



UNIVERSITI PUTRA MALAYSIA

***MORPHOLOGICAL AND GENETIC RELATIONSHIPS AMONG
SNAKEHEAD CHANNA STRIATA (BLOCH, 1793) POPULATIONS IN
PENINSULAR MALAYSIA***

MOHAMMAD HAFIZ BIN ABDUL RAHIM

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PENINSULAR MALAYSIA**

By

MOHAMMAD HAFIZ BIN ABDUL RAHIM

**Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia,
in Fulfilment of the Requirement for the degree of Master of Science**

January 2012

DEDICATED to

**My father, Abdul Rahim Bin Ismail...
who believe in the richness of learning,
his love and support has enabled me to achieve my goals
and finish what I have started**

Abstract of thesis presented to the Senate of University Putra Malaysia in fulfilment
of the requirement for the degree of Master of Science

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January 2012

Chairman: Professor Abdul Manan Bin Mat Jais, PhD

Faculty: Medicine and Health Sciences

Haruan, the local name for the snakehead *Channa striata*, is a tropical freshwater fish from the Channidae family that is valuable in terms of importance in economics, i.e. as food, and in medicine. Thus, this study was undertaken to determine morphological and genetic relationships among 6 populations of *C. striata* in Peninsular Malaysia.

The morphological and genetic variation of *C. striata* was studied based on polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) of the mitochondrial DNA cytochrome *b* gene (mtDNA *cyt b*) and morphometric and meristic analysis of samples collected from 6 *C. striata* locations around Peninsular Malaysia, namely Johor, Kedah, Kelantan, Pahang, Perak, and Terengganu. A total 120 specimens were used, with standard lengths ranging 17.50–37.50 cm, total lengths ranging 20.60–44.10 cm, and weights ranging 76.70–675.00 g.

Five meristic counts and 23 morphometric measurements were used for the morphological analysis. The quantitative data were analysed by univariate analysis of variance (ANOVA) and multivariate analysis of discriminant function analysis (DFA). The morphometric characteristics were more adequate than the meristic characters for good separation of the populations. In DFA, the results indicated that *C. striata* from different populations can be distinguished using several morphometric characters, namely the ratios of dorsal fin height to standard length (DFH/SL) and caudal peduncle height to standard length (CPH/SL). The findings for the morphological analysis refute an earlier hypothesis of there being no morphological variation between populations of *C. striata*.

For *C. striata* population genetics, 5 restriction endonucleases yielded enzyme-specific restriction morphs in a total of 29 composite mitochondrial DNA (mtDNA) haplotypes. The pairwise Φ_{ST} statistics (-0.01861 to 0.60629) and analysis of molecular variance (AMOVA) (37.57%), results indicated consistency, with sufficient information existing to construct a rational explanation of the snakeheads' genetic heterogeneity. This support for the findings showed that the *C. striata* populations were separated into 2 major clusters, namely clade I (containing the Johor population) and clade II (a mixture of the Kedah, Kelantan, Pahang, Perak, and Terengganu populations), based on unweighted pair group method with arithmetic mean (UPGMA) cluster analysis and the haplotype network of mtDNA *cyt b*. The patterns of high levels of haplotype diversity ($h = 0.6789-0.8737$) and moderate nucleotide diversity ($\pi = 0.008-0.0181$), the observed star or dumbbell-shaped structure of the haplotype network, neutrality tests ($D = -0.76304$ to 0.53277 , $P > 0.05$ and $F_s = -6.16223$ to -2.97325 , $P < 0.05$), and mismatch distribution (bimodal

or unimodal) tests demonstrated that whole populations of *C. striata* saw a recent population demographic expansion. The estimated population expansion in the mtDNA *cyt b* of *C. striata* populations occurred 1.41–1.69 million years ago. However, based on the net Tamura and Nei genetic distance, the divergence among regional populations occurred 0.72–2.6 million years ago.

