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RESEARCH PAPER

DNA Authentication of Asian Redtail Catfish *Hemibagrus nemurus* from Musi and Penukal River, South Sumatra Indonesia

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Abstract

Asian Redtail Catfish (*Hemibagrus nemurus*) is highly economic important catfish in South Sumatra. Investigating DNA authentication is of importance for species conservation and breeding selection. Cytochrome C Oxidase subunit I is one of mitochondrial DNA markers used for species barcoding in freshwater, brackishwater and marine fish. This research aims to explore the use of COI gene for species barcoding, constructing phylogenetic tree of Asian Redtail Catfish (ARC). The methods used in the research consisted of DNA extraction, PCR (Polymerase Chain Reaction) amplification and sequencing mtDNA COI gene of ARC obtained from Musi and Penukal Rivers (South Sumatra). A 572 and 596 base pairs of partial coding sequences were obtained representing each river. Phylogenetic analyses indicated that ARC was at the same cluster from Bagridae family, and different cluster from others catfish, *Pangasius* sp and *Oreochomis niloticus*. Further study using more species of Bagridae and habitat are needed to investigate the diversity of DNA from South Sumatra water resources

Keywords: Asian redtail catfish, COI gene, DNA authentication, phylogeny, South Sumatra.

Introduction

Catfishes are an important economic livelihood at water resource in South Sumatra-Indonesia. *Hemibagrus* has been cultured for food in East and Southeast Asia, mainly in Peninsular Malaysia and Thailand. It was widely dispersed throughout Sundaic and mainland Southeast Asia, which was thought to be conspecific due to consistent differences in morphology and morphometric (Ng & Kottelat, 2013). Throughout its geographical range, species of Hemibagrus are valuable food fishes and species identification is of importance for sustainably to utilize this species complex (Dodson & Lecomte, 2015). Asian redtail catfish (*Hemibagrus nemurus*) (Valenciennes, 1840), local people known as baung fish, is also one of popular raw material for delicacy food in Sumatra. ARC spread around Asia and Africa continent, while in Indonesia inhabit water resource in Sumatra, Kalimantan and Java for instance Jakarta, Karawang, Garut, Surabaya, Malang, Pasuruan, Palembang, Bengkulu, Muara Kampeh, Banyu Asin, Lake of Singkarak, Barito, Rasau, Kapuas and Sambas (Roberts, 1989). This species lives in most habitat types, but most frequent in large muddy rivers, with slow current and soft bottom (Kotellat, 1998). It is primarily an inhabitant of large rivers, and has been described as an opportunistic predator that feeds on fish, aquatic and terrestrial invertebrates (Rachmatika, 2003).

There are 60 species of Bagridae in Indonesia, but three of them (Beringit - *Mystus singaringan*, Baung – *Hemibagrus nemurus* Blkr., and baung buntut tikus – *Barcoides macropterus*) are inhabited at Musi River and Swamp Forest Merang-Kepayang (Hutan Rawa Gambut Merang-Kepayang/HRGMK, Banyuasin) (Iqbal, 2011). With many different species of Bagridae around Sumatra, there is a pressing need for better tools for species authentication through DNA molecular (Rajiv and Chauhan, 2010). Mitochondrial DNA can be used to investigate evolutionary process with high resolution (Brown, George & Wilson, 1979). Sequencing of this region has been widely used to discriminate species level (Nagl *et al*., 2001) and population study (Rognon & Guyomard, 1997; D'Amato, Esterhuyse, Waal, Brink & Volckaert, 2007). Cytochrome c oxidase subunit I is one of mtDNA gene, which is conserve, used for distinguishing species and population.

The conserved sequence of the 5' region of the mitochondrial gene cytochrome oxidase subunit I (COI or Cox1), a platform for the universal DNA barcoding of life, has been widely used for

distinguishing, for example, species in the Persian Gulf (Asgharian, Sahafi, Ardalan, Shekarriz & Elahi, 2011), Australian fish (Ward, Zemlak, Innes, Last, & Hebert, 2005), marine fishes in the northwest Atlantic Ocean, Canada (McCusker, Denti, Van Guelpen, Kenchington & Bentzen, 2013) and tilapia species (Shirak *et al*. 2009; Wu & Yang, 2012; Syaifudin Penman & McAndrew, 2015). The objective of the research described here was to distinguish between Bagridae species and to construct phylogeny tree.

