



UNIVERSITI PUTRA MALAYSIA

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

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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**

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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 fulfillment of the requirement for the degree of Doctor of Philosophy

GENETIC DIVERSITY OF *Boleophthalmus boddarti* AND OTHER MALAYSIAN GOBIES

By

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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 chrysopilos*, *Periophthalmus grasilos*, *Periophthalmus novemradiatus*, *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

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Ogos 2007

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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 chrysopilos*, *Periophthalmus grasilos*, *Periophthalmus novemradiatus*, *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 Polymorphic 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 menunjukkan *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 hepatic telah dijalankan 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 hepatic menunjukkan penghasilan EROD yang lebih tinggi ($P < 0.05$) dalam ikan dari Sungai Klang dan muaranya (purata = $24.55 \text{ pmol min}^{-1} \text{ mg protein}^{-1}$) berbanding dengan Sungai Kuala Muda (purata = $6.84 \text{ pmol min}^{-1} \text{ mg protein}^{-1}$). 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:

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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.

MEHDI MOHAMMADI

Date : 19 September 2007

TABLE OF CONTENTS

		Page
DEDICATION		ii
ABSTRACT		iv
ABSTRAK		vii
ACKNOWLEDGEMENTS		x
APPROVAL		xi
DECLARATION		xiii
LIST OF TABLES		xviii
LIST OF FIGURES		xxi
LIST OF ABBREVIATIONS		xxv
CHAPTER		
1	INTRODUCTION	1
	1.1 Background of study	1
	1.2 Significant of the study	2
	1.3 Statement of the problems	3
	1.4 Objectives of the Study	8
2	LITERATURE REVIEW	9
	2.1 Biology of Oxudercine Gobies	9
	2.1.1 Taxonomy and Evolution Gobiidae	9
	2.1.2 Oxudercinae	10
	2.1.3 Habitat (Common Mudskipper <i>Boleophthalmus boddarti</i>)	18
	2.1.4 Food	19
	2.1.5 Reproduction	19
	2.1.6 Adaptation of oxudercine gobies	20
	2.1.6.1 Respiratory organs	21
	2.1.6.2 Metabolic Rate	24
	2.1.7 Distribution of Oxudercine Gobies	27
	2.1.8 Morphometric Study	29
	2.1.8.1 Conventional Morphometric	30
	2.1.8.2 Truss morphometric	31
	2.2 Random Amplified Polymorphic DNA (RAPD)	32
	2.3 Mitochondrial DNA marker	35
	2.4 Polycyclic Aromatic Hydrocarbons (PAHs)	38
	2.4.1 Ecotoxicological Significance of PAHs Concentrations in Sediment and Porewater	43



	2.4.2	Mixed Function Oxidase (MFO) Enzyme	44
	2.4.3	Metabolism of xenobiotic in fish	46
	2.4.4	Ethoxyresorufin-O-deethylase (EROD)	48
3		MORPHOLOGICAL VARIATION AMONG SEVEN SPECIES OF OXUDERCINE GOBIES AND POPULATION DISCRIMINATION OF <i>Boleophthalmus boddarti</i>	49
	3.1	INTRODUCTION	49
	3.2	MATERIALS AND METHODS	51
	3.2.1	Sampling	51
	3.2.2	Conventional Morphometric	54
	3.2.2.1	Meristic Characters	55
	3.2.3	Truss Morphometrics	56
	3.2.4	Data Analysis	57
	3.3	RESULTS	60
	3.3.1	Morphometric Characters	60
	3.3.2	Stock identifications of <i>B. boddarti</i> Populations based on morphometric data	65
	3.3.3	Discriminant Function Analysis (DFA)	69
	3.3.3.1	Conventional morphometric	69
	3.3.3.2	Truss morphometric	71
	3.4	DISCUSSION	73
	3.5	CONCLUSIONS	76
4		GENETIC VARIATION AMONG <i>B. boddarti</i> POPULATIONS USING RAPD MARKER	77
	4.1	INTRODUCTION	77
	4.2	MATERIALS AND METHODS	78
	4.2.1	Sample collection	78
	4.2.2	Extraction of genomic DNA and DNA purity	80
	4.2.3	Polymerase Chain Reaction (PCR) for RAPD	81
	4.2.4	Data Scoring and Analysis	82
	4.3	RESULTS	84
	4.4	DISCUSSION	94
	4.4.1	Genetic variation	94
	4.5	CONCLUSIONS	97

