* maximum likelihood (Guindon *et al.*, 2010; Anisimova *et al.*, 2006).

**Results**

*Morphometric Characteristics*

Morphometric character related with TL (total length) showed indifferent percentage for each fish group (Table 2). Measurements of several body parts related with total length of *Hampala* indicated that the highest correlation with total length was PL (predorsal length) with correlation coefficient (r) of 0.980, followed by HL (head length) (r=0.975) and then head width (r=0.959). Linear relationship between total length and several morphometric characters is given in Fig. 2. All correlation coefficients (r) approached to 1 reflecting strong correlation with p >0.01, in which all morphometric characters directly rose their proportion between one to another.

**Table 2.** Morphometric measurements of *H. macrolepidota*: Kemencut (A), Arongan (B) and Sebarau (C) from Ranau Lake of South Sumatera and Lampung.

Measurement (mm)	A (n=5)			B(n=5)			C(n=5)		
	Min	Max	mean±SD TL(%) / mean	min	max	mean±SD TL(%) / mean	Min	max	mean±SD TL(%) / mean
Total Length (TL)	157	172	165.60±5.41	264	295	275.80±12.71	374	445	412±31.11
Fork Length (FL)	143	148	145.40±2.30	222	255	234.40±12.30	322	401	361±35.47
Standard Length (SL)	124	132	127.80±2.86	199	229	210.80±11.09	290	377	332.6±39.50
Body Depth (BD)	36.2	40.2	37.92±1.97	62.3	69.2	66.06±2.97	81	115.2	93.12±14.75
Caudal Peduncle Depth (CPD)	15.7	17.8	16.52±0.86	25.5	30.6	28.00±1.89	35.4	44.1	38.52±3.66
Caudal Peduncle Length (CPL)	20.1	21.7	20.82±0.70	33.6	40.6	36.14±2.88	45.9	69.6	55.44±10.62
Predorsal Length (PL)	65.8	67.6	66.88±0.67	97.1	118.6	106.74±10.32	147.3	196.1	169±19.65
Length of Dorsal Base (LDB)	16.7	19.4	18.38±1.11	29.6	35.6	32.02±2.40	39.1	49.8	43.68±4.14
Length of Anal Base (LAB)	10.7	11.8	11.40±0.45	17.3	20.6	18.56±1.27	22.6	29.1	25.58±2.54
Height of Dorsal Fin (HDF)	22.4	26.2	24.50±1.38	41.2	48.9	43.06±4.66	49.5	59.2	56.12±4.17
Height of Anal Fin (HAF)	15.8	19.9	18.28±1.52	29.3	35.1	33.00±2.46	41	52.6	47.40±5.07
Length of Pectoral Fins (LPF)	22.9	26.2	24.66±1.19	35.6	45.9	42.22±4.42	53.3	57.9	54.80±1.80
Length of Pelvic Fins (LPVF)	20.4	23.1	21.56±1.10	33.4	41.8	37.64±3.45	42.2	50.9	47.06±3.14
Longest Dorsal Spine (LLDS)	25.8	31.1	29.40±2.14	38.7	52.6	45.46±6.34	47.5	55.8	51.32±2.96
Head Length (HL)	32.9	40.8	37.26±3.66	55.9	66.9	60.70±4.44	87.4	119	102.46±12.37
Head Width (HW)	16.4	19.2	17.24±1.11	23.9	29.9	28.48±2.57	43.6	56.7	49.78±6.52
Snout Length (SL)	11.2	12.8	11.98±0.70	15.6	20.5	17.70±1.77	27.5	40.9	32.62±5.24
Suborbital Width (SW)	5.1	6.6	5.66±0.59	9.3	12.2	10.70±1.19	19.6	28.8	22.96±4.55
Preopercle Angle (OPA)	10.9	12.8	12.06±0.69	17.7	20.3	19.18±1.13	30.6	48.9	36.90±7.50
Eye Diameter (ED)	8.3	9.8	9.18±0.54	12.3	14.4	13.26±0.86	14.1	16.8	15.84±1.11
Upper Jaw Length (UJL)	12.9	15.4	14.44±1.15	19.3	23.4	21.94±1.75	35.7	46.1	38.80±4.17
Gape Width (GW)	9.0	11.9	10.22±1.08	15.8	19.8	17.12±1.66	31.2	37.9	33.62±2.77

Min= minimum; Max= maximum; SD= Standard Deviation; TL (%)= Percent total length

**Table 3.** Meristic counts of Hampala: Kemencut (A), Arongan (B) and Sebarau (C) from Ranau Lake, South Sumatera, Lampung.