Materials and Methods

Biological Materials

A total of 10 individuals of ARC were collected from two different rivers at South Sumatra Provinces (Musi & Penukal), Indonesia. All fin clips were preserved in 99% ethanol (1:10 w:v) and stored at -20°C until required.

Genomic DNA Extraction

Fin clips sample were used to extract DNA from all samples. Total genomic DNA was extracted using the Realpure Genomic DNA Extraction Kit (Durviz S.L) following the manufacturer's protocol. An RNAse incubation step was included to minimise RNA contamination, with each precipitated DNA sample being finally resuspended in 5 mM Tris, pH8.5. Those samples that passed quality control (no observable RNA and comprising predominantly high molecular weight DNA) were selected for use and diluted to a concentration of 50 ng/µL in 5 mM Tris; pH 8.5.

COI DNA Barcoding

In order to amplify 655 bp fragment, the DNA of ARC (2 individuals each) were used in PCR using primer cocktails of primer pairs FishF2-5' TCGACTAATCATAAAGATATCGGCAC 3' and FishR2-5' ACTTCAGGGTGACCGAAGAATCAGAA 3' (Ward *et al*. 2005). PCR was performed in 40 µl final volumes using KAPA HiFi HotStart ReadyMix from Kapa Biosystem. Each reaction contained 1.6 µl 10 µM FishF2 primer, 1.6 µl 10 µM FishR2 primer, 14.8 µl nuclease-free water, 20 µl 2x KAPA HiFi HotStart ReadyMix and 2 µl DNA template. The amplification conditions were: initial cycle of 95°C for 3 mins followed by 30 cycles of 98°C for 20 sec, 56°C for 40 sec, 72°C for 30 sec and final extension at 72°C for 1 min. The PCR products were run in 1% agarose gel at 75 V for 50min (Figure 1). Then, they were commercially sequenced (Sanger sequencing, GATC Biotech Ltd.) at 1st Base DNA Sequencing Service.

Sequences Alignment and Trimming

Two samples of sequencing from both directions were aligned using Mega 7 software. The resultant fragments were approximately 675 - 680 base pairs (bp). After trimming process with MEGA (Molecular Evolutionary Genetics Analysis) version 6, length of the aligned sequences were 596 – 572 bp and no gaps within sequences. Clustal Omega software from ebi.ac.uk was used for multiple sequence alignment in this study. The sequences were identified as COI fragments for *Hemibagrus nemurus* using BLAST (Basic Local Alignment Search Tool) procedure in NCBI (National Center for Biotechnology Information). The sequences have been deposited in

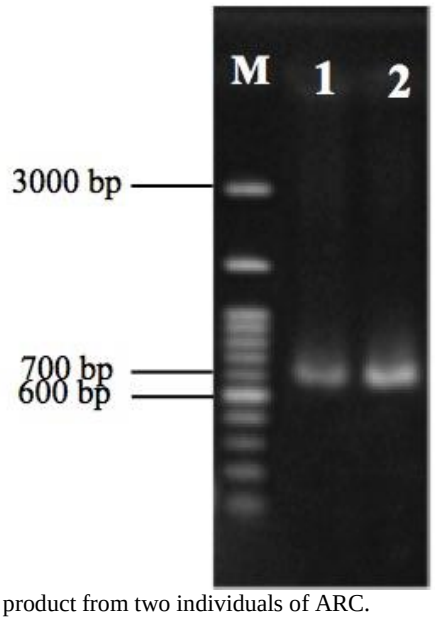

Figure 1. Gel electrophoresis of PCR product from two individuals of ARC.

the BOLD system under accession numbers ACS4799 and also submitted to the GenBank database with registration number MG521911 and MG521912.

