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PCR-RFLP analysis of mitochondrial DNA cytochrome *b* gene among Haruan (*Channa striatus*) in Malaysia

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ABSTRACT

Haruan (*Channa striatus*) is in great demand in the Malaysian domestic fish market. In the present study, mtDNA cyt *b* was used to investigate genetic variation of *C. striatus* among populations in Peninsular Malaysia. The overall population of *C. striatus* demonstrated a high level of haplotype diversity (h) and a low-to-moderate level of nucleotide diversity (π). Analysis of molecular variance (AMOVA) results showed a significantly different genetic differentiation among 6 populations ($F_{ST} = 0.37566$, $P = 0.01$). Gene flow (Nm) was high and ranged from 0.32469 to infinity (∞). No significant relationship between genetic distance and geographic distance was detected. A UPGMA tree based on the distance matrix of net interpopulation nucleotide divergence (d_A) and haplotype network of mtDNA cyt *b* revealed that *C. striatus* is divided into 2 major clades. The neutrality and mismatch distribution tests for all populations suggested that *C. striatus* in the study areas had undergone population expansion. The estimated time of population expansion in the mtDNA cyt *b* of *C. striatus* populations occurred 0.72–6.19 million years ago. Genetic diversity of mtDNA cyt *b* and population structure among Haruan populations in Peninsular Malaysia will be useful in fisheries management for standardization for Good Agriculture Practices (GAP) in fish-farming technology, as well as providing the basis for Good Manufacturing Practices (GMP).

1. Introduction

Haruan (*Channa striatus*) is in great demand in the Malaysian domestic fish market. Detailed knowledge of the genetic diversity and population genetics of *C. striatus* is needed for sound management, conservation, stock identification, and successful farming of the species. Haruan, the local name for the snakehead *C. striatus*, is an obligate freshwater fish of the family Channidae that has important economic value as a food fish, and has pharmacological properties as well as medicinal value (Mat Jais, 1991, 2007a, 2007b; Rahim et al., 2009; Jamaluddin et al., 2011). *C. striatus* can be morphologically distinguished based on coloration, meristics, and morphometrics (Mat Jais, 1991; Rahim et al., 2009), the distribution of scales on the underside of the lower jaw, the morphology of the suprabranchial organs, and a sharp pointed ridge at the mid-ventral part of the isthmus (Vishwanath and Geetakymari, 2009). The wild type is found in small rivers, lakes, pools, and shallow water bodies where agro-chemicals are applied in their natural habitats; they can survive in harsh environments with low levels of dissolved oxygen and high ammonia levels (Rahim et al., 2009; Jamaluddin et al., 2011). In particular, *C. striatus* has a diploid chromosome number of $2n = 42$, with a karyotype composed of 6 metacentric, 2 acrocentric, and 34 telocentric chromosomes, $NF = 50$, without heteromorphic sex chromosomes (Supiwong et al., 2009).

Cytochrome *b* (cyt *b*) is one of the most important protein encoding genes on the heavy strand of the mtDNA molecule. It has been widely used in molecular marker techniques for many applications to assess intra- or interspecies genetic diversity, genetic variation, phylogeography, species and hybrid identification, phylogeny in numerous species and genera, population genetic structure, conservation, and demographic history (Hsu et al., 2009; Li et al., 2009; Ma et al., 2010; Thangaraj and Lipton, 2010). Due to the potential of *C. striatus*, studies have analyzed the genetics of this species at the morphological, biological, dietary, physiological, biochemical composition, ecological, and chromosomal level for breeding programs, and the medical and pharmaceutical activities for its anti-microbial, anti-inflammatory, cell proliferation, induction of platelet aggregation, and anti-nociceptive properties (Mat Jais, 2007b; Rahim et al., 2009; Supiwong et al., 2009; Dahlan-Daud et al., 2010; Jamaluddin et al., 2011). In recent years, preliminary analysis of *C. striatus* populations has been carried out based on different molecular markers, such as analysis of mitochondrial mtDNA (Abol-Munafi et al., 2007; Lakra

Abbreviations: bp, base pairs; mtDNA, mitochondrial DNA; Myr, million years; cyt *b*, cytochrome *b*.

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et al., 2010; Jamaluddin et al., 2011), randomly amplified polymorphic DNA (RAPD; Ambak et al., 2006), and allozyme markers (Hara et al., 1998). Restriction fragment length polymorphism (RFLP) has proven to be a successful method for studying the population genetic structure and differentiation of many fish, such as skipjack tuna (Menezes et al., 2006), scad mackerel (Arnaud et al., 1999), tilapia (Espinosa-Lemus et al., 2009), arctic grayling (Redenbach and Taylor, 1999), and salmon (King et al., 2000).