These findings suggest that morphological and genetic studies provide the information required for increasing the efficiency of *C. striata* management in Peninsular Malaysia, which will be helpful in fisheries, and to biologists, and taxonomists studying these fascinating fishes.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

HUBUNGAN MORFOLOGI DAN GENETIK ANTARA POPULASI HARUAN *CHANNA STRIATA* (BLOCH, 1793) DI SEMENANJUNG MALAYSIA

Oleh

MOHAMMAD HAFIZ BIN ABDUL RAHIM

Januari 2012

Pengerusi: Profesor Abdul Manan Bin Mat Jais, PhD

Fakulti: Perubatan dan Sains Kesihatan

Haruan, nama tempatan untuk *Channa striata*, merupakan ikan air tawar tropika dari keluarga Channidae yang bernilai dari segi ekonomi, iaitu sebagai makanan, dan dari segi perubatan. Oleh itu, kajian ini dijalankan untuk menentukan hubungan morfologi dan genetik antara 6 populasi *C. striata* di Semenanjung Malaysia.

Variasi morfologi dan genetik *C. striata* dikaji berdasarkan tindak balas rantaian polimerase-polimorfisme panjang cebisan pemotongan (PCR-RFLP) gen sitokrom *b* mitokondria (mtDNA *cyt b*) dan analisis morfometrik dan meristik ke atas sampel yang di perolehi dari 6 lokasi *C. striata* di Semenanjung Malaysia, iaitu Johor, Kedah, Kelantan, Pahang, Perak dan Terengganu. Sejumlah 120 spesimen telah digunakan, dengan panjang piawai antara 17.50–37.50 sm, jumlah panjang antara 20.60–44.10 sm dan berat badan adalah 76.70–675.00 g.

Lima ciri meristik dan 23 ukuran morfometrik telah digunakan untuk analisis morfologi. Data kuantitatif dianalisa dengan analisis univariat varians (ANOVA) dan analisa multivariat analisis fungsi diskriminan (DFA). Ciri morfometrik adalah lebih mencukupi berbanding dengan ciri-ciri meristik untuk tujuan pemisahan terbaik di antara populasi yang dikaji. Keputusan DFA menunjukkan bahawa *C. striata* dari populasi yang berlainan boleh dibezakan dengan menggunakan beberapa ciri-ciri morfometrik, iaitu nisbah tinggi sirip dorsal ke panjang piawai (DFH/SL) dan tinggi kaudal pedunkel ke panjang piawai (CPH/SL). Hasil analisa morfologi telah menolak hipotesis awal yang menyatakan variasi tidak wujud di antara populasi *C. striata*.

Mengenai genetik populasi *C. striata*, 5 endonuklease pemotongan telah menghasilkan “morphs” pemotongan enzim-spesifik dalam jumlah 29 komposit haplotip mtDNA. Statistik Φ_{ST} berpasangan (-0.01861 ke 0.60629) dan analisis varians molekul (AMOVA) (37.57%) berpadan dengan maklumat yang ada, dan mencukupi untuk menghasilkan penjelasan yang rasional mengenai kepelbagaian genetik haruan. Oleh itu, keputusan yang diperolehi menyokong tanggapan bahawa populasi *C. striata* telah dipisahkan kepada 2 kelompok utama, iaitu klad I (yang mengandungi populasi Johor) dan klad II (yang merangkumi populasi Kedah, Kelantan, Pahang, Perak, dan Terengganu) berdasarkan analisis kelompok kaedah kumpulan pasangan tanpa penimbang dengan keertian aritmetik (UPGMA) dan rangkaian haplotip mtDNA sitokrom *b*. Corak paras kepelbagaian haplotip yang tinggi ($h = 0.6789-0.8737$) dan kepelbagaian nukleotida sederhana ($\pi = 0.008-0.0181$), struktur bentuk “star” atau “dumbbell” rangkaian haplotip, ujian neutraliti ($D = -0.76304$ ke 0.53277 , $P > 0.05$ dan $F_s = -6.16223$ ke -2.97325 , $P < 0.05$), dan ujian pengagihan tidak sepadan (berbentuk bimodal atau unimodal) menunjukkan

bahawa populasi keseluruhan *C. striata* mengalami pengembangan populasi demografi. Pengembangan populasi yang dianggarkan dalam mtDNA *cyt b* berlaku 1.41–1.69 juta tahun lalu. Walau bagaimanapun, berdasarkan net Tamura dan jarak genetik Nei, kecapahan pengembangan di populasi serantau berlaku 0.72–2.6 juta tahun lalu.