Meristic data	Counts		
	A(n=5)	B(n=5)	C(n=5)
Dorsal Fin Spines (DFS)	IV	IV	IV
Dorsal Soft Ray (DSR)	8	8	8
Anal Spines (AS)	III	III	III
Anal Soft Rays (ASR)	5	5	5
Total Pectoral Rays (TPR)	15-16	15-16	15-16
Scales Along LL (SALL)	28	27-28	27-28
Scales Above LL (SABL)	5½	5½	5½
Scales Below LL (SBLL)	3½	3½	3½
Scales Before Dorsal Fin (SBDF)	10	10	10
Scales Around Caudal Peduncle (SACP)	12	12	12

*Meristic Characteristic*

Calculation of several meristic characters of *Hampala* (*Kemencut*, *Arongan* and *Sebarau*) (Table 3), i.e. DFS (number of hard spines of dorsal fin), DSR (weak spines of dorsal fin), AS (hard spines of anal fin), ASR (weak spines of anal fin), TPR (total number of pectoral rays), SABL (number of scales on the lateral line), SBLL (number of scales on lower part of lateral line), SBDF (number of scales before dorsal fin), and SACP (number of scales around the tail rod) showed similar number range among three fish groups. The only difference was found on number of scales

(meristic character) along the SALL (lateral line), 27-28 and 15-16 scales, respectively.

*mtDNA*

Total DNA was isolated from muscle footage of all samples. The output of total DNA isolation was used fingerprints for *COI* gene amplification of mtDNA with PCR technique. *COI* gene amplification resulted in 679-702 pb-sized *COI* gene at the position of 5535-6249 pb based on the *GenBank* category. The DNA profile from amplification is give in Fig. 4.



**Fig. 4.** DNA profile of *H. macrolepidota* as amplification output using pair of *COI* F and *COI* R primers.

From 228 amino acids of 686 nucleotide translation output on partial *COI* gene of *Hampala macrolepidota*, total 223 situs of amino acid was enternal. The analysis of nucleotide base composition for *H. macrolepidota* did not identify the presence of varied nucleotide situs. For the four nucleotide bases, Adenine was evenly the most recorded (29.3%), while the least found was Guanine (17%). Mean composition of Adenine+Thymine in *H. macrolepidota* was totally more (56.1%) than average Guanine+Cytosine (43.9%).

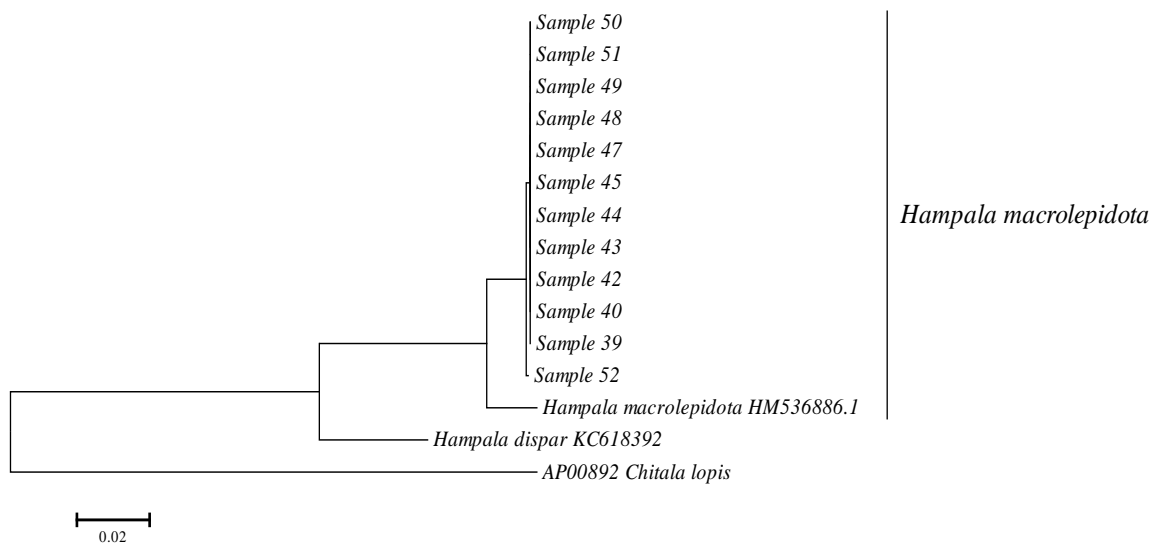
6176), Guanine of 290, 312, 381 (5820, 5832, 5901), and Thymine of 327, 393, 577 (5847, 5913, 6093).

