

Molecular phylogeny of Malaysian Pangasiid based on cytochrome C oxidase I (COI) DNA sequences

Azlina, Z.M.^a, Daud, S.K.^a, Siraj, S.S.^b, Aliabadian M.^{c,d}, Moghaddam, F.Y.^{c,d}

^aDepartment of Biology, Faculty of Science, Universiti Putra Malaysia, 43400 UPM Serdang Selangor.

^bDepartment of Aquaculture, Faculty of Agriculture, Universiti Putra Malaysia, 43400 UPM Serdang Selangor.

^cDepartment of Biology, Faculty of Science, Ferdowsi University of Mashhad, Mashhad, Iran.

^d Research Department of Zoological innovations (RDZI), Inisistute, of Applied Zoology, Ferdowsi University of Mashhad, Mashhad, Iran

The molecular phylogenetic trees of 42 representative samples of Malaysian pangasiid were constructed using cytochrome *c* oxidase subunit I (COI). The topologies resulting from molecular analyses, Maximum Parsimony, Maximum Likelihood, and Bayesian Inference, revealed the paraphyly of *Pangasius* genus and monophyly of *P. nasutus*, *P. bocourti*, *P. micronemus*, and *P. hypophthalmus* with high support values. The results clearly showed that COI gene can be used to isolate the native species of Malaysian pangasiids, including *P. nasutus*, *P. micronemus*, *P. bocourti* and *H. waandersii* from *P. hypophthalmus* that has been introduced from Thailand.

Key words: Pangasiidae, catfishes, COI gene, phylogeny

INTRODUCTION

Pangasiidae is a large family of tropical freshwater catfishes found throughout South and Southeast Asia. Since the groups were established as Pangasiini Bleeker, 1858; its content and classification have been greatly changed. Family Pangasiidae has a good potential for aquaculture and high values as flesh food at market in Malaysia. Morphologically, family Pangasiidae is recognized by a laterally compressed body, having two pairs of barbels, a short dorsal fin with two spines, a developed adipose fin, a long anal fin, and a strong pectoral spine (Gustiano, 2009).

In family Pangasiidae 5 genera, 30 species have been identified (Ferraris, 2007). According to Roberts and Vidthayanon (1991) 11 species are found in Thailand, 10 in Indonesia, 3 in Peninsular Malaysia and 4 species endemic to the Borneo Island. Most of the species in the Pangasiidae family are freshwater fishes. Five species also occur in brackish water: *P. pangasius* (Hamilton 1822), *P. krempfi* (Fang and Chau 1949), *P. kunyit* (Pouyaud et al. 1999), *P. sabahensis* (Gustiano et al. 2003), and *P. mekongensis* (Gustiano et al. 2003).

In a recent overview of Siluriform systematic, two genera are described for pangasiid by Teugel (2003), *Helicophagus* and *Pangasius*, each with 3 and 25 species, respectively (Diogo, 2007). Roberts and Vidthayanon (1991) in a systematic revision of Pangasiidae, recognized two genera: *Helicophagus* Bleeker, 1858 with two species and *Pangasius* Valenciennes, 1840 with 19 valid species. In a recent revision, *Helicophagus* genus has three species, namely *H. leptorhynchus*, *H. typus* and *H. waandersii* (Ferraris, 2007). *H. waandersii* is categorized as rare and endangered species in Malaysia (Murkherjee, 2001).

Following Vidthayanon and Roongthongbaisuree (1993), *Pangasius* can be divided in four subgenera: (1) *Pangasius* (*Neopangasius*) including four species, endemic to Borneo; *P. humeralis* Roberts, 1989, *P. kinabatanganensis* Roberts and Vidthayanon, 1991, *P. lithostoma* Roberts, 1989 and *P. nieuwenhuisii* Popta, 1904 subgenera (2) *Pangasius* (*Pteropangasius*) Fowler, 1937 including *P. pleurotaenia* Sauvage, 1878 and *P. micronema* Bleeker, 1847 subgenera (3) *Pangasius* (*Pangasianodon*) Chevey, 1930 including *P. gigas* Chevey, 1930 and *P. hypophthalmus* (Sauvage, 1878), and finally the fourth subgenera (4) *Pangasius* (*Pangasius*) Valenciennes, 1840, for which no diagnostic features are given and including all remaining species in *Pangasius* genus.

Helicophagus waandersii has a posterior nostril which located at the midway between the anterior nostril and eye. The eyes are located at the corner of the mouth and only vomerine teeth are present (Gustiano et al., 2006).

Pangasius nasutus has a sharp prominent snout. The tooth of upper jaw of this species is entirely exposed when the mouth is closed, which distinguishes *P. nasutus* from other species of *Pangasius* (Gustiano et al., 2006). *Pangasius micronemus* has vomero-palatine teeth in 4 more or less ovoid patches. It has large eyes and the snout is truncate (Kottelat et al., 1993). Beside that *P. bocourti* has truncate or rounded head and blunt rounded snout with a broad white band on muzzle (Robert and Vidthayanon, 1991).

Pangasius hypophthalmus has dark grey or black fins with 6 branched dorsal-fin rays. This species has a head relatively small and dark stripe on the middle of anal fin. They also have small gill rakers regularly interspersed with larger ones (Rainboth, 1996).

