



UNIVERSITI PUTRA MALAYSIA

***FISH LARVAE SPECIES IDENTIFICATION AND POPULATION GENETIC
STRUCTURE OF *Boleophthalmus boddarti* (Pallas, 1770) FROM
SELECTED MANGROVE SITES IN PENINSULAR MALAYSIA***

Izzati Adilah Azmir

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By

IZZATI ADILAH BINTI AZMIR

**Thesis Submitted to the School of Graduate Studies, Universiti Putra
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Philosophy**

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DEDICATED

To

My Mother and Father

Who always think highly of me, grace me with never ending prayers and help me with what they think best for the moment.

My Husband

For working so hard to support me both spiritually and financially as to ensure I can give full attention to my studies. For being so patient and a loyal listener who at the same time thinks that I am the smartest, brightest person on earth.

My Children

Who has become the sunshine during my bad days, who have taught me to be more patient and wanted to be a better person each day.

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May Allah bless all of you with His kindness.

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

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January 2018

Chairman : Associate Professor Yuzine bin Esa, PhD
Faculty : Agriculture

A study on fish larvae species identification from mangrove areas of Pendas Johor, Matang Perak, Pekan Pahang and Setiu Terengganu and population genetic assessment of Blue-spotted mudskipper *Boleophthalmus boddarti*, from similar sampling areas except Setiu Terengganu has been done. The fish larvae collection occurred from April 2015 to September 2015 by using a bongo net, towed at a depth of about 0.5 m from the surface for 5 min against the tidal flow. The mudskipper was collected with casting nets, hand net and bare hands at night time and especially when the tide is low, in between the month of January 2016 and September 2016. This study aims to identify fish larvae to species level by adopting integrative method; a combination of comparative and molecular method. The composition, abundance, diversity and phylogenetic relationship among fish larvae samples were also investigated. The lack of species identification work on fish larvae in Peninsular Malaysia has warrant this study to be conducted. The status of genetic population of mudskipper *B. boddarti* is investigated using two markers, mitochondrial and microsatellite. Specific microsatellite loci for the *B. boddarti* are absent and information on population structure and phylogenetic relationship among *B. boddarti* populations are lacking. Therefore, this study was conducted to characterize the genetic structure of *B. boddarti* in Peninsular Malaysia through mitochondrial Cytochrome c Oxidase I (COI) gene and new set of microsatellite marker developed using Next Generation Sequencing (NGS) method.

A total of 354 individuals of fish larvae were collected and through morphological analyses they were identified morphologically into 21 families, 20 genus and 18 species. Among the 21 families, the top 3 families, namely, Gobiidae (39.26%), Engraulidae (14.97%), and Clupeidae (14.40%), occurred in all sampling areas except in Setiu. The highest diversity of fish larvae was recorded for Pendas, Johor with Shannon Wiener index $H = 2.699$, and the lowest was for Setiu Terengganu with $H = 0.832$. Setiu Terengganu recorded the lowest evenness value, indicating high single-species dominance. From the total of 354 fish larvae collected, a representative of 177 fish larvae were selected and sequenced using Cytochrome c Oxidase I (COI) gene where they corresponded to 18 families, 33 genus and 41 species of larval fish. Results from BLAST and BOLD SYSTEM search showed all sequences have high percentage identity index and similarity (90% to 100%). The identification of fish larvae was mostly successfully confirmed through phylogenetics analysis showing monophyletic status between query sequences with reference sequences obtained from GenBank. However the *Sillago vittata* and *Sillago sihama* sequences was found to be in separate clusters despite their similar genus. A few strong match of specimens from different genus was found with high bootstrap value ($n > 90\%$) through Neighbour-Joining (NJ) and Maximum-likelihood (ML) analysis. The *Paramugil parmatus* was matched with *Liza melinoptera* (NJ = 100%, ML = 99%) and *Pseudogobius oligactis* was matched with *Eugnathogobius oligactis* (NJ = 92%, ML 94%).

A total of 11,957 short sequence repeats (SSR) motifs were identified via analysis of 6.87 Gb nucleotides conducted by using Illumina sequencing to produce a comprehensive transcript dataset for *B. boddarti* motif. The most abundant type of repeat motif was mono-nucleotide (63.66%), followed by tri-nucleotide (18.88%), di-nucleotide (15.54%), tetra-nucleotide (0.83%) and both penta- and hexanucleotide was found the least (0.11%). Experimental screening of 27 pairs of microsatellite primers detected 30% of microsatellite loci were polymorphic.

