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DNA BARCODING OF CAGED PANGASIIDS IN PAHANG RIVER MALAYSIA

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Abstract

The domestication aquaculture selection in farm environment can cause changes in phenotype and genotype of farmed fishes, which may later influence the demographic structure of wild populations if they accidentally escaped from cages. This is an alarming situation for conservation of native species of *Pangasius* sp. in Pahang River. Hence, the present study was aimed to investigate genetic variation among the fishes collected from different cages from Pahang River. We adopted conventional taxonomical approach to identify species and cross-examined using universal barcode gene Cytochrome Oxydase Subunit 1 (COX1) gene. Samples were collected from 6 commercial cages from Pahang River. Haplotype and genetic diversity among the fishes from different cages were determined. Results from Neighbor Joining tree showed that most of the samples were identified to be *Pangasianodon hypothalamus* despite having different morphometric character. This study also revealed that most of the caged *Pangasius* cultured in Pahang River are exotic and non-native to Malaysia. Thus, a continuous monitoring through studies on genetic variation of *Pangasius* sp. is an essential need for the sustainable development of this endangered fishes in Pahang River.

Keywords: Pangasidae, Pahang River, endangered fishes, COI

1.0 INTRODUCTION

An artificial selection in aquaculture can lead to phenotypic changes in characters such as body size, composition, and age at sexual maturity [1]. Consequently, it is almost certain that aquaculture organisms will differ genetically from those in the wild following direct and correlational selection for commercially desirable traits, as well as domestication selection caused by the farm environment. Changes in phenotype and genotype of farmed organisms can result in differences in fitness-related traits, which ultimately may alter the demographic structure of wild populations. In addition, the magnitude and direction of any changes in farmed populations will determine the degree to which they differ from those in the wild [2]. These potential differences become a concern for the maintenance of wild population size and structure if farmed fish enter the natural environment. As such, the extent of the effects of farmed fish on wild populations is contingent on a number of factors, including the population size of wild fish, the number and frequency with which farmed individuals enter the natural environment and the degree of interbreeding between farmed and wild fish [2].

As such, introgression of genes from hatchery fish may result in changes to behavior and life history of invaded natural populations. However, behavioral interactions between farmed and wild fish can occur even in the absence of interbreeding. Direct competition for territories, food, and mates between farmed and wild fish can also affect natural

populations. Genetic variation in a species enhances the capability of organism to adapt to changing environment and is necessary for survival of the species. Genetic variation arises between individuals leading to differentiation at the level of population, species and higher order taxonomic groups. Development of molecular genetic markers has powerful ability to detect genetic studies of individuals, populations or species. Molecular markers and their statistical analysis revolutionized the analytical power, which provide various scientific observations which have importance in aquaculture practice recently such as: Species identification, genetic variation and population structure study in natural populations, comparison between wild and hatchery populations, assessment of demographic bottleneck in natural population and propagation assisted rehabilitation programs [3].

In Malaysia, the *Pangasius* was introduced from Thailand in the 1980's and was successfully induced bred in captivity [4]. The success in gonadal maturation in captivity followed by induced breeding and mass seed production [5] resulted in the increased capacity by local hatcheries to produce various freshwater fish seeds to supply the local aquaculture industry. Recently the populations of wild endemic *Patin* in Pahang river have been reported declining. This can be seen by the lower number of yearly landed specimens as recorded by Department of Fisheries at Maran, Malaysia [6]. Due to an urgent need in identifying the genetic variation of cultured *pangasiid* in cultured cages, the present study was aimed to address the genetic variation among the cultured Pangasius in Pahang, Malaysia.