The name of *Helicophagus hypophthalmus* was first used by Kottelat (1984) and Burgess (1989), but later some researchers have used other generic names for species, such as *Pangasianodon hypophthalmus* and *Pangasius hypophthalmus* (Roberts and Vidthayanon, 1991; Pouyaud et al., 1999; Pouyaud and Teugels, 2000; Hogan et al., 2004; Gustiano, 2003; Ferraris, 2007), not all of them have been generally accepted.

A locally known 'Patin', is a large spiny fish. The size of these genera needs to be explained in terms of systematic. Adult fish is from 20 cm to 3 meters, but most are size 50 cm and more (Haslawati, 2004).

There are several hybrid *Pangasius* species such as *Patin Mas* (Hybrid species between *Pangasius sutchi* and *P. nasutus*), *Patin Kemboja* and *Patin Buah Kemboja* found in Sungai Pahang (Haslawati, 2004). These local cage cultured species breed with the wild *Pangasius* like a *P. nasutus* in the Sungai Pahang and produced suspected hybrid species. Some villagers also call the same species with different local name like *P. bocourti* as a *Patin Kemboja*, and *Patin Lawang*. Hence, it is crucial to know the phylogenetic relationships among species of the family Pangasiidae.

Considerable confusions have arisen in the systematics of this catfish group. Most of the previous studies described species without consulting existing type specimens. Nearly all authors have problems recognizing juveniles of the larger species, and junior synonyms are often based on small sized specimens (Gustiano, 2009). Available information on the phylogeny of the family of Pangasiidae is scarce (So et al., 2006) and some species of the family Pangasiidae have similar morphological features, such as *P. bocourti* with *P. nasutus*. The previous finding by Rainboth (1996) stated that there was misidentification on *P. bocourti* and *P. nasutus*. Study of Gustiano et al (2006) using biometric measurements enabled to distinguish four genera with seven species exist in four main river in Sumatra but the classification was solely based on morphology without any molecular evidence.

Molecular phylogeny performed by Pouyaud et al. (2000) provided support for the recognition of some *Pangasius* subgenera and, or species groups as distinct genera based on allozymes and mitochondrial DNA except for *P. (Neopangasius)*, which is polyphyletic and suggested to be included in *P. (Pangasius)*.

Molecular identification is considered important especially when there are morphologically similar or misidentified species (Marko et al., 2005). The mitochondrial genome is transmitted (primarily) maternally (Gyllensten et al., 1991) and mtDNA is haploid and clonally inherited with no recombination. In particular, the mitochondrial gene cytochrome-*c* oxidase subunit I (*COI*) can serve as a uniform target gene for a bio-identification system (Persis et al., 2009). Application of *COI* has proved to provide valuable information for species identification to complete the taxonomic data and global validation of systemic position, phylogeny (Khan et al., 2000). In the present study *COI* gene was used to give a clear picture of the phylogenetic relationships among different species of pangasiids found in Malaysia and examine the phylogenetic relationships among local species of the family Pangasiidae.

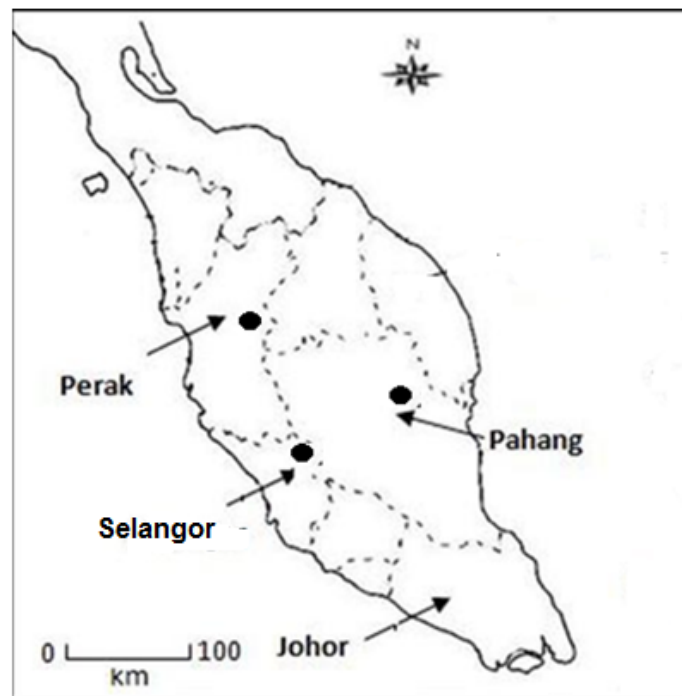


FIGURE 1. Map shows locality of collected samples of selected pangasiids in Peninsular Malaysia.