5	GENETIC DIVERSITY OF <i>Boleophthalmus boddarti</i> AND PHYLOGENETIC RELATIONSHIPS AMONG THE GENERA OF OXUDERCINAE SUBFAMILIES	98
	5.1 INTRODUCTION	98
	5.2 MATERIALS AND METHODS	101
	5.2.1 Population and Subfamily Samplings	101
	5.2.2 DNA Extraction and Purity Test	101
	5.2.3 Amplification and Sequencing of Cytb and 16S rDNA	102
	5.2.4 Data analysis	103
	5.3 RESULTS	108
	5.3.1 Genetic Diversity of <i>B. boddarti</i>	108
	5.3.2 Phylogenetic Analysis of Subfamily Oxudercinae	112
	5.3.2.1 Cytochrome b Gene	112
	5.3.2.2 Mitochondrial 16S rRNA	115
	5.4 DISCUSSION	117
	5.4.1 Genetic diversity of <i>B. boddarti</i>	117
	5.4.1.1 Diversity Index of <i>B. boddarti</i>	117
	5.4.2 Phylogenetic Relationship among Oxudercine Gobies	118
	5.5 CONCLUSIONS	120
6	EXPOSURE OF POLYCYCLIC AROMATIC HYDROCARBONS (PAHS) ON BLUE SPOTTED MUDSKIPPER	121
	6.1 INTRODUCTION	121
	6.2 MATERIALS AND METHODS	125
	6.2.1 Sampling	125
	6.2.2 Clean up and Extraction of Sediment and Porewater	127
	6.2.3 Extraction	127
	6.2.3.1 Sediment Sample Preparation	127
	6.2.3.2 Porewater Sample Preparation	129
	6.2.4 Column Chromatography Separations	131
	6.2.5 Gas Chromatography Mass Spectrometry (GC-MS) Analysis	133
	6.2.6 Chemicals in EROD assay	138
	6.2.7 EROD assay	138
	6.2.8 Protein assay	139
	6.2.9 Statistical methods	140
	6.3 RESULTS	141
	6.3.1 Polycyclic Aromatic Hydrocarbons	141
	6.3.2 Biological data	145
	6.3.3 Ethoxyresorufin-O-deethylase (EROD)	147
	6.4 DISCUSSION	152
	6.4.1 PAHs concentration in sediment and porewater	152

	6.4.2 EROD	155
	6.5 CONCLUSIONS	160
7	GENERAL DISCUSSION	162
8	CONCLUSIONS AND RECOMMENDATIONS	171
	REFERENCES	174
	APPENDICES	200
	BIODATA OF THE AUTHOR	206



LIST OF TABLES

Table		Page
2.1	Classification of Gobioid Fishes and the position of oxudercinae in this classification	10
2.2	Taxonomic Hierarchy of Subfamily Oxudercinae fish (Thacher 2003)	12
2.3	Geographical distribution of endemic Oxudercine gobies	28
2.4	Concentration of total PAHs in surface sediment of coastal, estuarine riverine area of North America, Europe, Africa and Asia	42
3.1	A summary of morphometric characteristics measured in cm (to nearest 0.01 cm) for seven species of oxudercine gobies	61
3.2	Range and mean \pm sd of the ratios of each morphometric characters to SL or HL and meristic character in seven Mudskipper gobies	63
3.3	Summary of one way ANOVA for each ratio of morphometric data to the SL in seven species of mudskippers	64
3.4	Summary of one way ANOVA for each meristic character in seven oxudercine gobies	65
3.5	Range mean \pm sd of <i>B. boddarti</i> in different state Peninsular Malaysia	67
3.6	Summary of one way ANOVA for each ratio of morphometric data to the SL between and within the six populations of <i>B. boddarti</i>	68
3.7	Summary of one way ANOVA for each meristic character in the six populations of <i>B. boddarti</i>	69
3.8	Structure matrix of morphometric characters	70
3.9	The values of the first three functions obtained through a multivariable discriminates analysis (MDA) performed on raw 33 Truss morphometric data of <i>B. boddarti</i>	72