2.0 EXPERIMENTAL

The areas covered were from Kuala Tembeling to Temerloh until Kuala Pahang (Table 1). Eight stations were picked randomly alongside the river. Samples were identified morphologically and revalidated the reliability of identification using universal gene barcoding. DNA extraction was done by using DNeasy® Blood & Tissue DNA Extraction Kit from Qiagen and quantified using Nanodrop 2000c spectrophotometer. CO1-3 primer set designed previously [7] was used in this experiment to amplify Cytochrome C Oxidase Subunit 1 (CO1) gene in the Pangasiids. The forward and reverse primers named as FishF2_t1;5'TGTAAAACGACGGCCAGTCGACTAATCATAAAGATATCGGCAC-3' and FishR2_t1:5'-CAGGAAACAGCTATGACACTTCAGGGTGACCGAAGAATCAGAA-3' respectively. PCR cycle profile consisted of 94°C for 2 minutes, 35 cycles of 94°C for 30 seconds, 50°C for 40 seconds and 72°C for 1 minute, with a final extension at 72°C for 10 minutes [7]. The Sequencing service was provided by outsourcing in First Base Sdn. Bhd., Malaysia.

2.1 Data Analysis

The *CO1* gene sequences of 11 samples collected from cages, two (2) samples from wild population of Pahang river and another 16 CO1 gene sequences of Pangasiids derived from GenBank were aligned. Pair-wise evolutionary distance among them was estimated by Tamura-3 parameter method [8], using software programme MEGA 6 (Molecular Evolutionary Genetic Analysis). Tamura-3 method was chosen as the best parameter based on the best-fit model calculated by MEGA 6. Tamura-3 Phylogenetic trees were constructed using neighbor-joining method to verify the closely related between Pangasiid species and also within species in Pahang river using 1000 replicate bootstrap value. *Tor tambroides*(KC905024.1) and *Helichophagus wandersii* (HQ641127) were used as outgroups.

Table 1 Sampling Sites with coordinates along Pahang River and its Farming techniques applied for each cage

Checkpoint	Sampling Site	Latitude	Longitude	Farming Technique	Fingerling Sources
1	Kuala Lipis Border	4°04'14.8"N	102°18'53.1"E	Polyculture ; with Tilapia in different cages	-Kg. Baru -Jln. Benta
2	Kg. Batu Lada	3°57'37.9"N	102°25'36.6"E	Monoculture	-Jengka 25 -Felda Perlok -Kg. Baru
3	Kuala Tembeling	4°04'21.4"N	102°18'58.6"E	Polyculture ; with Tilapia and Lipur in different cages	-Kuala Lumpur
4	Kuala Krau	3°41'09.9"N	102°22'56.5"E	Polyculture ; with Tilapia in different cages	-Jengka -Rawang -Jerantut
5	Temerloh	3°23'47.7"N	102°25'31.6"E	Polyculture; with Tilapia in different cages	-Felda Peroi -Felda Perlok
6	Triang	3°20'03.4"N	102°30'18.8"E	Polyculture; with Tilapia and Jelawat in different cages	-Felda Purun, Maran
7	Chenor	3°30'37.3"N	102°36'28.7"E	Polyculture with Tilapia in a cage	-Pusat Bandar Triang -Felda Simpang Lepah
8	Kampung Kemboja, Pekan	3°31'52.0"N	103°17'55.4"E	Polyculture ; - Patin Hitam - Patin Buah - Kerai - Patin Lawang - Patin Emas	-Kemboja -Thailand -Pekan

3.0 RESULTS AND DISCUSSION

3. 1 PCR Product Gel Elusion

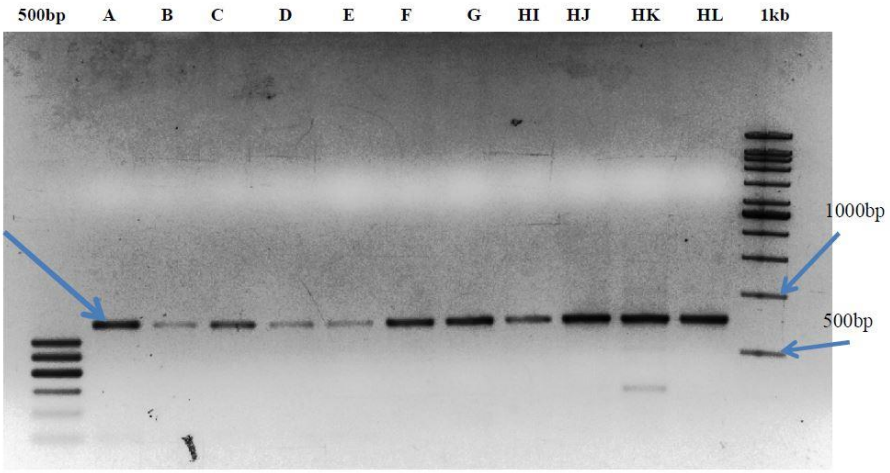

Figure 1 Ethidium bromide stained image of amplified CO1 gene as visualized on 1% (w/v) Agarose gel. Samples A-G sampled from K.Lipis, K.Tembeling, Kg. BatuLada, K.Krau, Temerloh, Triang and Chenor respectively. HI, HJ,HK,HL were sampled from Pekan. 1kb DNA ladder was used as a reference