Keputusan yang diperolehi menunjukkan bahawa kajian morfologi dan genetik membekalkan maklumat yang diperlukan untuk meningkatkan kecekapan pengurusan *C. striata* di Semenanjung Malaysia, yang dan berkemungkinan dapat membantu dalam bidang perikanan serta ahli biologi dan taksonomi yang mengkaji spesies menarik ini.

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I certify that a Thesis Examination Committee has met on 17 January 2012 to conduct the final examination of Mohammad Hafiz Abdul Rahim on his thesis entitled "**Morphological and genetic relationships among snakehead *Channa striata* (Bloch, 1793) populations in Peninsular Malaysia**" in accordance with Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Master of Science.

Members of the Thesis Examination Committee were follows:

Sabrina Sukardi, PhD

Associate Professor
Faculty of Medicine and Health Sciences
University Putra Malaysia
(Chairman)

Aziz Arshad, PhD

Professor
Faculty of Agriculture
University Putra Malaysia
(Internal examiner)

Siti Khalijah Daud, PhD

Associate Professor
Faculty of Science
University Putra Malaysia
(Internal examiner)

Vijay Kumar, PhD

Associate Professor
Biotechnology Research Institute
University Malaysia Sabah
(External examiner)

SEOW HENG FONG, PhD

Professor and Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:

This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

Abdul Manan Mat Jais, PhD

Professor
Faculty of Medicine and Health Sciences
University Putra Malaysia
(Chairman)

Patimah Ismail, PhD

Professor
Faculty of Medicine and Health Sciences
University Putra Malaysia
(Member)

Norwati Muhamad, PhD

Forestry Biotechnology Division,
Forest Research Institute Malaysia
(Member)