From 228 amino acids translated from 686 nucleotides on partial *COI* gene of *H. macrolepidota* compared with *GenBank* data, several amino acids could be taken as genetic markers to distinguish Indonesian *H. macrolepidota* from *Hampala* outside Indonesia. *H. macrolepidota* from Indonesia specifically possesses isoleucine genetic marker on the 94<sup>th</sup> amino acid position (1941).

From 686 *COI* gene nukleotides of *H. macrolepidota* compared with those of the *GenBank* data, some nucleotide base could be taken as genetic markers to distinguish Indonesian *H. macrolepidota* from *Hampala* kinship outside Indonesia. *H. macrolepidota* from Indonesia specifically possessed Cytosine genetic marker on the nucleotide base position of 276, 519, 558 (5814, 6036, 6074), Adenine of 282, 357, 402, 531, 660 (5820, 5877, 5922, 6047,

The kinship relationship reconstruction of nucleotide base trace of *H. macrolepidota* and its kinship is shown in Fig. 5. The *COI* gene nucleotide-based phylogram exhibited that the intraspecific *Hampala* sketchly created a kinship supported by bootstrap value of 100%. Based upon genetic analysis of mtDNA from the kinship relationship, there was no haplotypic variation formed, single haplotype for sample collection. This group possesses the closest

kinship with *H. macrolepidota* supported with in this study is clearly *H. macrolepidota*.  
 bootsrap value of 100%. It means that *Hampala* used



**Fig. 5.** Phylogram PhyML maximum likelihood (branch length indicates number of substitution per situs) based on COI gene nucleotide 669 pb of *Hampala* and comparative relative from the *Genbank*.

**Discussion**

Based on the morphological characteristics (morphometric and meristic) and total DNA isolation, *GenBank* analysis and kinship phylogram, *Hampala* (*Kemencut*, *Arongan* and *Sebarau*) from Ranau Lake waters is *H. macrolepidota* (Kuhl & Van Hasselt 1823). *H. macrolepidota* from Ranau Lake possesses genetic distance of 100% or does not have difference.

Based on phylogenetic tree, sample fish (*H. macrolepidota*) from Ranau Lake and *H. macrolepidota* from the *GenBank* registered by Yang *et al.* (2010) possesses genetic distance (similarity) from 97.4%-100%, meaning that both species belong to single same species, and the presence of genetic distance (not 100%) could result from that the fish does not come from the same location or different geographic position. Two different species (one Genus), *H. macrolepidota* from Ranau Lake and *Hampala dispar* from the *GenBank*, possess 92% similarity or reach 8% difference. Even to see further genetic distance, it could be seen from the relationship between *H. macrolepidota* and *Chitala lopsis* (*GenBank*) having only about 30% similarity.

There are many different local names of *H. macrolepidota* so that it is confusing the actual species name. People-known different local names of *Hampala* species result from different size groups and fishing gears used and are to easily give information and transaction. To know the species name of the local different names, detail identification was done through morphological characteristics and even mtDNA analysis. Species identification is traditionally done through morphological character observations. According to Straruss and Bond (1990), fish morphology has historically become major information for taxonomic study. The detail and accurate identification could be done through barcoding mtDNA genetic analysis (Meyer and Paulay, 2005; Turan *et al.*, 2006; Alo *et al.*, 2013).

Morphologically, the three fish groups do not show any difference, such as morphometric character measurements in relation to total length (TL). There are only slight differences in number of scale counts along the lateral line (SALL) with a range of 27-28 scales. Based on Weber and Beaufort (1916), number of scales along the lateral line of *H. macrolepidota*

was 28-29 scales. Whereas according to Ryan and Esa (2006), *Hampala* in Malaysia waters (peninsular), number of scales along the lateral line ranged from 26-29 scales.

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2. Research Team of Ranau Lake, Research Institute for Inland Fisheries, Palembang, South Sumatera, Indonesia, 2012.

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