Our previous study provided information of the physical–chemical and morphological properties, including morphometrics and meristic variations, that have been reported in *C. striatus* populations, suggesting significant heterogeneity among populations. However, the variation of morphological characteristics is probably influenced by genetic and environmental factors, so discrimination of populations based on morphological variation should be confirmed by evidence to verify that morphological variation reflects a true degree of reproductive isolation, rather than environmental isolation (Tzeng, 2004; Tzeng et al., 2008). Therefore, in the present study, we used mtDNA cyt *b* from Haruan to investigate the genetic diversity and population structure of *C. striatus* populations in Peninsular Malaysia and to provide a preliminary assessment of genetic diversity patterns of *C. striatus* in order to improve knowledge. Our findings have potential implications for the management, conservation, and stock identification as important resources for the fisheries industry. Such information for this species will be useful in the management of different populations of *C. striatus* for Good Agriculture Practices (GAP) in farming technology, as well as providing the basis for Good Manufacturing Practices (GMP).

2. Materials and methods

2.1. Sample collection

We collected 120 wild Haruan from 6 sampling stations throughout Peninsular Malaysia: Sungai Petani, Kedah (N 5°38'33.33", E 100°28'09.27"); Bagan Datoh, Perak (N 3°57'51.31", E 100°45'13.51"); Kuantan, Pahang (N 3°49'48.45", E 103°19'15.53"); Pontian, Johor (N 1°29'14.93", E 103°23'57.85"); Kuala Terengganu, Terengganu (N 5°19'29.40", E 103°08'27.29"), and Tanah Merah, Kelantan (N 5°48'21.80", E 102°09'17.17") (Fig. 1). These stations were selected

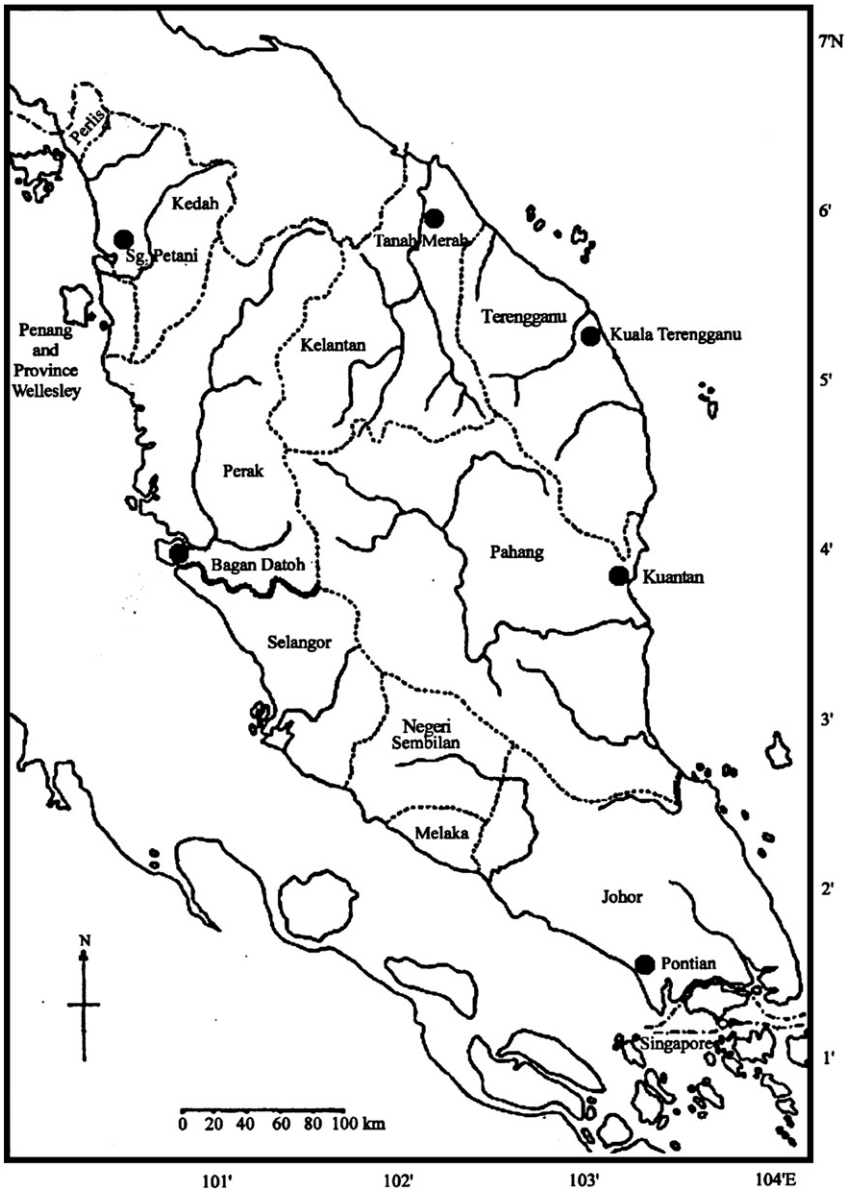

Fig. 1. Map showing the distribution of Peninsular Malaysia and the sampling locations (black dots) of *C. striatus*.