Both markers showed strong genetic differentiation between populations with result from mitochondrial COI analysis revealed haplotype sharing between Pekan Pahang and Pendas Johor populations (KY754661) and Pendas Johor and Matang Perak populations (MF572075). Zero connectivity between Matang Perak and Pekan Pahang populations ($F_{st} = 0.53086$; N_m was 0.44186) due to huge biogeographical barrier. Microsatellite marker analysis detected minimal differentiations (F_{st}) among Pendas Johor and Matang Perak populations ($F_{st}=0.26461$) and between Pekan Pahang and Matang Perak populations ($F_{st}=0.38423$, $N_m=0.80129$) reflecting isolation between populations. However the species was found to be locally adapted where genetic variation was more likely to occur within populations rather than among populations (ratio of 6:3). The mtDNA marker further showed no evidence of recent population expansion through Tajima's D , F_u F_s' and Bayesian skyline plots but possible occurrence

in population expansion did occurred long before present years during Pleistocene era.

High haplotype diversity (0.680 to 0.819) and moderate nucleotide diversity (0.00657 to 0.05886) was found from *COI* analysis showing genetic variation was in moderation even though Pendas Johor possessed highest number of sample. Thus this reflect possibility of environmental degradation and further supported by microsatellite marker analysis where moderate genetic variation was also seen (mean H_o = 0.4337, mean H_e = 0.4535). Deviated Hardy-Weinberg equilibrium was mainly due to heterozygote deficiencies and possibility of inbreeding especially in Pekan Pahang with small sample size (lzz 4-6, H_o = 0.280, H_e = 0.497). The high H_o compared to expected heterozygosity (H_e) value documented in Pendas Johor population particularly, signal recently bottleneck event showed through Wilcoxon test with probability value less than 0.05 after applying three mutational model (IAM, SMM and TPM) and Mode-Shift indicator further confirmed the population was under shifted mode. The population structure of *B. boddarti* inferred based on the two types of markers was not showing significant differences. In conclusion, integrative identification provide better results to phenotypically ambiguous specimen like the fish larvae and application of mitochondrial and nuclear gene markers were able to characterize genetic structure and evolutionary kinship of *B. boddarti*.

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

**IDENTIFIKASI SPESIES LARVA IKAN DAN STRUKTUR POPULASI
GENETIK *Boleophthalmus boddarti* (Pallas, 1770) DARIPADA KAWASAN
PAYA BAKAU TERPILIH DI SEMENANJUNG MALAYSIA**

Oleh

IZZATI ADILAH BINTI AZMIR

Januari 2018

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Fakulti : Pertanian

Kajian ke atas identifikasi spesies larva ikan di kawasan paya bakau di Pendas Johor, Matang Perak, Pekan Pahang dan Setiu Terengganu serta semakan populasi genetik ikan belacak berbintik biru *Boleophthalmus boddarti*, dari kawasan kajian yang sama kecuali Setiu Terengganu telah dijalankan. Pengumpulan larva ikan telah dilakukan antara April 2015 sehingga September 2015 menggunakan jaring bongo, ditunda di kedalaman 0.5m dari permukaan air laut selama 5 minit secara melawan arus. Ikan belacak telah dikumpul menggunakan jala, penyauk tangan dan juga tangan pada waktu malam semasa air surut, di antara bulan Januari 2016 hingga September 2016. Kajian ini bertujuan untuk mengidentifikasi larva ikan hingga ke tahap spesies dengan menggunakan kaedah integratif; kombinasi kaedah perbandingan dan molekular. Komposisi, kelimpahan, kepelbagaian dan hubungan filogenetik antara larva ikan turut dikaji. Didapati terdapat kekurangan kajian identifikasi spesies larva ikan di Semenanjung Malaysia maka menjadikan kajian ini harus dijalankan. Status populasi genetik ikan belacak *B. boddarti* telah dikaji menggunakan dua penanda, mitokondria dan mikrosatelit. Penanda mikrosatelit khusus untuk *B. boddarti* masih tidak wujud dan informasi tentang populasi genetik serta hubungan filogenetik di antara populasi *B. boddarti* masih kurang. Maka dengan itu, kajian ini dijalankan adalah untuk mencirikan struktur genetik *B. boddarti* di Semenanjung Malaysia menggunakan gen *sitokrom c oksides I* dan penanda baru mikrosatelit yang dibina khas melalui kaedah 'Next Generation Sequencing' (NGS).