Table 2 BLAST analysis results of generated CO1 gene sequences

Samples	Product size (bp)	Sequences producing significant alignments	Percentage of similarity	Identities	E value	Accession Number
A	666	*Pangasianodon hypophthalmus* voucher AUPH15 cytochrome oxidase subunit I (*COI*) gene, partial cds; mitochondrial Length : 651bp	100	651/651	0.00	JF292405
B	660		100	651/651	0.00	
C	669		100	651/651	0.00	
D	671		100	651/651	0.00	
E	667		99	650/651	0.00	
F	667		99	650/651	0.00	
G	676		100	651/651	0.00	
HI	664		100	651/651	0.00	
HJ	674		100	651/651	0.00	
HL	661		100	651/651	0.00	
HK	670	*Pangasius nasutus* voucher SLM-PN(PH)-04 cytochrome c oxidase subunit 1 (*COI*) gene, partial cds; mitochondrial Length : 582bp	100	582/582	0.00	JF781175

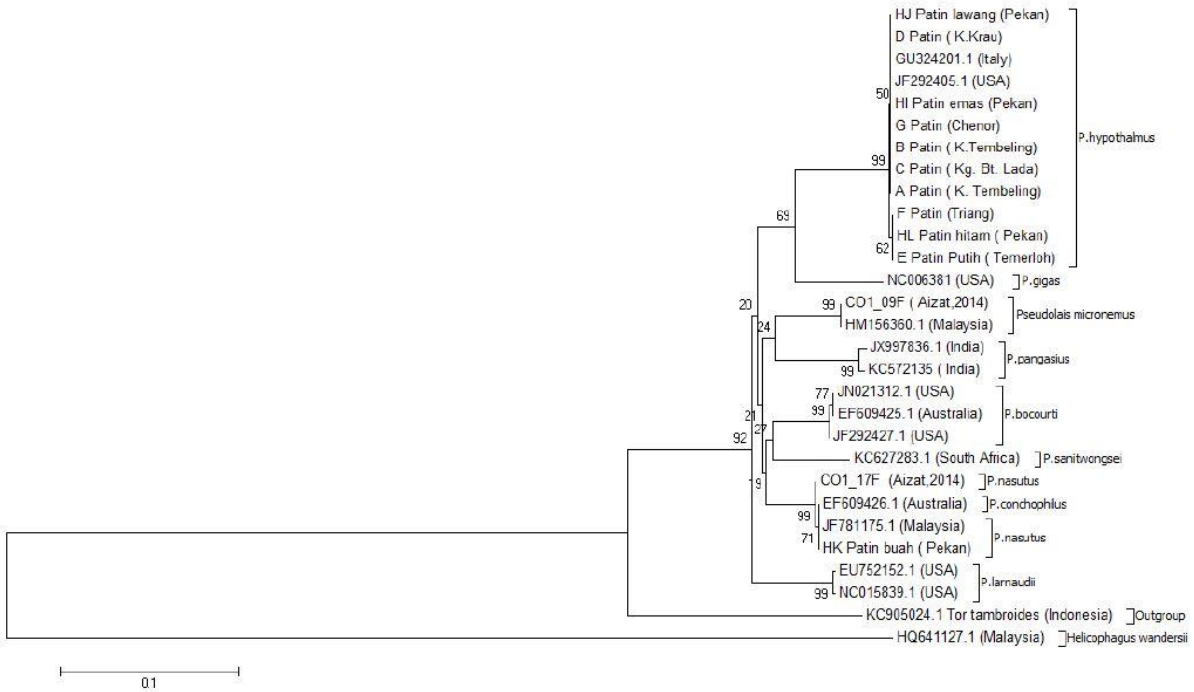

Figure 2 Phylogenetic analysis of CO1 gene from Pangasiids in Pahang River and their near neighbors based on NJ tree. Higher bootstrap value in internal nods of every clad showed the reliability of constructed phylogenetic tree