MATERIAL AND METHODS

A total of 42 samples of Pangasiidae were collected from three parts of Peninsular Malaysia, namely Pahang, Perak and Selangor (Fig. 1). Sungai Pahang is the longest river in peninsular Malaysia and has a diversity of wildlife, including pangasiid species compare to other area. The samples belong to different pangasiid species, namely *Pangasius hypophthalmus* (*Pangasianodon hypophthalmus*), *Pangasius nasutus*, *Pangasius bocourti*, *Pangasius micronemus* and *Helicophagus waandersii*. The sample size (n) and location of selected samples are shown in Table 1. Samples of muscle tissues were amplified with primers *COI*_f, 5'-CCTGCAGGAGGAGGAGAYCC-3' and *COI*_e 5'-CCAGAGATTA GAGGGAATCAGTG-3', previously described by Palumbi et al. (1991) and Esa et al (2008). The final concentration of 20 μ L PCR reaction containing 2.0 μ L of extracted DNA (\pm 50ng/ μ L), 1.4 μ L

Mgcl₂ (1.5mm), 4.0μL (1x) PCR buffer, 0.4μL dNTP (200μM), 0.4μL Taq DNA polymerase (2 Unit) (Promega), 0.08μL for forward and reverse primers, and 11.4μL water. PCR profile was performed with predenaturation at 95°C for 3 min, followed by 35 cycles at 95°C for 40 Sec, 45°C for 30sec and 72°C for 1 min (Nadiatul et al., 2012). The sequences were deposited in GeneBank under accession numbers (HQ641125- HQ641166) (Table 2).

The *COI* sequences were aligned using BioEdit7.0.5 (Hall, 1999) and checked by eye for the presence of stop codons or insertions/deletions that would have disrupted the reading frame (Alaie Kakhk and Aliabadian, 2012).

The 42 nucleotide sequences were subjected to phylogenetic analyses by Maximum parsimony (MP), and Maximum likelihood (ML) using the PAUP 4.0b10 program (Swofford, 2001). To test the robustness of the nodes we ran 500 and 2000 bootstrap replicates under ML and MP, respectively, with a single random addition sequence replicate per bootstrap replicate (Dyanat et al., 2010). The aligned sequences were also used to estimate Bayesian inference (BI) using MrBayes 3.1 (Ronquist and Huelsenbeck, 2003). The Markov Chain Monte Carlo (MCMC) process was set to 4 X 10⁶ generations with trees being sampled every 1000 generations.

To assess the nodal support for each branch, 100 and 1000 bootstrap replicates with parameters estimated automatically were run under ML and MP, respectively. Evaluation of the data for appropriate models for BI and ML analyses was done using the Akaike Information Criterion (Akaike, 1973) as implemented in the ModelTest 3.7 (Posada and Crandall, 1998); the best model for the data was GTR+C+I. The nucleotide and haplotype diversity indices implemented in the DNaSP (ver. 4.0) program (Rozas et al., 2003).

The Kimura-2 parameter (K2P) distance was used to calculate the genetic distances and to quantify sequence divergences between individuals (Kimura, 1980).

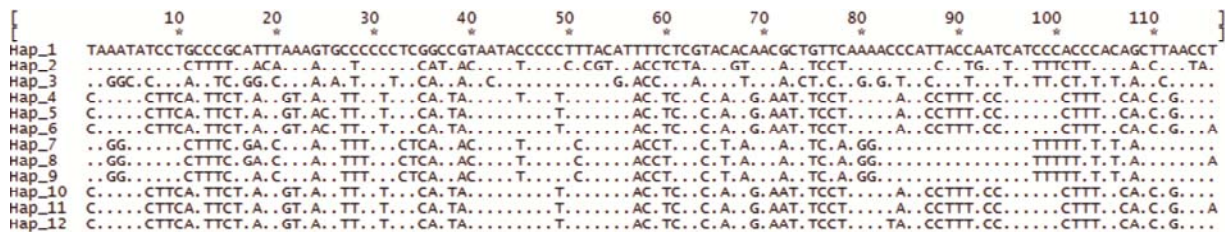


FIGURE 2. Partial sequences of mitochondrial *COI* for pangasiids haplotypes.

TABLE 1. Sample size and total length of pangasiid samples collected from various locations in Malaysia.

Code	Local name	Scientific name	Mean and SD of TL (cm)	Number of sample(n)	Location
PNR	Patin Buah	<i>Pangasius nasutus</i>	41.80 ± 2.9	10	Rawang, Selangor.
PNP	Patin Buah	<i>Pangasius nasutus</i>	33.00 ± 3.4770	8	Sungai Pahang.
PHKK	Patin Hitam	<i>Pangasius hypophthalmus</i>	28.65 ± 2.9	10	Kuala kangsar, Perak.
PHR	Patin Hitam	<i>Pangasius hypophthalmus</i>	29.8 ± 1.0723	10	Rawang, Selangor.
PB	Patin Lawang	<i>Pangasius bocourti</i>	64 ± 0	1	Sungai Pahang.
PM	Patin Juara	<i>Pangasius micronemus</i>	37 ± 1	2	Sungai Pahang.
HW	Patin Muncung	<i>Helicophagus waandersii</i>	47 ± 0	1	Sungai Pahang.
NC 003489		<i>Ictalurus punctatus</i> (outgroup)		1	
AF482987		<i>Ictalurus punctatus</i> (outgroup)		1	

Note: AF482987 and NC 003489 were used as an Outgroup for *COI*, TL - Total length, SD - Standard deviation, n = sample size The location for collected samples of selected pangasiids Abbreviations: A = Kuala Kangsar, Perak (n = 10, PHKK – *Pangasius hypoptthalmus*), B = Rawang, Selangor (n = 10, PHR – *Pangasius hypoptthalmus* and n = 10, PNR – *Pangasius nasutus*), C = Pahang (n = 1, HW - *Helicophagus waandersii*, n = 1, PB – *Pangasius bocourti*, n = 2, PM – *Pangasius micronemus* and n = 8, PNP – *Pangasius nasutus*), n = sample size.