Sejumlah 354 larva ikan telah dikumpul dan dikenalpasti tergolong ke dalam 21 famili, 20 genus dan 18 spesies melalui analisis morfologi. Antara 21 famili yang dikenalpasti, 3 famili iaitu Gobiidae (39.26%), Engraulidae (14.97%) dan Clupeidae (14.40%), dijumpai di semua kawasan kajian kecuali di Setiu Terengganu. Kepelbagaian larva ikan paling tinggi dicatatkan di Pendas Johor melalui Indeks Shannon Wiener $H = 2.699$, manakala kepelbagaian paling rendah dicatatkan di Setiu Terengganu dengan $H = 0.832$. Setiu Terengganu juga mencatatkan nilai kesamaan paling rendah menunjukkan Setiu Terengganu di dominasi oleh satu jenis spesies. Daripada jumlah terkumpul sebanyak 354 larva ikan, sebanyak 177 individu dipilih sebagai wakil, di jujuk dan mendapati tergolong kepada 18 famili, 33 genus dan 41 spesies larva ikan. Keputusan dari pencarian BLAST dan BOLDSYSTEM menunjukkan semua jujukan DNA mempunyai peratusan identiti dan kesamaan yang tinggi (90% hingga 100%). Identifikasi larva ikan didapati berjaya dibuktikan melalui analisis filogenetik apabila status monofiletik terhasil diantara jujukan yang dikaji dengan jujukan rujukan yang diperolehi daripada GenBank. Bagaimanapun, jujukan *Sillago vittata* dan *Sillago sihama* didapati berada di kumpulan berlainan sedangkan tergolong di dalam kumpulan genus yang sama. Beberapa sampel berlainan genus menunjukkan padanan dengan nilai 'bootstrap' yang tinggi ($n > 90\%$) melalui analisis 'Neighbour-joining' (NJ) dan analisis 'Maximum-likelihood' (ML) termasuklah *Paramugil parmatus* berpadanan dengan *Liza melinoptera* (NJ = 100%, ML = 99%) dan *Pseudogobius oligactis* berpadanan dengan *Eugnathogobius oligactis* (NJ = 92%, ML 94%).

Sebanyak 11, 957 jujukan pendek berulang dikenalpasti melalui analisis ke atas 6.87 Gb nukleotid yang dilakukan menggunakan jujukan Illumina yang mampu menghasilkan motif dataset transkrip meyeluruh untuk *B. boddarti*. Didapati jenis ulangan motif yang paling tinggi adalah mono-nukleotid (63.66%), diikuti oleh tri-nukleotid (18.88%), di-nukleotid (15.54%), tetra-nukleotid (0.83%) manakala penta- dan heksanukleotid didapati paling sedikit (0.11%). Sebanyak 30% lokus mikrosatelit menunjukkan polimorfik setelah ujian saringan dilakukan ke atas 27 penanda mikrosatelit.

Kedua-dua jenis penanda menunjukkan perbezaan genetik yang besar di antara populasi yang dikaji dengan hasil daripada penanda mitokondria menunjukkan haplotip didapati dikongsi diantara populasi Pekan Pahang dan Pendas Johor (KY754661) dan di antara populasi Pendas Johor dan Matang Perak (MF572075). Tiada perkaitan didapati diantara populasi Matang Perak dan Pekan Pahang ($F_{st} = 0.53086$; $N_m = 0.44186$) disebabkan perpisahan biogeografi. Penanda mikrosatelit menunjukkan perbezaan minimal nilai F_{st} di kalangan populasi Pendas Johor dan Matang Perak ($F_{st} = 0.26461$) dan diantara populasi Pekan Pahang dan Matang Perak ($F_{st} = 0.38423$, $N_m = 0.80129$) mencerminkan pengasingan berlaku diantara populasi kajian. Walaubagaimanapun, spesies ikan belacak ini didapati lebih beradaptasi dengan kawasan asal mereka dimana variasi genetik dilihat berlaku dikalangan populasi tempatan berbanding dengan populasi yang berlainan kawasan

(nisbah 6:3). Penanda mikrosatelit turut menunjukkan tiada bukti berlakunya pengembangan populasi ikan belacak menerusi Tajima's D , F_u F_s' and 'Bayesian skyline plots' tetapi berkemungkinan telah berlaku pada Pleistocene era sebelum tahun masihi.

