UNIVERSITI PUTRA MALAYSIA

# GENETIC DIVERSITY OF *Boleophthalmus boddarti* AND OTHER MALAYSIAN GOBIES

MEHDI MOHAMMADI

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# GENETIC DIVERSITY OF *Boleophthalmus boddarti* AND OTHER MALAYSIAN GOBIES

**By**

**MEHDI MOHAMMADI**

**Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of Requirements for the Degree of Doctor of Philosophy**

**August 2007**

# DEDICATION

**Dedicated to the memories of my father, mother and brother**

**Dedicated to my wife, son and daughter**
**Milad and Fatemeh**

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy

**GENETIC DIVERSITY OF *Boleophthalmus boddarti* AND OTHER MALAYSIAN GOBIES**

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**Chairman : Associate Professor Siti Khalijah Daud, PhD**

**Faculty : Science**

## ABSTRACT

Mudskippers (Family Gobiidae: Subfamily Oxudercinae) are residents of tidal mudflat shores, tidal muddy zone of estuaries, rivers, and mangrove swamps. The aims of this study were to describe the morphological and genetic variations among populations of *Boleophthalmus boddarti*, phylogenetic of Malaysian Oxudercine gobies, and the exposure of PAHs on *Boleophthalmus boddarti*. The samples of *B. boddarti* were collected from six locations, namely Pulau Pinang, Selangor (Kuala Selangor), Negeri Sembilan (Pasir Panjang), Melaka, Johor and Pahang (Cherating). Conventional and Truss morphometrics analyses were carried out on 300 individuals belonging to 7 species, namely *B. boddarti*, *Periophthalmus chrysospilos, Periophthalmus grasilos, Periophthalmus novemoradiatus, Periophthalmodon schlosseri, Pseudapocryptes elangatus* and *Scartlaos histophoris.* Using the Discriminate Function Analysis (DFA), the conventional morphometric

discriminate the populations of *B. boddarti* into 2 groups while Truss morphometrics into 3 groups, whereby geographically closer populations were grouped together. Of the 29 Randomly Amplified Polymorphic DNA (RAPD) primers tested, only 12 primers gave clear bands and showed polymorphisms. Eleven bands were identified as RAPD markers in the six populations (N=155) of *B. boddarti*. The dendrogram from RAPD data revealed three major groups of B*. boddarti*, in which the first group consisted of the central population (Selangor, Negeri Sembilan and Melaka populations), the second group made up the southern (Johor) and eastern (Pahang) populations, and the third group was the northern populations (P. Pinang), which was distinctly separated from the rest of the population, with a genetic distance of 0.698. The mitochondrial cytochrome b (cytb) sequences in 6 populations of *B. boddarti* revealed a total of 26 haplotypes. Based on haplotype analyses, the populations were grouped into two clades, I and II. Clade I was divided into two subclades consisting of the northern (P. Pinang) and the central (Selangor) populations as subclade IA, and the central population (N. Sembilan and Melaka) as subclade IB. Clade II consisted of the southern (Johor) and the eastern (Pahang) populations of *B. boddarti*. Both cytb (345bp) and 16S rDNA (550bp) gene sequences were carried out for phylogenetic studies on 11 species of the subfamily Oxudercinae. Four phylogenetic trees were constructed using maximum parsimony (MP) and neighbor-joining (NJ) methods, using Kimura-2-Parameter (K2P) and Jukes and Cantor models. Based on parsimony analysis, Oxudercinae subfamily was divided into two main clades consisting of *Oxuderces* in one clade and the rest were in another clade. The distributions of polycyclic aromatic