TABLE 2. Pangasiidae species, samples code and GeneBank accession numbers used in this study.

Species	Code	Accession numbers
<i>Pangasius bocourti</i>	PB	HQ641125
<i>Pangasius micronemus</i>	PM1	HQ641166
<i>Pangasius micronemus</i>	PM2	HQ641126
<i>Helicophagus waandersii</i>	HW	HQ641127
<i>Pangasius hypoptthalmus</i>	PHKK1	HQ641128
<i>Pangasius hypoptthalmus</i>	PHKK2	HQ641129
<i>Pangasius hypoptthalmus</i>	PHKK3	HQ641130
<i>Pangasius hypoptthalmus</i>	PHKK4	HQ641131
<i>Pangasius hypoptthalmus</i>	PHKK5	HQ641132
<i>Pangasius hypoptthalmus</i>	PHKK6	HQ641133
<i>Pangasius hypoptthalmus</i>	PHKK7	HQ641134
<i>Pangasius hypoptthalmus</i>	PHKK8	HQ641135
<i>Pangasius hypoptthalmus</i>	PHKK9	HQ641136
<i>Pangasius hypoptthalmus</i>	PHKK10	HQ641137
<i>Pangasius hypoptthalmus</i>	PHR1	HQ641156
<i>Pangasius hypoptthalmus</i>	PHR2	HQ641157
<i>Pangasius hypoptthalmus</i>	PHR3	HQ641158
<i>Pangasius hypoptthalmus</i>	PHR4	HQ641159
<i>Pangasius hypoptthalmus</i>	PHR5	HQ641160
<i>Pangasius hypoptthalmus</i>	PHR6	HQ641161
<i>Pangasius hypoptthalmus</i>	PHR7	HQ641162
<i>Pangasius hypoptthalmus</i>	PHR8	HQ641163
<i>Pangasius hypoptthalmus</i>	PHR9	HQ641164
<i>Pangasius hypoptthalmus</i>	PHR10	HQ641165
<i>Pangasius nasutus</i>	PM1	HQ641138
<i>Pangasius nasutus</i>	PM2	HQ641139
<i>Pangasius nasutus</i>	PNP3	HQ641140
<i>Pangasius nasutus</i>	PNP4	HQ641141
<i>Pangasius nasutus</i>	PNP5	HQ641142
<i>Pangasius nasutus</i>	PNP6	HQ641143
<i>Pangasius nasutus</i>	PNP7	HQ641144
<i>Pangasius nasutus</i>	PNP8	HQ641145
<i>Pangasius nasutus</i>	PNR1	HQ641146
<i>Pangasius nasutus</i>	PNR2	HQ641147
<i>Pangasius nasutus</i>	PNR3	HQ641148
<i>Pangasius nasutus</i>	PNR4	HQ641149
<i>Pangasius nasutus</i>	PNR5	HQ641150
<i>Pangasius nasutus</i>	PNR6	HQ641151
<i>Pangasius nasutus</i>	PNR7	HQ641152
<i>Pangasius nasutus</i>	PNR8	HQ641153
<i>Pangasius nasutus</i>	PNR9	HQ641154
<i>Pangasius nasutus</i>	PNR10	HQ641155

TABLE 3. Haplotype distribution of each sequence of *COI*.

Haplotype	n	Sequences	PP	PN	PM	PB	HW	OG
<i>COI_1</i>	1	PB				√		
<i>COI_2</i>	2	PM1,PM2			√			
<i>COI_3</i>	1	HW					√	
<i>COI_4</i>	3	PHKK1,PHKK6,PHKK9	√					
<i>COI_5</i>	7	PHKK2,PHKK4,PHKK5,PHKK7,PHK K8,PHKK10,PHKK3	√					
<i>COI_6</i>	1	PHKK3	√					
<i>COI_7</i>	7	PNP1,PNP3,PNP4,PNP5,PNP6,PNP7,P NP8		√				
<i>COI_8</i>	1	PNP2		√				
<i>COI_9</i>	10	PNR1,PNR2,PNR3,PNR4,PNR4,PNR5, PNR6,PNR7,PNR8,PNR9,PNR10		√				
<i>COI_10</i>	4	PHR1,PHR2,PHR7,PHR10	√					
<i>COI_11</i>	1	PHR4	√					
<i>COI_12</i>	4	PHR5,PHR6,PHR8,PHR9	√					
<i>COI_13</i>	2	AF 482987- <i>Ictalurus punctatus</i> NC 003489- <i>Ictalurus punctatus</i>						√

Note: PH = *P. hypophthalmus*, PN = *P. nasutus*, PM = *P. micronemus*, PB = *P. bocourti*, HW = *H. waandersii*, OG = Outgroup, n = sample size.

TABLE 4. Intraspecific pair-wise distances of mitochondrial *COI* gene based on Kimura 2 parameter methods.