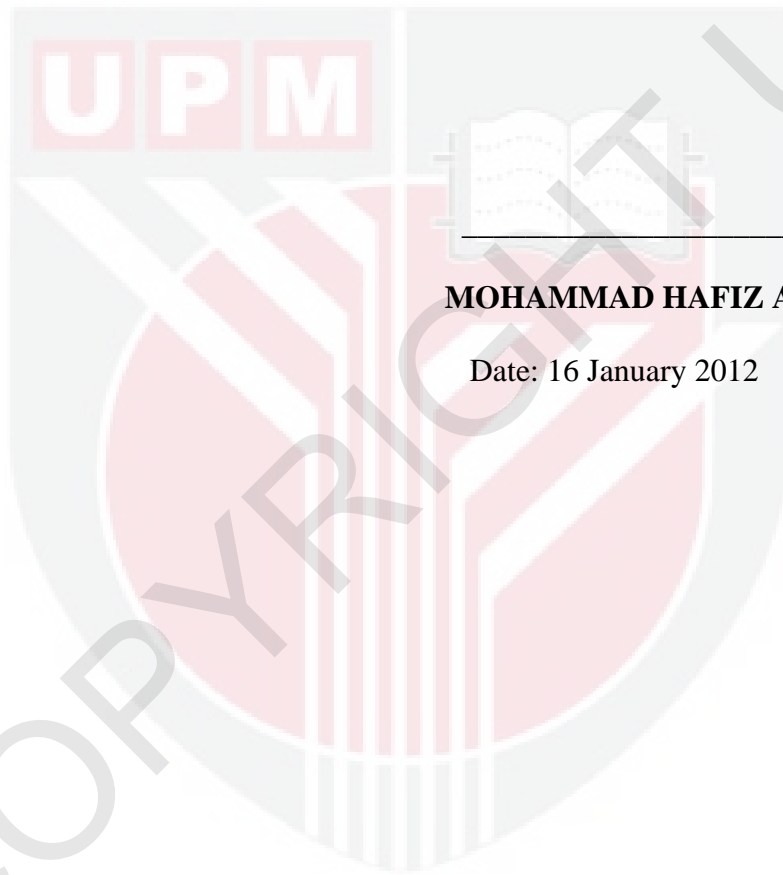
BUJANG BIN KIM HUAT, PhD

Professor and Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:

DECLARATION

I declare that the thesis is my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously, and submitted for any other degree at Universiti Putra Malaysia or any other institution.



MOHAMMAD HAFIZ ABDUL RAHIM

Date: 16 January 2012

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LIST OF ABBREVIATIONS

AA	Arachidonic acid
AFLP	Amplified Fragment Length Polymorphism
AMOVA	Analysis of molecular variance
ANOVA	Analysis of variance
CV	Canonical Variance
Cyt <i>b</i>	Cytochrome <i>b</i>
DFA	Discriminant Function Analysis
DNA	Deoxyribonucleic acid
DO	Dissolved oxygen
EPA	Eicosapentaenoic acid
FRIM	Forest Research Institute Malaysia
GAP	Good Agriculture Product
GMP	Good Manufacture Practices
HSD	Honestly Significant Difference
IBD	Isolation by distance
LWR	Length Weight Relationship
mtDNA	Mitochondrial DNA
<i>N_m</i>	Gene flow
NTU	Nephelometric Turbidity Units
PCR	Polymerase Chain Reaction
PIC	Polymorphic Information Content
ppt	Parts per thousand
RAPD	Random Amplified Polymorphic DNA

REAP	Restriction Enzyme Analysis Package
RFLP	Restriction Fragment Length Polymorphism
SNPs	Single Nucleotides Polymorphisms
SSRs	Simple Sequence Repeats
STS	Sequence Tagged Sites
UNISEL	Universiti Selangor
UPGMA	Unweighted Pair Group Method with Arithmetic Mean
UPM	Universiti Putra Malaysia



CHAPTER 1

INTRODUCTION

1.1 General Introduction

Haruan, the local name for snakehead *Channa striata*, is an obligate freshwater fish from the Channidae family that possesses a 42 pair diploid (2n) chromosome karyotype. The fish possesses important economic value as a food fish, pharmacological properties, as well as medicinal value. In Malaysia, there is a demand for freshwater fish, *C. striata* in particular, as it has a very good composition of amino acids and fatty acids; a fatty acid compositional study of *C. striata* flesh revealed high levels of arachidonic acid, which is beneficial in the healing of internal and external wounds, an absence of eicosapentaenoic acids, as well as low cholesterol content and a high amount of good-quality protein (Yaakob and Ali, 1992; Mat Jais *et al.*, 1998, 2002, 2007b; Gam *et al.*, 2006; Zuraini, 2006; Supiwong *et al.*, 2009).

Morphologically, *C. striata* is a unique group of freshwater fish. Compared to other species, the fish has distinctively small pelvic fins, while a few others lack them completely. The fish can be distinguished from Channidae family members by their elongated cylindrical body; flattened head; long, entirely soft-rayed dorsal and anal fins; large mouth with well-developed teeth on both upper and lower jaws; tube like anterior nostrils; round to somewhat truncated caudal fins; cycloid or ctenoid body scales; and shield-like scales on the head, where the shape of the head resembles that of a snake (Musikasinthorn, 2003). Moreover, *C. striata* can be distinguished based

on coloration, meristics and morphometrics, as well as the distribution of scales on the underside of the lower jaw, shape of the head, morphology of the suprabranchial organs, and a sharp-pointed ridge at the mid-ventral part of the isthmus (Vishwanath and Geetakymari, 2009).