hydrocarbons (PAHs) in both sediments and porewaters (N=8) in the Klang River and its estuaries were higher than those in the Kuala Muda River. Hepatic EROD activities were carried out on *B. boddarti* (N=62) collected from the Klang River and its estuaries as polluted and Kuala Muda River as less polluted rivers. Hepatic EROD activities showed significantly ($p<0.05$) higher induction of EROD in fish from the Klang River and its estuaries (mean=24.55 pmol $min^{-1}$ mg $protein^{-1}$) than those in the Kuala Muda River (mean=6.84 pmol $min^{-1}$ mg $protein^{-1}$). There were close relationships between the log EROD activities in *B. boddarti* and the total PAHs concentration in sediments ($r^2$= 0.68) and porewaters ($r^2$= 0.66), implying that this fish can be used as an early signal of PAHs exposure in estuarine areas. In conclusions, morphological and molecular markers using both RAPD mtDNA were able to discriminate the populations of *B. boddarti* in Peninsular Malaysia. MtDNA sequences (cytb and 16S rDNA) were found to be useful tools for phylogenetic studies of the subfamily Oxudercinae. For future endeavour, the use of the other molecular markers is recommended to study the population structure of mudskippers in Malaysia.

Abstrak tesis yang dikemukakan kepada untuk Senat Universiti Putra Malaysia sebagai memenuhi keperluan ijazah Doktor Falsafah

**KEPELBAGAIAN GENETIK PADA *Boleophthalmus boddarti* DAN IKAN BELACAK LAIN DI MALAYSIA**

**Oleh**

**MEHDI MOHAMMADI**

**Ogos 2007**

**Pengerusi : Profesor Madya Siti Khalijah Daud, PhD**

**Fakulti : Sains**

Ikan Belacak (Famili Gobiidae: subfamili Oxudecinae) adalah penghuni pantai dataran berlumpur, zon pasang surut muara berlumpur, sungai dan paya bakau. Tujuan kajian ini adalah untuk menghuraikan variasi morfologi dan genetik antara populasi *Boleophthalmus boddarti,* filogenetik ikan belacak Oxudercine di Malaysia, dan pendedahan PAHs ke atas *Boleophthalmus boddarti.* Sampel ikan belacak, *Boleophthalmus boddarti*, telah dipungut dari enam lokasi, iaitu Pulau Pinang, Selangor (Kuala Selangor), Negeri Sembilan (Pasir Panjang), Melaka, Johor and Pahang (Cherating). Analisis morfometrik konvensional dan Truss dijalankan ke atas 300 individu daripada 7 spesies ikan belacak, iaitu *B. boddarti*, *Periophthalmus chrysospilos, Periophthalmus grasilos, Periophthalmus novemoradiatus, Periophthalmodon schlosseri, Pseudapocryptes elangatus* and *Scartlaos histophoris.* Berdasarkan Analisis Fungsi Diskriminan (DFA), morfometrik konvensional dapat mengelaskan populasi *B. boddarti* kepada 2 kumpulan, manakala morfometrik Truss mengelaskan populasi *B. boddarti*

kepada 3 kumpulan, di mana populasi digolongkan mengikut kawasan geografi. Daripada 29 primer *Randomly Amplified Polymophic DNA* (RAPD) yang diuji, hanya 12 primer sahaja yang menghasikan jalur yang jelas dan menunjukkan polimorfisme. Sebelas jalur dikenalpasti sebagai penanda RAPD dalam 6 populasi (N=155) *B. boddarti*. Dendrogram daripada RAPD menujukkan *B. boddarti* boleh digolongkan kepada tiga kumpulan utama, di mana kumpulan pertama terdiri daripada populasi kawasan tengah (Selangor, Negeri Sembilan dan Melaka), kumpulan kedua terdiri daripada populasi dari kawasan selatan (Johor) dan timur (Pahang), dan kumpulan ketiga populasi dari kawasan utara (P.Pinang), yang terpisah jauh daripada kumpulan yang lain dengan jarak genetik sebanyak 0.698. Analisis jujukan mitokondria sitokrom b (cytb) ke atas 6 populasi *B. boddarti* menghasilkan sejumlah 26 haplotip. Berdasarkan analisis haplotip, populasi *B. boddarti* dikelaskan kepada 2 klad, I dan II. Klad I dibahagikan pula kepada dua subklad yang terdiri daripada populasi utara (P. Pinang) dan tengah (Selangor) sebagai subklad 1A, dan populasi tengah (N.Sembilan dan Melaka) sebagai subklad 1B. Klad II pula terdiri daripada populasi selatan (Johor) dan kawasan timur (Pahang). Kedua-dua jujukan gen cytb (345bp) dan 16S rDNA (550bp) dijalankan untuk kajian filogenetik ke atas 11 spesies dalam Subfamili *Oxudercinae*. Empat pokok filogeni telah dibina menggunakan kaedah neighbour-joining (NJ) dan parsimoni maksimum (MP), di mana kedua-duanya menggunakan model Kimura-2-Parameter (K2P) dan model Jukes dan Cantor. Berdasarkan analisis parsimoni, subfamili *Oxudercinae* dibahagikan kepada 2 klad utama, yang mana *Oxuderces* dikelaskan dalam satu klad, dan yang selebihnya dalam klad yang lain. Taburan hidrokarbon