Species	K2P%
<i>P. bocourti</i> (PB)	0.000
<i>P. micronemus</i> (PM)	0.001
<i>P. nasutus</i> (PN)	0.000
<i>P. hypophthalmus</i> (PH)	0.002
<i>H. waandersii</i> (HW)	0.000

TABLE 5. Interspecific pair-wise distances of mitochondrial *COI* gene based on Kimura 2 parameter methods.

Species	PB	PM	PN	PP	HW	IP
<i>P. bocourti</i> (PB)	-					
<i>P. micronemus</i> (PM)	0.114	-				
<i>P. nasutus</i> (PN)	0.100	0.088	-			
<i>P. hypophthalmus</i> (PP)	0.126	0.121	0.122	-		
<i>H. waandersii</i> (HW)	0.099	0.105	0.083	0.128	-	
<i>Ictalurus punctatus</i> (IP)	0.193	0.170	0.174	0.165	0.164	-

RESULTS

The characteristics of *COI* dataset of pangasiids

A total of 462 bp of the mtDNA *COI* gene was successfully sequenced. Out of 462 nucleotide sites, 116 were variable sites, 346 bp conserved sites and 103 bp parsimony informative sites. *Ictalurus punctatus* was used as outgroup. Method Base composition in these sequences contained low G (mean: 18.2%) and almost equal T, C and A (mean: 32.3%, 21.9% and 27.7%, respectively). It is due to the low average content of G in the third codon position at 3.9%. In the second codon, the mean for G (16.2%) was lower than A (16.9%), T (43.5%) and C (23.3%). In the first codon, the mean for G (34.4%) was higher than A, T and C (mean: 26.0%, 23.7% and 15.9% respectively).

The highest rates of different transition substitution occurred between thymine and cytosine with a frequency of 50.98% and the transversion frequency was almost the same for all bases. The transition/transversion bias for the 42 individuals computed by MEGA 4.0 was $R=5.089$. This showed that the occurrence transition was much higher at least 5 fold than the occurrence of transversion events.

Haplotype distribution and genetic distance using *COI*

In total, 12 haplotypes were identified from 42 samples of pangasiids in which 5 unique (singleton) haplotypes namely (*COI_1*, *COI_3*, *COI_6*, *COI_8* and *COI_11*) were found. (Table 3 and Fig. 2). The haplotype diversity (HD) was 0.8901. The nucleotide diversity (Π) for the *COI* sequences was 0.07237. In general, intraspecific K2P distances ranged from 0 to 0.2% (Table 4) and interspecific K2P distances for *COI* gene ranged from 8.3 to 12.8 % (Table 5). The highest genetic distance occurred between *P. bocourti* and *H. waandersii* (0.128) and the closest genetic distance was between *P. nasutus* and *H. waandersii* (0.083)..,

Phylogenetic relationships among pangasiids using *COI*

Based on MP, BI and ML almost similar trees were produced. The populations were divided into 2 major clades (Fig. 3). The first clade consisted of *P. nasutus*, *H. waandersii*, *P. bocourti* and *P. micronemus* while the second clade included *P. hypophthalmus*.

In all constructed trees, the first clade was further divided into 2 subclades, in which *P. nasutus*, *P. bocourti* and *H. waandersii* placed in the first subclade while *P. micronemus* formed the second subclade. All produced tree with different methods were supported with high bootstrap values of >85%. The first clade from MP, ML and BI tree analyses was supported by 85%, 99% bootstraps and 100% posterior probability and the second clade were supported by 100%, 99% % bootstraps and 100% posterior probability respectively (Fig. 3).

DISCUSSION

The results of nucleotide composition exhibit anti-G (Guanine) bias for *COI* gene, because of the value of G was lower (mean: 18.2%) than other bases. Out of 462 bp segments, 116 (25.1%) nucleotide positions were variable sites. Most variable sites (107, 92.24% of the total variable sites) were found at the third codon position. Seven (6.9%) were found at the first codon position and one (0.9%) was the second codon position. This was similar to the findings by Steinke et al. (2009) that worked on the family Liparidae from Canadian Pacific waters with the nucleotide frequency for G that was 18.45% (lower than other bases).