The Percentage of Sequences Similarity and Phylogenetic Analyses

The percentage of similarity of CO-I sequences from Musi River and Penukal River were analysed using nucleotide BLAST (Basic Local Alignment Search Tool). All species names used are in accordance with The Catalogue of Life (Roskov *et al*. 2014). Accession numbers (GenBank) for voucher species which were used to construct a gene tree are listed in Table 1. *Pangasidae* and *Tilapia* were also used as species outgroup in the analysis. The robustness of the Maximum Likelihood (ML) tree was indicated by performing bootstrapping analysis with 1000 replicates with values along branches reported as Bayesian posterior probabilities for GTR-CAT model (Randomized Axelerated Maximum Likelihood/RAxML) and visualized by Figure 2.

Results

The length of the COI partial sequences that were retrieved from *H. nemurus* species were 572 and 596 base pairs, representing Penukal and Musi River at South Sumatra Province. We can develop and distinguish COI gene for use in differentiating ARC with *Oreochromis niloticus* (Stirling collection) and *Pangasidae* from Germany, India and Thailand. It also discriminate others Bagridae from Indonesia, India, Malaysia, Thailand, China and USA. Nucleotide BLAST analyses showed COI sequences in the present study agreed with those in the NCBI GenBank Database, with 100% identity to *H. nemurus* from Indonesia (KM213068.1), 99% to *Mystus* sp (KT001043.1) and *H. capitulum* (KT001043.1) from Malaysia. The phylogenetic tree (Figure 3) showed that all the Bagridae were separated from *Pangasidae* and *Oreochromis*. *H. macropterus* (USA) formed a distant with high supported bootstrap value (95) from one big clade of Bagridae which consisting of three sub-clades.

The first sub-clades consisted of *H. nemurus* (China), *H. menoda*, *M. cavasius*, *M. oculatus*, and *M. bleekeri*, all of them were from India. However, the first sub-clade indicated low bootstrap value (28) with others two clades. Meanwhile, the second sub-clade showed a high bootstrap value (89) with the third clade. As expected, the COI sequences of *H. nemurus* from Penukal River (PALI) and Musi River, Indonesia in this study were clustered tightly in the

Table 1. Accession numbers (GenBank) of voucher species used to build a phylogenetic tree

No	Species	Accession Number	Sample Origin
1	*H. nemurus*	KM213068.1	South Sumatera
2	*H. capitulum*	KP856825.1	Indonesia
3	*Mystus* sp	KT001043.1	Malaysia
4	*H. fortis*	KT799807.1	Malaysia
5	*Mystus cavasius*	JN628905.1	India
6	*H. menoda*	JN697600.1	India
7	*M.oculatus*	HQ009493.1	India
8	*H. macropterus*	JF292350.1	USA
9	*M. bleekeri*	KP939357.1	India
10	*P. macronema*	KT289892.1	Vietnam
11	*H. nemurus*	JQ289148.1	Thailand
12	*H. nemurus*	JN646095.1	Malaysia
13	*H. nemurus*	JN020074.1	China
14	*H. nemurus*	JN020075	China
15	*H. nemurus*	JF781228.1	Malaysia
16	*H. nemurus*	JF781229.1	Malaysia
17	*H. nemurus*	JF781230.1	Malaysia
18	*H. nemurus*	KM213067.1	Palembang, Indonesia
19	*H. nemurus*	KM213068.1	Palembang, Indonesia
20	*H. nemurus*	KF805368.1	Thailand
21	*H. nemurus*	KF805369.1	Thailand
22	*H. nemurus*	KF805370.1	Thailand
23	*H. nemurus*	KF805371.1	Thailand
24	*H. nemurus*	KT001039.1	Malaysia
25	*H. nemurus*	KT001040.1	Malaysia
26	*H. nemurus*	KU692545.1	Java & Bali
27	*Pangasidae*	KJ531383.1	Germany
28	*Pangasius Hyphoptalmus*	KR080263.1	Thailand
29	*Pangasius*	KX685193.1	Indonesia
30	*P. macronema*	KT289892.1	Vietnam
31	*O. niloticus*	KM438538.1	Stirling collection