aromatik polisiklik (PAHs) dalam sediment dan air liang (N=8) di Sungai Klang dan muaranya adalah lebih tinggi berbanding dengan yang terdapat di Sungai Kuala Muda. Aktiviti EROD hepatik telah dijialankan ke atas *B. boddarti* (N=62) yang diambil dari Sungai Klang dan muaranya yang mewakili kawasan tercemar, dan Sungai Kuala Muda yang mewakili kawasan kurang tercemar. Aktiviti EROD hepatik menunjukkan penghasilan EROD yang lebih tingi ($P<0.05$) dalam ikan dari Sungai Klang dan muaranya (purata = 24.55 pmol $min^{-1}$ mg $protein^{-1}$) berbanding dengan Sungai Kuala Muda (purata = 6.84 pmol $min^{-1}$ mg $protein^{-1}$). Terdapat hubungan yang rapat antara log aktiviti EROD dengan jumlah kepekatan PAHs dalam sedimen ($r^2$= 0.68) air liang ($r^2$= 0.66) dalam *B. boddarti* menunjukkan bahawa ikan ini boleh digunakan sebagai pengesan awal kepada pendedahan PAHs di kawasan muara sungai. Sebagai kesimpulan, ciri morfologi dan penanda molekul yang menggunakan kedua-dua RAPD dan mtDNA berkebolehan untuk mengelaskan populasi *B. boddarti* di Semenanjung Malaysia. Jujukan mtDNA (cytb dan 16s rDNA) didapati amat berguna untuk kajian filogenetik bagi subfamili *Oxudercinae*. Untuk kajian akan datang, penggunaan penanda molekul yang lain disyorkan untuk kajian populasi ikan belacak di Malaysia.

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I certify that an Examination Committee met on 13th August 2007 to conduct the final examination of Mehdi Mohammadi on his Doctor of Philosophy thesis entitled "Genetic diversity of *Boleophthalmus boddarti* and other Malaysian Gobies" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follow:

**Aziz Bin Arshad, PhD**
Associate Professor
Faculty of Science
Universiti Putra Malaysia
(Chairman)

**Tan Soon Guan, PhD**
Professor
Faculty of Science
Universiti Putra Malaysia
(Internal Examiner)

**Ahmad Ismail, PhD**

Assoc. Professor
Faculty of Science
Universiti Putra Malaysia
(Internal Examiner)

**Mahani Mansor Clyde, PhD**
Professor
Faculty of Science
University of Kebangastan Malaysia
(External Examiner)

**HASANAH MOHD. GHAZALI, PhD**
Professor/Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date : 27 September 2007

This thesis submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirements for the degree of Doctor of Philosophy. The members of the Supervisory Committee are as follows:

**Siti Khalijah Daud, PhD**
Associate Professor
Faculty of Sciences
Universiti Putra Malaysia
(Chairman)

**Siti Shapor Siraj, PhD**
Associate Professor
Faculty of Sciences
Universiti Putra Malaysia
(Member)

**Mohad Pauzi Zakaria, PhD**
Associate Professor
Faculty of Environmental Studies
Universiti Putra Malaysia
(Member)

**AINI IDERIS, PhD**
Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

Date : 15 November 2007

## DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.

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**MEHDI MOHAMMADI**

Date : 19 September 2007

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