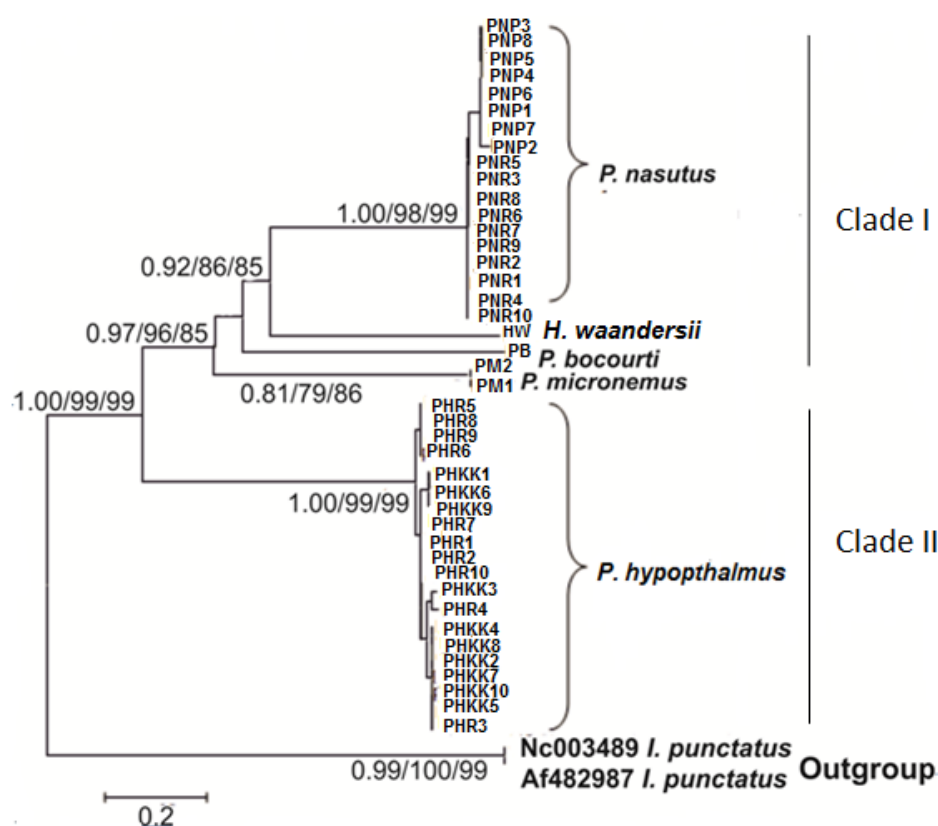


FIGURE 3. Bayesian consensus tree resulting from analysis data of partial *COI* gene among 5 species of pangasiids. Values at nodes indicate posterior probability, maximum likelihood bootstrap and parsimony bootstrap (all $>0.50/50\%$); * denote posterior probability or bootstrap value of 1.00/100%. Abbreviations: PNP – *Pangasius nasutus*, Maran Pahang, PNR – *Pangasius nasutus*, Rawang Selangor, HW – *Helicophagus waandersii*, Pahang, PB-*Pangasius bocourti*, Pahang, PM – *Pangasius micronemus*, Pahang, PHR – *Pangasius hypophthalmus*, Rawang Selangor, PHKK – *Pangasius hypophthalmus*, Kuala Kangsar, Perak.

The values of variable and parsimony informative sites for *COI* were 92.24% and 75.45%. According to Ward et al. (2007) this characteristic are valuable to be used for DNA barcoding. In addition, the strong bias in the base composition is a feature typical of the *COI* and of other mitochondrial protein-coding genes (Brown, 1985; Irwin et al., 1991).

The nucleotide substitution of *COI* showed that the occurrence of transition was fivefold higher than transverse with the R value of 5.089. In mitochondrial DNA always showed transition dominant over tranversion. According to Ward et al. (2005), for bony fish mtDNA, a much larger excess of transitions related to the observed transversions. Rewrite the sentence

The haplotype diversity observed in *COI* was 0.8901. The Haplotypes of *P. hypophthalmus* and *P. nasutus* have been distinguished based on *COI* variation and by locations (Table 3). Each haplotype was specific for a particular species and no shared haplotype was found between different species.

The genetic distances between pangasiid species based on *COI* genes ranged from 0.083 to 0.128. Although the genetic distance values were small between pangasiid species, the species were still well differentiated. Using cytochrom b gene, Pouyaud et al. (2000) also found a small genetic distance

values for the pangasiid species in their study (ranged from 0.004 to 0.149), but nominal species were still differentiated.

The *COI* tree divided the pangasiid samples to two clades, the first clade consisted of *P. nasutus*, *H. waandersii*, *P. bocourti* and *P. micronemus* while the second clade included *P. hypophthalmus*. *H. waandersii* was found to be closer to *P. nasutus*, *P. bocourti*, and *P. micronemus* than to *P. hypophthalmus* the phylogenetic tree results are congruent with previous morphological description and identification of the species based on Robert and Vidthayanon (1991). According to Haslawati et al. (2011) *H. waandersii* and *P. micronemus* proved to be closer than other pangasiids.

The results of *COI* gene have placed *H. waandersii* in Clade 1 with other *Pangasius* genera, while *H. waandersii* was from a different genus with different morphological characteristics of other pangasiid species (Haslawati, 2004).

Interestingly the genetic *COI* distances isolate the native species from local species. In this study, the local species, *P. nasutus*, *P. bocourti*, *P. micronemus* and, *H. waandersii* were distantly related to *P. hypophthalmus* that introduced from Mekong, in Thailand.

The most significant finding in this study was the paraphyly of the *Pangasius* genus in Malaysia with high bootstrape value. The tree from *COI* gene revealed that *P. nasutus*, *P. bocourti*, *P. micronemus* and *H. waandersii* were aggregated together and constituted in a monophyletic group with high bootstrap values. Our result confirmed the monophyly of *P. hypophthalmus* which is congruent with previous morphological studies that introduced *Pangasius hypophthalmus* (Sauvage, 1878) as *Pangasianodon hypophthalmus* (Striped catfish) (Rainboth, 1996); Kottelat, 2001; Gustiano, 2003, and Ferraris, 2007). Monophyly of *P. hypophthalmus* also confirmed the subgeneric classification proposed for *Pangasius* by Vidthayanon and Roongthongbaisuree (1993).