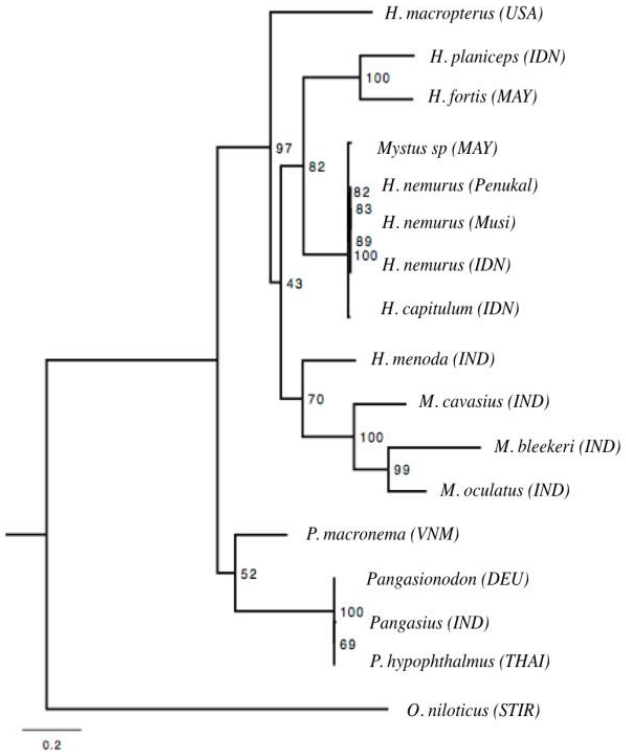

Figure 2. Maximum Likelihood (ML) tree.