Kepelbagaian haplotaip didapati tinggi (0.680 hingga 0.819) manakala kepelbagaian nukleotid adalah sederhana (0.00657 hingga 0.05886) melalui analisis *sitokrom c oksides I* menandakan variasi genetik berada di tahap sederhana walaupun Pendas Johor mempunyai bilangan sampel yang paling banyak. Ini menyimpulkan kemungkinan berlakunya degradasi persekitaran kajian dan seterusnya disokong oleh analisis penanda mikrosatelit dimana variasi genetik turut berada dalam keserdahanaan (min H_o = 0.4337, min H_e = 0.4535). Keseimbangan Hardy-Weinberg menyimpang adalah disebabkan kekurangan heterozigositi dan kemungkinan berlakunya pembiakan dalam populasi yang sama terutama di Pekan Pahang yang mempunyai bilangan sampel kecil (Izz 4-6, H_o = 0.280, H_e = 0.497). Ketinggian nilai H_o berbanding nilai purata heterozigositi terjangka (H_e) disaksikan di populasi Pendas Johor terutamanya, memberi isyarat telah berlakunya kesesakan genetik dan dibuktikan menerusi ujian Wilcoxon dengan nilai kemungkinan kurang dari 0.05 setelah mengaplikasi tiga model mutasi (IAM, SMM and TPM) dan penunjuk 'Mode-shift' juga menunjukkan keputusan yang sama. Kajian struktur populasi *B. boddarti* melalui dua jenis penanda menyimpulkan keputusan yang hampir sama. . Kesimpulannya identifikasi secara integratif memberi keputusan yang lebih baik kepada sampel kajian yang sifat fizikalnya meragukan seperti contoh larva ikan. Aplikasi penanda gen mitokondria dan nuklear terbukti mampu mencirikan struktur genetik dan hubungan kekeluargaan serta evolusi *B. boddarti*.

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

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TABLE OF CONTENTS

	Page
ABSTRACT	i
ABSTRAK	iv
ACKNOWLEDGEMENTS	vii
APPROVAL	viii
DECLARATION	x
LIST OF TABLES	xvi
LIST OF FIGURES	xix
LIST OF ABBREVIATIONS	xxiii
 CHAPTER	
 1 INTRODUCTION	 1
1.1 Background of the study	1
1.2 Problem statement	3
1.3 Significant of the study	4
1.4 Objectives	4
 2 LITERATURE REVIEW	 5
2.1 Fish larvae	5
2.1.1 Fish larvae identification	7
2.1.2 Fish larvae ecosystem and habitats	10
2.1.2.1 Mangrove and seagrass habitat	10
2.1.2.2 Coral reef habitat	11
2.1.2.3 Estuaries and coastal habitat	11
2.1.3 Fish larvae distribution and abundance in Malaysia	11
2.2 Mudskipper	16
2.2.1 Taxonomic description and morphology	16
2.2.2 Habitat and distribution	17
2.2.3 Importance of mudskippers	18
2.3 Genetic Identification of Fish larvae	19
2.3.1 Mitochondrial DNA as marker	19
2.3.2 DNA barcoding	19
2.3.3 Advantages of DNA Barcoding in Fish larvae Identification	20
2.3.4 Arising Issues on DNA Barcoding	24
2.4 Microsatellite development	25
2.4.1 General strategies for microsatellite development	25
2.4.2 Next-Generation Sequencing (NGS)	25