The phylogenetic relationships based on molecular markers among family Pangasiidae in Malaysia are in concordance with their morphological information, therefore, *COI* gene seems to provide a powerful source of information and marker for solving some taxonomical problems arising from solely based on morphological characteristics. It is vital to have additional data on the phylogenetic relationships and molecular evidence of this family. Then for future studies, more indigenous pangasiids with large sample size and genes, both mitochondrial and nuclear are suggested.

ACKNOWLEDGMENTS

We wish to thank all the staff of Department of Biology, Universiti Putra Malaysia for their contribution in this research project. We are also indebted to Dr. Khairul Adha A. Rahim, Rahman Zulkefli, Nor Hasnita Othman and Nadiatul Hafiza Hassan for helpful assistance.

LITERATURE CITED

Akaike, H., 1973. Information theory as an extension of the maximum-likelihood principle. In: Petrov, B., Csake, F., Editors, *Second International Symposium on Information Theory*, pp. 267-281. Akademiai kiado, Budapest.

Alaie Kakhki, N., Aliabadian, M., 2012. Mitochondrial DNA (CYTB) divergences in two distinct, Old World and New World Barn Owls. *Iranian Journal of Animal Biosystematics (IJAB)* 8, 47-55

Brown, W.M., 1985. *The mitochondrial genome of animals*. In "Molecular Evolutionary Genetics" (R. J. MacIntyre, Eds.). pp. 95-130. Plenum, New York.

Burgess, W.E., 1989. *An Atlas of Freshwater and Marine Catfishes*. A preliminary survey of the Siluriformes. T.F.H. Publications, Neptune City, New Jersey, U.S.A. 1-784, Pls. 1-285.

- Chaux, J., Fang, P.W., 1949. Catalogue des Siliroidei d'Indochine de la collection du laboratoire des peches colonials au Museum, avec la description de six especes nouvelles. *Bulletin du Museum D'Histoire Naturelle*, Paris 2, 194-201.
- Diogo, R., 2007. Osteology and Myology of the Cephalic Region and Pectoral Girdle of *Pangasius macronema*, With a Discussion on the Synapomorphies and Phylogenetic Relationships of the Pangasiidae (Teleostei: Siluriformes). *International Journal of Morphology* 25(3), 453-466.
- Dianat, M., Tarahomi, M., \ Darvish, j., Aliabadian, M., 2010 Phylogenetic analysis of the five-toed Jerboa (Rodentia) from the Iranian Plateau based on mtDNA and morphometric data. *Iranian Journal of Animal Biosystematics (IJAB)* 6, 49-59.
- Esa, Y., Shapor, S.S., Daud, S.K., A.Rahim, K.A., Japning, J.R.R., Tan, S.G., 2008. Mitochondrial DNA Diversity of *Tor tambroides* Valenciennes (Cyprinidae) from Five Natural populations in Malaysia. *Zoological Studies* 47, 360-367.
- Ferraris, C. J., 2007. Checklist of catfishes, recent and fossil (Osteichthyes:Siluriformes), and catalogue of siluriform primary types. *Zootaxa* 1418, 1-628.
- Gustiano, R., 2003. *Taxonomy and phylogeny of Pangasiidae catfishes from Asia (Ostariophysi, Suliformes)*. Karholieje Universiteit Leuven. 1-296.
- Gustiano, R., Pouyaud, L., 2006. Diversity of pangasiid catfishes from Sumatra. *Buletin Plasma Nutfab* 12, 83-88.
- Gustiano, R., 2009. Pangasiid catfishes of Indonesia. *Buletin Plasma Nutfab* 15, 91-100.
- Gyllensten, U., Wharton, D., Josefsson, A., Wilson, A.C., 1991. Paternal inheritance of mitochondrial DNA in mice. *Nature* 352, 255-257.
- Hamilton, F., 1822. An account of the fishes found in the river Ganges and its branches. Viii+405. Edinburgh.
- Haslawati, B., 2004. Mengenal Ikan Patin. Berita perikanan. <http://www.pppat.gov.my/fish/kenalipatin.pdf>. Accessed 12 October 2011.
- Haslawati, B., Rizman-Idid, M., Muniandy, S., Zakaria-Ismail, M., 2011. <http://www.dnabarcodes2011.org/conference/program/abstract>. Accessed 9 July 2012.
- Hogan, A.B., Moyle, P.B., May P.B., Vander Zanden, M.J., Baird, I.G., 2004. The imperiled giants of the Mekong. *American Scientist* 92, 228-237.
- Irwin, D.M., Kocher, T.D., Wilson, A.C., 1991. Evolution of cytochrome *b* gene of mammals. *Journal of Molecular Evolution* 32, 128-144.
- Khan, M.Z., 2000. *The fishery and Resource Characteristics of Pomfrets*. In: Pillai VN, Menon NG (eds) Marine fisheries research and management. Central Marine Fisheries Research Institute, Kochi, pp 364-373.
- Kimura, M., 1980. A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. *Journal of molecular Evolution* 16, 111-120.