PCR product gel elution has clearly shown the efficiency of selected primers in amplifying the target gene. The apparent bands were appeared on 1% (w/v) agarose gel at ~650bp position (Figure 1). NJ tree showed the best bootstrap value and clad all the generated Patin in single clad with *Pangasius hypothalamus* (Figure 2). However, those samples were known by locals with different names. This might be happen due to different morphological characteristic. For example, Patinemas *(local name)* is a hybrid species between *P. hypothalmus*and *P. nasutus* [9]. Abdul Muneer *et al*., (2011) [10] observed morphological characteristics of a same species might be varied due to the environmental factor such as, water quality, nutrition and geographical distribution. Similarly, sample HJ locally known as Patin lawing was genetically identified as *P. hypothalamus* which is supporting the findings of previous study by Mohd Zafri *et al*., (2005) [6]. Sample HK locally known as *Cambodia Patin buah* was claimed to be brought from Cambodia and has been cultured in Pahang River. Our analysis identified HK sample as *P. nasutus* which is the native Pahang river fish (Table 2). This might probably be due to cross breeding efficiency and highest possibility of cage fish escaping to the wild population. Tajima Neutrality test showed low nucleotide diversity among the caged fishes (***π:*** 0.032617 and negative D value: -1.161562) representing rare alleles present at low frequency. Besides, negative D value also shows that lower heterozygosity might occur due to purifying selection or also known as negative selection, where mutations can occur and accumulate at silent sites (Loewe, 2008). Thus, there are likely to be lots of segregating sites, but not much heterozygosity. The main consequence of negative selection is the extinction of less-adapted variants.

Previous study shown that 11 species are found in Thailand, 10 in Indonesia, 3 in Peninsular Malaysia and 4 species endemic to the Borneo Island [11, 12]. In Pahang River, cage cultured *Pangasius* can be found all along the river started from Kuala Tembeling until Kuala Pahang, Pekan.

Pouyad and co-workers [13] had listed three species of catfishes which are native to the Pahang River which are "Patin buah" or *Pangasius nasutus, Pangasius bocourti,* and *Pangasius polyuranodon*, other than that local fishermen have also listed another two species that can be found there which are "Patin juara" (*Pangasius micronema*) and "Patin muncung" (*Helicophagus wandersii*) [6]. However, the most commonly bred catfish species in Malaysia is not from the local catfish, but it is "Patins angkar" or *Pangasius sutchi*, which is later known as *Pangasiu shypophthalmu s*after the reclassification by [12]. This species are originated from Thailand and brought to Malaysia for aquaculture purpose [14].

Nevertheless, *Pangasius hypophthalmus* is more preferred by the locals to be domesticated than the native catfishes this is because, this omnivorous catfish can grow very quick, easy to be bred in captive, easy to formulate the feed, and has high immunity system and can adapt to surrounding easily [3]. However, *Pangasius hypophthalmus* which is reared in cages might have escaped during rainy season in Pahang that occurs in November annually. As consequences, *Pangasius hypophthalmus* might have cross-breeding with the wild Pangasiids.

Interbreeding can give direct effect by reducing fitness and indirect effects occur through competitive, disease and parasite. Previous studies have shown that farm fish in the wild environment have severely reduced lifetime fitness, genetic effects compared to native populations with intermediate hybrid fitness. For example, "*Patinemas*" is the product of cross-breeding between *Pangasius hypophthalmus* and *Pangasius nasutus.*

4.0 CONCLUSION

It can be concluded that most of the Pangasius cultured in cages were *P. hypothalamus* in Pahang. The low rate of rare allele frequency in caged fishes suggests that the caged fishes are genetically less diverse. The possibility of accidental escape of caged fishes is also reflected in the analysis. Further studies are needed to compare the genetic diversity of wild and cultured Pangasius in Pahang river.

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