4.1	Geographical region, sampling sites and sample size of Malaysian mudskipper	79
4.2	Primers codes Sequence of RAPD Primers used for population variation	81
4.3	Number of bands, number of polymorphic bands, and percentage of polymorphism revealed by the eight RAPD primers in six populations of <i>B. boddarti</i>	85
4.4	Number of polymorphic /total number of bands generated per primer in six populations of <i>Boleophthalmus boddarti</i> in Malaysia	88
4.5	Genetic distance of RAPD marker in six populations of <i>B. boddarti</i> based on Lynch and Miligan (1994)	93
5.1	Haplotype Distribution in six populations of <i>B. boddarti</i> from 344 bp cytb	110
5.2	Values for nucleotide diversity (π), haplotype diversity (h), Tajima's distance, Fu's and Lie Fs and θ for <i>B. boddarti</i> populations	110
5.3	Distance between populations of <i>Boleophthalmus boddarti</i> based on K2P models	111
6.1	The sample location and Geographical position of Klang River and its estuaries and Kuala Muda River	126
6.2	Selected character ions and time interval for developing SIM mode in GC-MS for fifteen PAHs target compounds analysis	135
6.3	Fifteen PAHs Targets compounds with Molecular information and their corresponding information	136
6.4	PAHs concentration $\text{ng g}^{-1} \text{dw}^{-1}$ in sediment and ng l^{-1} porewater; AHs $\mu\text{g/g dw}^{-1}$ in sediment and $\mu\text{g/l}$ in porewater of the Klang River and Kuala Muda Stations	142
6.5	Mean \pm standard error of EROD ¹ , T.Length, Weight, GSI ² , LSI ³ & CF ⁴ in blue spotted mudskipper from Kalang River and its estuary and Kuala Muda River	146
6.6	Mean and standard deviation (SD) of hepatic EROD activities in Blue spotted mudskipper	148

6.7	Ethoxyresorufin-O-deethylase (EROD) activities in different marine species with varieties of PAHs profile concentrations. NA: not available	151
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LIST OF FIGURES

Figure		Page
2.1	SEM photographs of mudskipper gill filaments. A: Branched gill filaments of <i>P. schlosseri</i> (scale 700µm). B: Interlamellar fusions in <i>P. schlosseri</i> (scale 100µm). By Mehdi 22.7.03 UPM.	22
2.2	Subdivision of indo-west pacific and west coast of Africa based on maximal oxudercine endemism. The number shows oxudercine species diversity in that subdivision adapted from (Murdy 1989).	29
3.1	<i>Scratelous histophoris</i> (A), <i>Periophthalmodon schlosseri</i> (B), <i>Periophthalmus gracilus</i> (C), <i>Periophthalmus novamoradiatus</i> Puala Pinang, <i>Periophthalmus chrysospilios</i> (gold spotted mudskipper)(E), <i>Pseudapocryptes elangatus</i> (F) from Kuala Muda coastal mudflats.	52
3.2	Blue spotted mudskipper, <i>Boleophthalmus boddarti</i> , (A,B), Fin print (C), view of outside burrow and habitat (C, D) a pair of <i>Bleophthalmus boddarti</i> from Kuala Selangor riverine area (E).	53
3.3	Sampling locations of the mudskipper in six locations in Peninsular Malaysia.	54
3.4	Conventional morphometric and meristic character of <i>Boleophthalmus boddarti</i> .	56
3.5	Location of the 12 body landmarks used to calculate the truss networks (lines).	57
3.6	location of superior and lateral landmarks used to calculate the Truss lines.	57
3.7	Plots of the coordinates of individuals of <i>B. boddarti</i> according to the first two discriminant functions, obtained from morphometric data.	71
3.8	Plots of the coordinates of individuals of <i>B. boddarti</i> According to the first two discriminant functions, obtained from truss morphometric data.	73