	2.4.3	Application of microsatellites	26
2.5		Population Genetic Study	28
	2.5.1	Population structure and connectivity	28
	2.5.2	Molecular Markers in Population Genetics Studies	29
	2.5.2.1	Mitochondrial markers	31
	2.5.2.2	Nuclear DNA marker	31
	2.5.2.3	Microsatellites marker	33
	2.5.3	Genetic Variation and Population Structure Study in Mangrove/Seagrass Ecosystem.	34
3		MORPHOMETRIC DESCRIPTIONS, DIVERSITY AND DISTRIBUTION QUANTIFICATION OF FISH LARVAE FROM SELECTED MANGROVE AREAS IN PENINSULAR MALAYSIA	38
	3.1	Introduction	38
	3.2	Materials and Method	39
	3.2.1	Sampling method	39
	3.2.2	Sampling stations	40
	3.2.3	Morphological identifications of fish larvae	41
	3.2.4	Biodiversity analysis	42
	3.3	Results	43
	3.3.1	Identification of fish larvae	43
	3.3.2	Taxonomic description of fish larvae	44
	3.3.3	Fish larvae composition and abundance according to study sites	58
	3.3.4	Diversity and distribution of fish larvae	60
	3.4	Discussions	63
	3.5	Conclusion	68
4		DNA BARCODING APPLICATION AND PHYLOGENETIC ANALYSIS IN IDENTIFICATION OF FISH LARVAE	69
	4.1	Introduction	69
	4.2	Materials and methods	70
	4.2.1	Sampling of fish larvae and adult samples	70
	4.2.2	DNA Extraction	71
	4.2.3	DNA Purity	72
	4.2.4	PCR Amplification and Agarose Gel Electrophoresis	72
	4.2.5	DNA Purification and DNA Sequencing	73
	4.2.6	Data Analysis	74
	4.2.7	Phylogenetic Assessment	74
	4.3	Result	75
	4.3.1	Species assignment of fish larvae using direct sequence comparison.	75

4.3.2	Haplotype assignment	78
4.3.3	Fish larvae <i>COI</i> gene assessment	78
4.3.4	Characteristics of <i>COI</i> dataset	80
4.3.5	Phylogenetic relationship between fish larvae	82
4.3.6	Confirmation on fish larvae species assignment through phylogenetic relationship with adult fish samples	86
4.4	Discussion	88
4.5	Conclusion	93
5	POPULATION GENETIC STRUCTURE OF BLUE-SPOTTED MUDSKIPPER, <i>Boleophthalmus boddarti</i> FROM MANGROVE AREAS OF PENINSULAR MALAYSIA INFERRED FROM ANALYSIS OF THE MITOCHONDRIAL CYTHOCHROME C OXIDASE I (<i>COI</i>) GENE.	95
5.1	Introduction	95
5.2	Material and methods	96
5.2.1	Sample Collection	96
5.2.2	DNA Extraction	97
5.2.3	PCR amplification and sequencing	98
5.2.4	DNA Purification and DNA Sequencing	99
5.2.5	Data analysis	99
5.2.6	Phylogenetic analysis	99
5.2.7	Population genetic analysis	100
5.3	Result	101
5.3.1	Species assignment and <i>COI</i> gene efficiency	101
5.3.2	Genetic diversity and mtDNA polymorphism	102
5.3.3	Phylogenetic relationship among haplotypes	105
5.3.4	Phenotype characteristics among haplotypes	114
5.3.5	Population genetic structure of <i>B. boddarti</i>	115
5.4	Discussion	118
5.5	Conclusion	124
6	MICROSATELLITE DEVELOPMENT THROUGH NEXT GENERATION SEQUENCING (NGS) TECHNIQUE AND POPULATION STRUCTURE ANALYSIS ON BLUE-SPOTTED MUDSKIPPER, <i>Boleophthalmus boddarti</i> FROM MANGROVE AREAS OF PENINSULAR MALAYSIA	125
6.1	Introduction	125
6.2	Materials and method	126
6.2.1	Fish sample	126
6.2.2	RNA extraction and cDNA library preparation	127
6.2.3	Liver transcriptome assembly	130
6.2.4	Gene Functional Annotation	133

