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# A PRELIMINARY GENETIC BASELINE DATA OF CHACUNDA GIZZARD SHAD, *Anodontostoma chacunda* IN SETIU WETLANDS, TERENGGANU, MALAYSIA

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**Abstract:** The Setiu Wetlands and its surrounding is an important ecosystem especially in providing services and commodities to the local community. Many indigenous fishes inhabiting this Wetland are harvested for food and medicinal remedy but research on its biodiversity specifically the genetic distribution of the commercially important food fish species like chacunda gizzard shad *Anodontostoma chacunda* or is limited. Locally known as *selangat*, its population genetics was investigated by using mitochondrial DNA CO1 gene target. DNA template obtained from a total of 15 individuals revealed nine putative haplotypes. Our preliminary genetic baseline data revealed pair-wise genetic divergence between haplotypes ranged from 0.20% to 1.70% with an average of 0.90%.Haplotype and nucleotide diversity was relatively high ($H_d = 0.8857 \pm 0.0169$; $\pi = 0.0064 \pm 0.0038$), suggesting the populations of the *A. chacunda* at Setiu Wetland are in relatively good 'health'. Haplotype sharing between the different localities indicates gene flow that lead to low level of population structure which is difficult to detect. More specimens from different locations of Setiu Wetlands should be included in future study, to elucidate genetic variation and distribution of the *A. chacunda* in Setiu Wetlands, and ultimately to assist in specific sustainable fisheries management and planning.

Keywords: *Anodontostoma chacunda*, chacunda gizzard shad, mitochondrial DNA CO1 gene, population genetics, Setiu Wetlands

## Introduction

Setiu Wetlands is a 1,350 hectare wetland located in Kuala Besut, Terengganu. It is the largest natural wetland in the East Coast of Malaysia which encompasses a diverse ecosystem that includes freshwater, brackish water, seawater and a 14 km lagoon. However, research on its biodiversity specifically the genetic distribution of the commercially important fish species like chacunda gizzard shad, *Anodontostoma chacunda* or is limited.

Locally known as ikan selangat, it is an important and common food fish in the market (Vidthayanon, 2008), where it is consumed fresh, smoked and dried (Ambak *et al.,* 2010). It can be found from inshore waters, lagoon, marine coastal and estuaries of the Setiu Wetlands (Matsunuma *et al.,* 2011) but also up rivers; present all year round but is noticeably abundant after the monsoon season during the months of March until May when the sea is less turbulent. *A. chacunda* is a small to medium sized fish (average size about 20 cm) with compressed body and a large black spot is present behind the gill cover. It presents at Indo-West pacific region.

This study aims to characterize the genetic diversity and to provide a preliminary insight into the population structuring of *A.*

*chacunda* in Setiu Wetlands, Terengganu. The genetic data is a crucial piece of information in indicating the current population health prior to any management and conservation efforts. The gene pools have to be determined in order to further understand the genetic characteristics, effective size and productive efficiency of each population (Altukhov & Salmenkova, 1987). This can be achieved by characterizing the extent of genetic variation within species and account for this variation. The amount of variation can be determined by the frequency of alleles (Griffiths *et al.,* 2012). This study is expected to characterize the preliminary baseline data on the genetic diversity of the species specific of *A. chacunda* from Setiu Wetlands that will benefit the conservation and fisheries management strategy of this species.

## Materials and Methods

### *Sample collection and mtDNA extraction*

A total of 15 specimens were collected from two sampling locations in Setiu Wetlands, Site A (N 5º 31.34', E 103º 0.75') (n = 13) and B (N 5º 35.99, E 102º55.81') (n= 2) (Figure 1). Fin clips were excised from the dorsal fin rays (approximately 1cm x 1cm) and stored in 1.5 ml centrifuge tubes containing 95% ethanol and labeled accordingly. DNA template was isolated using an AquaGenomic™ DNA isolation kit (MultiTarget Pharmaceuticals, Salt Lake City, Utah 84116) according to the manufacturer's protocol.


Figure 1: Sampling localities (A &B) of *Anodontostoma chacunda* populations analyzed in the present study.
*Insert shows map of Peninsular Malaysia.