The wild species can be found in small rivers, lakes, pools, and shallow water bodies where agrochemicals are applied. In their natural habitats, *C. striata* are able to survive in harsh environments with low dissolved oxygen and high ammonia levels (Marimuthu and Haniffa, 2007; Cong *et al.*, 2008; Jamaluddin *et al.*, 2011).

Morphometrics (quantitative) and meristics (qualitative) are 2 types of morphologic characters that have been most commonly used to delineate fish stock. Morphometric characters are continuous characters describing aspects of the body shape. Meristic characters are the number of discrete, serially repeated, countable structures that are unchanged in embryos or larvae (Turan, 2004a). Morphometrics and meristics analysis is considered more appropriate than the use of single morphological characters to investigate and determine the relationship between populations or closely related species. Differences in morphometric and meristic characters among populations of a species are based on the consideration that morphometric characters are usually more related to genetic causes, while meristic characters are much more dependent on environmental differences (Costa *et al.* 2003; Remerie *et al.*, 2005).

Cytochrome *b* is one the most important proteins encoding genes on a heavy strand of mtDNA molecules which has been widely used as a molecular marker technique for many applications to access intra- and 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).

In recent years, preliminary analysis of *C. striata* populations has been carried out based on different molecular markers, such as analysis of mitochondrial DNA (mtDNA) (Abol-Munafi *et al.*, 2007; Lakra *et al.*, 2010; Jamaluddin *et al.*, 2011), randomly amplified polymorphic DNA (RAPD; Ambak *et al.*, 2006), microsatellite DNA markers (Jamsari *et al.*, 2011), and allozyme markers (Hara *et al.*, 1998). Restriction fragment length polymorphism (RFLP) combined with polymerase chain reaction (PCR) has been proven to be a successful method for studying the population genetic structure and differentiation of many fishes, 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).

Due to the potential of *C. striata*, studies have analysed its genetics at the morphological, biological, dietary, physiological, biochemical composition, ecological, and chromosomal level for breeding programs, and medical and pharmaceutical activities for its anti-microbial, anti-inflammatory, cell proliferation, induction of platelet aggregation, and anti-nociceptive properties (Mat Jais, 2007a; Supiwong *et al.*, 2009; Dahlan-Daud *et al.*, 2010; Jamaluddin *et al.*, 2011).

These findings would have potential implications for stock management, conservation, and identification as important resources for the fisheries industry. Such information on this species will be useful in the management of different populations of *C. striata* for Good Agriculture Practices (GAP) in farming technology, as well as provide the basis for Good Manufacturing Practices (GMP).

1.2 Justification of the Study

C. striata is a commercially important freshwater fish in Malaysia which has come to play an important economic role as a food resource, and possesses potential medical values and pharmacological properties. To ensure a constant supply of fresh *C. striata* in accordance with strict biochemical quality requirements and mineral, protein, and lipid composition specifications for biomedical and pharmaceutical products, researchers need to i) provide a preliminary assessment of the unit stock of *C. striata* in Peninsular Malaysia, and ii) investigate which population is of superior genetic quality in order to maintain a high percentage of genetic variability. The present study has potential application in fish stock estimation and proposes measures for sustainable management. If the extent of gene flow were low enough for the stocks to be managed as a single panmictic population, it would be advisable to manage them as a single unit.

1.3 Objective of the Study

The general objective of this thesis was to provide biological information on morphological, stock identification, genetic structure, relationship, and demographic

histories on different geographical scales from major regions of the natural distribution areas of the species in Peninsular Malaysia. The specific objectives were:

- 1) To determine the morphometric and fin rays variations among 6 populations of *C. striata* in Peninsular Malaysia.
- 2) To determine the length weight relationship of *C. striata*.
- 3) To determine the level of genetic diversity between and within *C. striata* populations.
- 4) To estimate the degree of genetic differentiation between *C. striata* populations

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