6.2.5	Simple Sequence Repeat (SSR) detection and validation	134
6.2.6	Data deposition	134
6.2.7	DNA extraction	134
6.2.8	Optimization of PCR condition for selected microsatellite markers	134
6.2.9	PCR amplification of microsatellites	135
6.2.10	Fragment Analysis	136
6.2.11	Genotypic data preparation and genotyping accuracy assessment	136
6.2.12	Data Analysis	137
6.3	Results	138
6.3.1	<i>De novo</i> Illumina sequencing data analysis and microsatellites characterization.	138
6.3.2	Functional annotation of unigenes	140
6.3.3	Experimental screening of microsatellite markers	144
6.3.4	Genotyping errors	146
6.3.5	Genetic polymorphism	147
6.3.6	Hardy Weinberg equilibrium (HWE)	148
6.3.7	Genetic variations and inbreeding coefficient	150
6.3.8	Linkage disequilibrium (LD)	151
6.3.9	Genetic differentiation among population	152
6.3.10	Population structure	153
6.3.11	Population bottleneck	154
6.4	Discussion	156
6.5	Conclusion	164
7	GENERAL DISCUSSION AND CONCLUSION	165
7.1	Discussion	165
7.2	Conclusion	167
	REFERENCES	168
	APPENDICES	214
	BIODATA OF STUDENT	232
	LIST OF PUBLICATIONS	233

LIST OF TABLES

Table	Page
2.1 Characteristics observed during early life stages of fish larvae	6
2.2 Body shape classification based on body depth (BD) to body length (BL) ratio	8
3.1 List of families of fish larvae recorded in selected mangrove areas in Peninsular Malaysia	43
3.2 Occurrence of fish larvae from the four mangrove areas surveyed in Peninsular Malaysia	59
3.3 ANOVA table for fish larvae specimens from selected mangrove areas in Peninsular Malaysia	60
3.4 Fish larvae abundances, indices of diversity, and evenness at stations sampled between April 2015 to September 2015 in mangrove areas of Peninsular Malaysia	62
4.1 Species assignment of 153 barcoded fish larvae from the four mangrove areas surveyed in Peninsular Malaysia	77
4.2 Nearest match in the identification of the fish larvae specimens from mangrove areas of Peninsular Malaysia, Malaysia	78
4.3 Genetic divergences of Kimura 2-parameters (K2P) at different taxonomic level for fish larvae specimens from mangrove areas of Peninsular Malaysia, Malaysia	79
5.1 List of mitochondrial DNA haplotypes observed from 96 <i>B. boddarti</i> specimens sampled from mangrove areas of Peninsular Malaysia, Malaysia	102
5.2 Haplotype distribution, nucleotide diversity, number of haplotypes, haplotypes diversity and number of polymorphic sites in <i>B. boddarti</i>	104
5.3 Pairwise genetic distance among 22 haplotypes of <i>B. boddarti</i>	109
5.4 The phenotype characteristics among <i>B. boddarti</i> haplotypes	114

5.5	One-way ANOVA on haplotype groups of <i>B. boddarti</i> comparing their phenotype	115
5.6	Hierarchical analysis of molecular variance (AMOVA) in <i>B. boddarti</i>	115
5.7	Pairwise genetic distance (F_{ST}) and gene flow among 3 populations of <i>B. boddarti</i>	116
5.8	Neutrality test and mismatch distribution parameter estimates (significance level: $P < 0.01$) for three populations of <i>B. boddarti</i> in mangroves of Peninsular Malaysia * θ =theta, τ =tau, CI-confidence interval	116
6.1	Summary of the transcriptome analysis on <i>B. boddarti</i>	138
6.2	Annotation of unigenes from the transcriptome	139
6.3	Functional annotation of unigenes from the transcriptome	141
6.4	Microsatellite markers development and their application in a tested population. Ta: annealing temperature; Produce amplicons (y) is for yes, (-) is for no amplicons, (+) for monomorphic amplicons and (++) is for larger than expected size amplicons	144
6.5	Eight microsatellite primers tested on <i>B. boddarti</i> and their estimated size and temperature	145
6.6	Allele frequencies between allele size of the loci and the population of <i>Boleophthalmus boddarti</i>	148
6.7	Genetic variation in three populations of <i>B. boddarti</i> in Peninsular Malaysia from eight microsatellites loci	149
6.8	Test of the linkage disequilibrium on each locus for three populations	152
6.9	Hierarchical analysis of molecular variance (AMOVA) in <i>B. boddarti</i>	153
6.10	Pairwise F_{ST} estimations and gene flow from 3 populations of <i>B. boddarti</i> generated from eight microsatellites loci	153
6.11	Membership ratio estimated for each population of <i>B. boddarti</i>	154

- 6.12 Eight microsatellite loci analysis reveals bottleneck event in the population of Blue-spotted mudskipper from Peninsular Malaysia