***Polymerase chain reaction (PCR) amplification***

Genomic DNA was PCR amplified with mitochondrial cytochrome c oxidase subunit 1 (CO1) gene primer pair CO1F (5‟-TCGACTAATCATAAAGATATCG GCAC-3‟) and CO1R (5‟-ACTTCAGGGTG ACCGAAGAATCAGAA-3‟) (Ward *et al.*, 2005). The reaction mixture consisted of 50-100ng of genomic DNA, 0.24 µM of each primer, 0.20 mM of dNTP. 1x PCR buffer, 1 mM $MgCl_2$ and 0.08 U of *Taq*polymerase. Each PCR will be amplified in a 25 µl reaction volume in an MJ PTC-200 Thermal Cycler. The temperature profile for the amplification consists of initial incubation at 95°C for 4 mins, 35 cycles of 94°C for 60s, 60°C for 60s, 72°C for 120s, final extension at 72°C for 20 mins and final hold at 4°C. After amplification, products were visualized on 1.7% agarose gels stained with SyBr Safe. PCR productswere then sent for DNA sequencing (First BASE Laboratories Sdn Bhd, Selangor Malaysia) using forward primer only.

***Data analysis***

Multiple CO1 sequences were aligned and compiled carefully using ClustalW implemented in MEGA v. 6 (Tamura *et al*., 2013). DNA sequences were translated into protein to ensure accurate alignment and detection of nuclear mtDNA (numt), if present. The complete aligned dataset were analyzed for nucleotide variable sites, parsimony informative sites, number of haplotypes, number of transitions and trans-versions, synonymous and non-synonymous amino acid substitution and nucleotide frequencies. Using the same program, genetic divergence between haplotypes based on Kimura 2-parameter genetic distance (Kimura, 1980) was calculated. We calculated two estimations of diversity measurement to describe DNA polymorphism at each sampling site using Arlequin v. 3.5(Excoffier & Lischer, 2010). The first, haplotype/gene diversity (Hd), measures the probability of uniqueness of a haplotype in a given population. The second, nucleotide diversity (p), is the mean number of pair-wise nucleotide differences among individuals in a sample.

Selective neutrality tests that evaluate deviation from neutral expectation which may arise from historical population range expansion or mutation-drift disequilibrium were examined through Tajima's D (Tajima, 1989) and Fu's Fs (Fu, 1997) statistics for each locality using Arlequin 3.5 (Excoffier & Lischer, 2010). Tajima's D that uses information on mutation (segregating sites) frequency detects deviation from neutrality due to population bottleneck or expansion, directional selection or introgression. The null hypothesis for Tajima's D test is neutral evolution in an equilibrium population, which implies no selection bias and the population has not experienced growth or size reduction (Tajima, 1989). A positive value of Tajima's D indicates balancing selection or population sub-structuring or recent bottleneck while a negative value indicates recent directional selection (selection sweep) or recent population growth with excess of rare alleles (Tajima, 1989). Fu's Fs statistics that is based on Ewens' sampling distribution (Ewens, 1972) and uses information on haplotype distribution is a powerful test to detect past population size fluctuation (Ramos-Onsins &Rozas, 2002).

To view the haplotype relationships, a phylogenetic network of all haplotypes was constructed based on median joining (MJ) calculation in Minimum Spanning Network (MSN) (Bandelt *et al*., 1999).

**Results and Discussion**

The 591 bp segment of the 15 CO1 gene sequences from two localities revealed 16segregating sites defining nine haplotypes. All unique sequences have been deposited in GenBank with accession number KU569892 to 569900. DNA sequence blast performed in MEGA v. 6 returned 99% similarity to *A. chacunda* species, confirming the specimens used in the study were the *A.*

*chacunda*. Of the 16 variable sites occurring at only the third codon position (all nucleotide mutations resulted to synonymous substitution), which is a common property of mitochondrial DNA (Seligmann, 2012), seven (43.8%) were parsimony informative. Ratio of transition to trans-version substitution for the entire dataset was 1:3.