4.1	Sampling location of <i>B. boddarti</i> populations in Malaysia.	79
4.2	RAPD pattern obtained from P. Pinang population (1-15) of <i>B. boddarti</i> generated by primer OPA1 (780 bp marker for P. Pinang population). Lane M is 100 bp DNA markers.	85
4.3	RAPD pattern obtained from K. Selangor population (1-15) of <i>B. boddarti</i> generated by primer OPA7 (815 bp marker for K. Selangor). Lane M is 100 bp DNA markers.	86
4.4	RAPD patterns obtained from P. Dickson population (1-15) generated by primer OPA-15 in <i>B. boddarti</i> , lane M is 100 bp DNA marker.	86
4.5	RAPD patterns obtained from Melaka population (1-15) of <i>B. boddarti</i> genotypes generated by primer OPA17 (600bp marker for Melaka population). Lane M is 100 bp DNA markers.	87
4.6	RAPD patterns obtained from Johor population (1-13) of <i>B. boddarti</i> generated by primer OPA-18 (670 marker for Johor and 430 shared marker with Pahang population) . Lane M is 100 bp DNA markers.	87
4.7	RAPD patterns obtained from Pahang population (1-15) of <i>B. boddarti</i> generated by primer OPA 9. Lane M is 100 bp DNA markers.	88
4.8	UPGMA cluster analysis of RAPD data for six populations of <i>B. boddarti</i> .	93
5.1	mtDNA patterns obtained from 16S using L2510 and H3080 primers for phylogenetic studies of <i>Periophthalmus schlosseri</i> (1-6) and Melaka population (7-11) <i>Boleophthalmus boddarti</i> generated by primer L 0021 and H0494. Lane M is 100 bp DNA markers.	107
5.2	The amplification of whole cytb gene in <i>B. boddarti</i> (1-4) and <i>Periophthalmus chrysopilos</i> (5-8) generated by primers L 0021 and H956. Lanes M are molecular weight marker (100 bp Plus).	107
5.3	DNA sequence of partial cytb of <i>B. boddarti</i> generated by L0021 and H0494 primers.	108
5.4	Neighbour-Joining dendrogram based on genetic Jukes-Cantor distance for the 27 haplotypes identified from the six populations <i>B. boddarti</i> . Number beside internal	112

branches showed bootstrap probabilities (>79%) based on 500 pseudoreplicates.

- 5.5 Molecular phylogeny of Oxudecinae subfamily based on NJ. This hypothesis based on partial sequenced of cytb gene. The number on nodes indicates decay index values. The shape on the left part of species name indicate genus name.
(■ =Periophthalmus, ● = Perophthalmodon and ◆ =Boleophthalmus). 114
- 5.6 Molecular phylogeny of Oxudecinae subfamily based on MP (B). This hypothesis based on partial sequenced of cytb gene. The number on nodes indicates decay index values. The shape on the left part of species indicate genus name
(■ =Periophthalmus, ● = Perophthalmodon and ◆ =Boleophthalmus). 114
- 5.7 Molecular phylogeny of Oxudercinae subfamily based on NJ. This hypothesis was based on partial sequenced of 16S gene. The number on nodes indicates decay index values. The shape on the left part of species indicate genus name
(■ =Periophthalmus, ● =Periophthalmodon and ◆ =Boleophthalmus). 116
- 5.8 Molecular phylogeny of Oxudercinae subfamily based on MP (B). This hypothesis was based on partial sequenced of 16S gene. The number on nodes indicates decay index values. The shape on the left part of species indicate genus name (=Periophthalmus, =Periophthalmodon and ◆ =Boleophthalmus) 116
- 6.1 Sampling location of Klang River and its estuary and Kuala Muda River. 126
- 6.2 Some Molecular structures of Polycyclic Aromatic Hydrocarbons (PAHs) analysed in this study. 137
- 6.3 Total PAHs concentration in sediment and porewater in the Klang River and estuary (A, B and C) and Kuala Muda River 143
- 6.4 PAHs profile of sediment (dark colour) and pore water (light colour)of Klang River and estuary station A and B. 144
- 6.5 PAHs profile of sediment (dark colour) and pore water (light colour) of Klang River and estuary station C. 144

6.6	PAHs profile of sediment (dark colour) and pore water (light colour) of Kuala Muda estuary.	145
6.7	Liver somatic index (LSI) of blue spotted mudskipper collected from Klang River and estuary and Kuala Muda River.	147
6.8	EROD activity in $\text{pmol min}^{-1} \text{mg protein}^{-1}$ of the blue Spotted mudskipper collected from Klang River (KLRA-C) and its estuaries and K. Muda River.	149
6.9	Correlation between EROD activity to fish weight (a) and fish Weight Correlation is significant at the 0.01 level. Log transforms data were used.	149
6.10	Correlation between EROD activity to fish weight (a) and liver Weight (b) Correlation is significant at the 0.01 level. Log transforms data were used.	150
6.11	Correlation between EROD to PAHs in different stations in Klang and Kuala Muda. Significant at 0.01 levels. Log transforms data were used.	150
6.12	Ethoxyresorufin-O-deethylase (EROD) activities $\text{pmol min}^{-1} \text{mg protein}^{-1}$ in different marine species in the world compared with the blue spotted mudskipper in Malaysian rivers and estuaries.	151