Evidence of geographical structuring in the Malaysian Snakehead, *Channa striata* based on partial segment of the CO1 gene

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Abstract

*Channa striata*, locally known as “haruan”, is economically important in fisheries and aquaculture industries in several Asian countries. DNA sequencing, based on a partial segment of the Cytochrome oxidase c subunit 1 (CO1) gene, was used to determine genetic variation in *C. striata* samples from four different populations on the west coast of Peninsular Malaysia. The highest nucleotide and haplotype diversities were observed in the Linggi population (\(\pi = 0.0067, h = 0.835\)), and the lowest in the Timah Tasoh population (\(\pi = 0.0008, h = 0.286\)). Apart from Kajang-Linggi, which was insignificant, FST values were significant (\(p < 0.05\)) in all pairwise-population comparisons. Consequently, it is inferred that genetic structuring *C. striata* populations in this region was largely shaped by a common origin, with secondary influences from geographical factors and isolation.

Key words: *Channa striata*, snakehead, mtDNA, CO1, isolation by distance.

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*Channa striata*, locally known as “haruan”, is a carnivorous freshwater air-breather (Jianguang and Fast, 1997). A popular food-fish in Malaysia (Ali, 1999; Mat Jais, 2007), Thailand and Indo-China, it is also cultivated in India, Pakistan and Thailand (Hossain *et al.*, 2008). Very hardy, it can stay alive out of water for a long time, whereby is the normal marketing procedure (Hossain *et al.*, 2008). It is also widely used in traditional medicine for wound-healing (Zakaria *et al.*, 2004). Studies by Ng *et al.* (2004) showed that a *C. striata* extract is useful as an alternative treatment in, osteoarthritis, a joint-disorder involving the softening and disintegration of articular cartilage, with subsequent changes in the underlying bones.

Due to its higher mutation rate of base substitution compared to nuclear DNA (Qiongying *et al.*, 2006), maternally inherited mitochondrial DNA is an efficient genetic marker in genetic-differentiation studies. Mitochondrial cytochrome c oxidase 1 (CO1) being the prime DNA barcoding region for taxonomically identifying animals, such as fishes, insects, amphibians and sponges (Erpenbeck *et al.*, 2005; Seifert *et al.*, 2007; Alessandrini *et al.*, 2008; Rock *et al.*, 2008; Smith *et al.*, 2008), is sometimes useful for investigating phylogeographic groups within a single species (Yu *et al.*, 2009). The objective herein was to determine the genetic variation in *C. striata* populations from four different locations on the west coast of Peninsular Malaysia, through DNA sequencing of CO1 of mtDNA.

Forty-five samples were collected from two northern sites, Timah Tasoh (Perlis) and Jeniang (Kedah) and two southern Linggi (Negeri Sembilan) and Kajang (Selangor) (Figure 1). With the exception of Kajang, a cultured species of unknown origin, but believed to be from the southern region itself, all were wild populations. Prior to DNA extraction, fin tissues were preserved in TNES-Urea (100 mM Tris-HCL pH 7.5, 125 mM NaCl, 10 mM EDTA pH 7.5, 1% SDS, 3 M Urea), modified according to Valles-Jimenez *et al.* (2004).

DNA extraction was with an Aquagenomic Kit (Bio-Syntech, Salt Lake City, Utah, USA), according to manufacturer’s instructions. A segment of the CO1 mtDNA gene was amplified using a pair of primers - forward primer L6154 (5’-AYC ARC AYY TRT TYT GRT TCT-3’) and reverse primer H6556 (5’ TGR AAR TGI CGI ACW ACR TA-3’) (Telechea *et al.*, 2006). PCR was done in a Peltier thermal cycler (MJ Research Waltham, MA, USA), with the following profile: pre-denaturation at 95 °C for 5 min, 30 cycles of denaturation at 94 °C, annealing at 50 °C and extension at 70 °C for 1 min each, followed by final extension at 72 °C for 5 min. The PCR products were then purified using Wizard® SV Gel and a PCR Clean-Up System by Promega (Promega Madison, WI) and sequenced on an ABI3730XL Genetic Analyzer (Applied Biosystems, Foster City, CA, USA).

Sequences were edited using MEGA 4.0 (Tamura *et al.*, 2007) and aligned with Clustal W 1.6, implemented in same software. Arlequin version 3.1 (Schneider *et al.*, 2000) was used for calculating nucleotide and haplotype
diversities, haplotype frequency and FSTS values. Bonferroni correction was applied, with global significance level at 0.05 to correct for multiple comparison. The relationships among all haplotypes were constructed using the Network program (Bandelt et al., 1999).