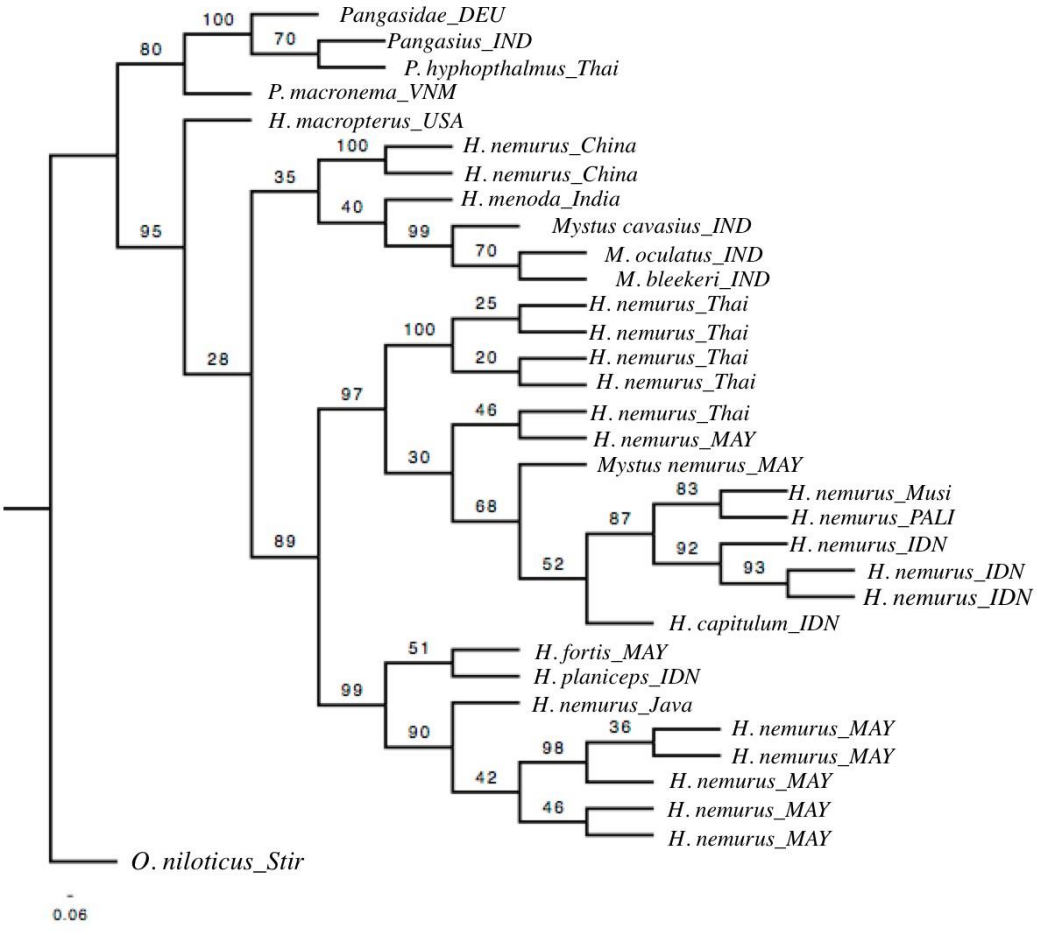

Figure 3. Gene tree of Asian redtail catfish (*H. nemurus*) derived from Cytochrome c oxidase subunit I sequences.

second sub-clade with the same species from Thailand, Malaysia and Palembang (Indonesia) and also others species *Mystus* sp (Malaysia), and *H. capitulum* (Indonesia). The third clade was consisted of *H. nemurus* from Java and Malaysia, together with *H. fortis* (Malaysia) and *H. planiceps* (IDN), where *H. nemurus* from Java was separated from the same species from Malaysia with high bootstrap value (90). It was also confirmed that *H. fortis* and *H. planiceps* were clearly distinct relative to *H. nemurus*.

Discussion

Based on phylogenetic tree, ARC derived from Penukal River was in the same group with the same species from Musi river (Figure 2), due to geographically those two rivers were connected each others, and no hybridization occurence with others species. The sequences of COI gene indicated that it can be used in differentiating ARC with others Bagridae from India, Malaysia, Thailand, China and USA. The sequence of COI gene of ARC in this study agreed to previous research by Wibowo, Sloterdijk & Ulrich, (2005) with sample origin from Musi Banyuasin, South Sumatera Province, however they all were separated from the same species from Java, Indonesia. It is confirmed that molecular species authentication techniques have the potential for rapid, accurate assessment of proper labelling (Wong *et al*., 2011).

A high score of supported bootstrap value (95) between *H. macropterus* (USA) and one big clade of Bagridae which consisting of three sub-clades indicated high confidence level of an outgroup of this species. *H. nemurus* (China) showed a relative distinct from the same species in the same clades, however there was not enough supported bootstrap value (35) to conclude. This result also accordance with previous research based on the mitochondrial cytochrome b where *H. nemurus*, *H. capitulum*, and *Mystus* sp were considered to be one group as it was shown as the presence of the Sundaic clade on the west coast of the Thai-Malay Peninsula as well as on the Sundaic Islands reflects the historical connection between east Sumatra and West Peninsular Malaysia across the Strait of Malacca (Dodson & Lecomte, 2015). However, one voucher species of *Mystus* sp (Accession number: KT001043.1) seems to be *H. nemurus* due to very tight closely in the gene tree.

The third clade was consisted of *H. nemurus* from Java and Malaysia, together with *H. fortis* (Malaysia) and *H. planiceps* (IDN). There was a separation of *H. fortis* (Malaysia) and *H. planiceps* (IDN) from others Bagridae. *H. planiceps*, considered endemic to Java, occurs in the upper parts of river basins, indicating a preference for clear, fast-flowing habitats with substrates of rocks and sand (Ng & Kottelat, 2013). The separation of *H. planiceps* from *H. nemurus* have been hypothesized at previous study from Dodson & Lecomte (2015) where the phylogeography of the genus appears to have been influenced by two geological histories, the formation of North Borneo and the emergence of the Sunda Islands.

Conclusions

DNA barcoding is an invaluable tool for species authentication of Asian Redtail Catfish (ARC) from Musi and Penukal River, however more individuals of species/subspecies on Bagridae and habitats are needed in better understanding structure of species.

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