- Kottelat, M., 1984 . A review of the species of Indochinese fresh-water fishes described by H.E. Sauvage. Bulletin du Museum National d'Histoire Naturelle Ser. 4: Section A: *Zoologie, Biologie et Écologie Animales* 6(4), 791-822.
- Kottelat, M., 2001. *Fishes of Laos*. WHT Publications, Colombo. 1-198, Pls. 1-48.
- Kottelat, M., Whitten, .A.J., Kartikasari, S.N., Wirjoatmodjo, S., 1993. *Freshwater Fishes of Western Indonesia and Sulawesi*. Periplus Edition (HK) Ltd, Republic of Indonesia. pp. 91-102.
- Marko, P.B., Lee, S.C., Rice, A.M., Gramling, J.M., Fitzhenry, T.M., McAlister, J.S., Harper, G.R., Moran, A.L., 2005. Mislabelling of a depleted reef fish. *Nature* 430, 309-310.
- Murkherjee, T.K., 2001. Genetics for Improvement of Fish in Malaysia. Fish genetics research in member countries and institutions of the International Network on Genetics in Aquaculture. pp. 65-70.
- Nadiatul, H.H., Daud, S.K., Siraj, S.S.b., Sungan, S.c., Moghaddam, F.Y., 2011. Genetic diversity of Malaysian indigenous Mahseer, *Tor douronensis* in Sarawak river basins as revealed by cytochrome c oxidase I gene sequences. *Iranian Journal of Animal Biosystematics (IJAB)*, 17, 119-127
- Palumbi, S.R., Martin, A.P., Romano, S., Mcmillan, W.O., Stice, L., Grabowski, G., 1991. The simple fool's guide to PCR. Honolulu: Department of Zoology and Kewalo Marine Laboratory, University of Hawaii.
- Persis, M., Reddy, A.C.S., Rao, L.M., Khedkar, G.D., Ravendar, K., Nasruddin, K., 2009. COI (cytochrome oxidase-I) sequence based studies of Carangid fishes from Kakinada coast, India. *Molecular Biology Reports* 36, 1733-1740.
- Posada, D., Crandall, K.A., 1998. Modeltest: testing the model of DNA substitution. *Bioinformatics* 14, 817-818.
- Pouyaud, L., Teugels, G.G., Gustiano, R., Legendre, M., 2000. Contribution to the phylogeny of pangasiid catfishes based on allozymes and mitochondrial DNA. *Journal of Fish Biology* 56, 1509-1538.
- Pouyaud, L., Teugels, G.G., Legendre, M., 1999. Description of a new pangasiid catfish from South-East Asia (Siluriformes). *Cybium* 23 (3), 247-258.
- Pouyaud, L., Teugels, G.G., 2000. Description of a new pangasiid catfish from east Kalimantan, Indonesia (Siluriformes: Pangasiidae). *Ichthyological Exploration of Freshwaters* 11(3), 193-200.
- Rainboth, W.J., 1996. *Fishes of the Cambodian Mekong*. FAO species identification field guide for fishery purposes Rome: Food and Agriculture Organization Publication. pp. 37.
- Roberts, T.R., Vidthayanon, C., 1991 Systematic revision of the Asian catfish family Pangasiidae, with biological observations and descriptions of three new species. *Proceedings of the Academy of Natural Sciences of Philadelphia* 143, 97-143.
- Ronquist, F., Huelsenbeck, J.P., 2003. MrBayes3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19, 1572-1574
- Rozas, J., Sanchez-Delbarrio, J.C., Messeguer, X., Rozas, R., 2003. DnaSP, DNA polymorphism analyses by the coalescent and other methods. *Bioinformatics* 19, 2496-2497.

- So, N., Houdt, J.K.J.V., Volckaert, F.A.M., 2006. Genetic diversity and population history of the migratory catfishes *Pangasianodon hypophthalmus* and *Pangasius bocourti* in the Cambodian Mekong River. *Fisheries Science* 72, 469-476.
- Steinke, D., Zemlak, T.S., Boutillier, J.A., Hebert, P.D.N., 2009. DNA barcoding of Pacific Canada's fishes. *Marine Biology* 156, 2641-2647.
- Swofford, D.L., 2001. PAUP*: Phylogenetic Analysis Using Parsimony (* and other methods), v 4.0b10. Senior Associates, Sunderland, MA.
- Teugels, G.G., 2003. *State of the Art of Recent Siluriform Systematics*. In Arratia, G.;Kappor, B.G.; Chardon, M., Diogo, R. eds. *Catfishes*. Enfield, Science Publishers. pp. 317-52.
- Vidthayanon, C., Roongthongbaisuree, S., 1993. Taxonomy of Thai riverine catfishes family Schilbeidae and Pangasiidae. *National Inland Fisheries Institute* 150, 1-57.
- Ward, R.D., Zemlak, T.S., Innes, B.H., Last, P.R., Hebert, P.D.N., 2005. DNA barcoding Australia's fish species. *Biological Science* 360, 1847-1857.
- Ward, R.D., Holmes, B.H., 2007. An analysis of nucleotide and amino acid variability in the barcode region of cytochrome c oxidase I (cox1) in fishes. *Molecular Ecology* 7, 899-907.