Aligned sequences of 364 bp in the mtDNA CO1 gene were obtained. Twelve unique haplotypes were identified from the four populations of 45 individuals. The sequences were all submitted to the Genbank under accession numbers GQ204314, GQ204319, GQ204321, GQ204323, GQ204326, GQ204330, HM192913 and HM192914. The highest nucleotide and haplotype diversities were observed in Linggi (π = 0.007, h = 0.835) and the lowest in Timah Tasoh (π = 0.0008, h = 0.286). Linggi presented 13 polymorphic sites, Jeniang and Kajang 7 each, and Timah Tasoh only one (Table 1).

The minimum spanning-network relationships among all the haplotypes are represented in Figure 1. Except for Timah Tasoh, Hap1, the most common haplotype, was found in the remaining three populations. From this common haplotype, two clades emerged, the northern and the southern. The spanning network showed that geographically close populations, i.e. Timah Tasoh and Jeniang (north) and Kajang and Linggi (south), were also genetically close.

The highest inter-population genetic distance occurred between Linggi and Jeniang, at 0.011, and the lowest between Timah Tasoh and Jeniang, at 0.004 (Table 2). Genetic differentiation, as revealed by pairwise FST analysis, was insignificant between the Kajang and Linggi populations, and significant between the remaining two (Table 2). The scatterplot revealed a highly positive correlation of genetic isolation versus geographical distance (Figure 2).

Table 1 - Summary of variable sites and the distribution of haplotypes, nucleotide diversity, number of haplotypes and number of polymorphic sites among the four populations of Channa striata (n = sample size).

<table>
<thead>
<tr>
<th>Haplotypes</th>
<th>Variable sites</th>
<th>Timah Tasoh (n=7)</th>
<th>Jeniang (n=10)</th>
<th>Kajang (n=1)</th>
<th>Linggi (n=1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hap1</td>
<td>T G G G TTTTAATTGAATCTC</td>
<td>0.100</td>
<td>0.714</td>
<td>0.286</td>
<td>0.286</td>
</tr>
<tr>
<td>Hap2</td>
<td>...... C .... A . G ....</td>
<td>0.800</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hap3</td>
<td>...... C ...... G ....</td>
<td>0.857</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hap4</td>
<td>.............. C ...</td>
<td>0.286</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hap5</td>
<td>............ G . C ...</td>
<td>0.214</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hap6</td>
<td>..... C .. G ..... C ...</td>
<td>0.142</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hap7</td>
<td>. . A . . C . . . . .... C ...</td>
<td>0.143</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hap8</td>
<td>. . . G . . . G G . . . . G . . . .</td>
<td>0.1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hap9</td>
<td>......... G .... G ....</td>
<td>0.071</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hap10</td>
<td>A A T . . ..... C .... C A C .</td>
<td>0.071</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hap11</td>
<td>.......... G ... C .. A</td>
<td>0.071</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hap12</td>
<td>.... C . C ...... G ....</td>
<td>0.143</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Nucleotide diversity 0.0008 0.0047 0.0039 0.0067
No. of haplotypes 12 13 2 1
Haplotype diversity 0.286 0.378 0.483 0.835
No. of polymorphic sites 13 3 1 1

Figure 1 - A. Sampling localities of the four Channa striata populations analyzed in the study. B. The minimum spanning-network relationships among the twelve haplotypes of mtDNA CO1 in the four populations of Channa striata. Each bar with a crossed connection between haplotypes represents one mutation site. The size of each circle is an approximate indication of haplotype frequency (black circle found in northern region populations; grey circle found in southern region populations; open circle is the intermediate (mv)).
The absence of the common haplotype Hap1 in the Timah Tasoh population is the evidence of its isolation. The presence of a dam could be a likely factor, through its construction possibly changing the original riverine environment into a lacustrine. Notwithstanding, although Hap1 is missing, the main Timah Tasoh haplotype (Hap3) only differed from it by two bases. Thus, the most probable scenario for this population would be a shared origin which evolved independently, due to its isolation. Wang et al. (2004) described the common haplotype as having immense potentiality for producing mutational derivatives. Through being more recently derived, the other haplotypes were unique.

Based on this preliminary analysis, it can be inferred that the genetic structuring of the Peninsular Malaysia *C. striata* population was largely shaped by a common origin, with secondary influences from geographical factors and isolation. Further studies, with more efficient markers and larger populations, especially from the northern and southern regions, are required to corroborate the findings.

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