Pair-wise genetic divergence between haplotypes ranged from 0.20% to 1.70% with an average of 0.90%. Overall haplotype and nucleotide diversity was relatively high ($H_d$ = 0.8857 ± 0.0169; $\pi$ = 0.0064 ± 0.0038), suggesting a healthy population size that is still fit for commercial harvest. A similar observation by Tan *et al*. (2012; 2015) who studied on the population genetics of the striped snakehead, *Channa striata* in Malaysia based on mitochondrial DNA ND5 and CO1 gene, respectively. However, low level of genetic variation was found in population of *Tor* spp. from Kelantan (located at east coast of Peninsular Malaysia), probable of habitat destruction and over-exploitation (Esa & Abdul Rahim, 2013). The current study was the first initiative in providing genetic baseline data to fishery managers to understand the population status of the economically important *A. chacunda* from the Setiu Wetlands for the sustainable fishery management and planning. A negative value of neutrality test Tajima's D (-0.97) and Fu's Fs (-2.04) indicates recent population expansion, resulting substantial rare alleles.

The median joining network based on nucleotide distance among haplotypes depicted close relationship among haplotypes, with many adjacent haplotypes differing from each other by one to three nucleotides (Figure 2).

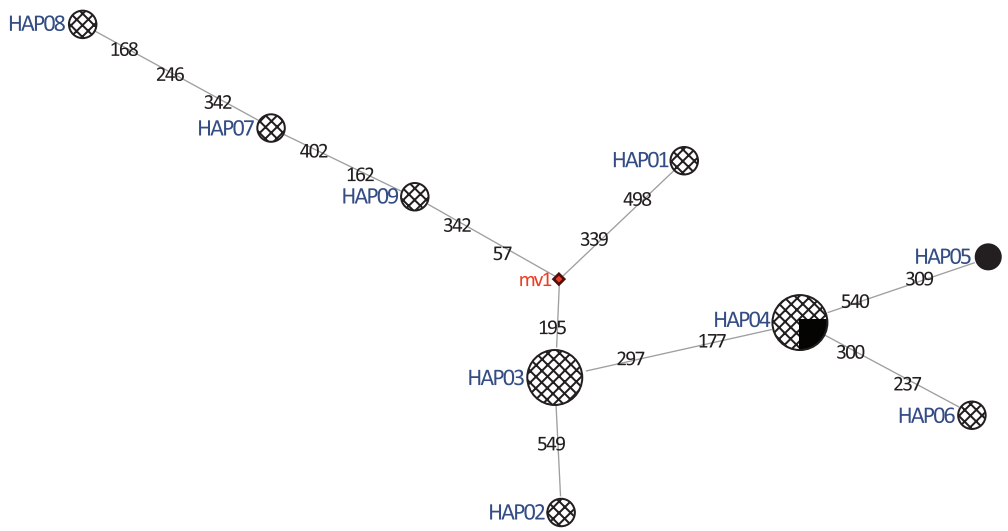


Figure 2: Minimum spanning network diagram of haplotypes inferred from mtDNA CO1 gene. Diagonal cross pattern represents haplotype from population A, solid pattern from population B. mv=median vector. Numbers in between the haplotype nodes are nucleotide mutation sites.

One intermediate hypothetical haplotypes (mv1) was detected, probable of haplotype extinction (Malhi *et al*., 2002) or un-sampled specimen. Hap03 and Hap04 were equally abundant at the study sites, with Hap04 was also found in population B. Most of the haplotypes (77.78%) are singletons, indicating a recent population expansion, in support with the negative values in neutrality test of Tajima's D and Fu's Fs (de Jong *et al*., 2011).

**Conclusion**

The preliminary genetic baseline study revealed that the genetic diversity of chacunda *A. chacunda* populations at Setiu Wetlands was relatively high, suggesting that they are in relatively good "health" and are still fit for commercial use. Common haplotype was present at different localities, suggesting gene flow within the area due to absence of effective geographical barriers. However, more specimens from different locations of Setiu wetlands should be included in future studies, to elucidate genetic variation and structure of the *A. chacunda* in Setiu Wetlands, and ultimately to assist in specific sustainable fisheries management and planning.

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