156



LIST OF FIGURES

Figure	Page
2.1 Development stages (Early life history stage of <i>Trachurua symmetricus</i> from Ahlstrom from and Ball, 1954	5
2.2 Diagrammatic representations for early and late yolk-sac larvae modified from Kramer (1960)	7
2.3 Diagrammatic representation of morphology of a teleost larva	8
2.4 Postflexion stage of a fish larva showing anatomical and morphometric features	9
2.5 The Blue-spotted mudskipper <i>Boleophthalmus boddarti</i>	17
3.1 Sampling stations of fish larvae from four selected mangrove areas in Peninsular Malaysia	40
3.2 The Sillaginidae fish larva collected from the selected mangrove areas in Peninsular Malaysia	44
3.3 The Blenniidae fish larva collected from the selected mangrove areas in Peninsular Malaysia	45
3.4 The Tetradontidae fish larva collected from the selected mangrove areas in Peninsular Malaysia	46
3.5 The Apogonidae fish larva collected from the selected mangrove areas in Peninsular Malaysia	46
3.6 The Mullidae fish larva collected from the selected mangrove areas in Peninsular Malaysia	47
3.7 The Eleotridae fish larva collected from the selected mangrove areas in Peninsular Malaysia	48
3.8 The Leiognathidae fish larva collected from the selected mangrove areas in Peninsular Malaysia	48
3.9 The Gerreidae fish larva collected from the selected mangrove areas in Peninsular Malaysia	49

3.10	The Clupeidae fish larva collected from the selected mangrove areas in Peninsular Malaysia	50
3.11	The Scatophagidae fish larva collected from the selected mangrove areas in Peninsular Malaysia	50
3.12	The Monacanthidae fish larva collected from the selected mangrove areas in Peninsular Malaysia	51
3.13	The Sparidae fish larva collected from the selected mangrove areas in Peninsular Malaysia	52
3.14	The Lutjanidae fish larva collected from the selected mangrove areas in Peninsular Malaysia	52
3.15	The Sciaenidae fish larva collected from the selected mangrove areas in Peninsular Malaysia	53
3.16	The Sygnathidae fish larva collected from the selected mangrove areas in Peninsular Malaysia	54
3.17	The Mugilidae fish larva collected from the selected mangrove areas in Peninsular Malaysia	55
3.18	The Ambassidae fish larva collected from the selected mangrove areas in Peninsular Malaysia	55
3.19	The Engraulidae fish larva collected from the selected mangrove areas in Peninsular Malaysia	56
3.20	The Gobiidae fish larva collected from the selected mangrove areas in Peninsular Malaysia	57
3.21	The Callionymidae fish larva collected from the selected mangrove areas in Peninsular Malaysia	57
3.22	The Pomacentridae fish larva collected from the selected mangrove areas in Peninsular Malaysia	58
3.23	Number of fish larvae species and individuals caught at different mangrove stations	61
3.24	A dendrogram showing a cluster of the fish larvae species occurrence among the mangrove areas studied	62

4.1	Gel electrophoresis (1% agarose gel) visualization of selected fish larvae specimens amplified using <i>COI</i> gene.	76
4.2	Frequency distributions of <i>COI</i> Kimura 2-paramater (K2P) genetic distances (%) intraspecies and intragenus of 18 families, 33 genus and 41 species fish larvae in Peninsular Malaysia, Malaysia	79
4.3	Neighbour-Joining (NJ) tree showing relationships among 98 haplotypes of fish larvae sampled from selected mangrove areas in Peninsular Malaysia. Each node represented with bootstrap value (%) after 1000 pseudoreplications of NJ analysis	84
4.4	Maximum likelihood (ML) tree showing relationships among 98 haplotypes of fish larvae sampled from selected mangrove areas in Peninsular Malaysia. Each node represented with bootstrap value (%) after 1000 of pseudoreplications	85
4.5	Bayesian inference (BI) showing relationships among 98 haplotypes of fish larvae sampled from selected mangrove areas in Peninsular Malaysia	86
5.1	Sampling stations of Blue-spotted mudskipper from three selected mangrove areas in Peninsular Malaysia	97
5.2	The PCR amplification gel using <i>COI</i> gene on 11 samples of random <i>B. boddarti</i> samples viewed using 1% agarose gel producing approximately 650bp bands	101
5.3	Neighbour-Joining relationship between 22 sequences of <i>B. boddarti</i> representing three populations from selected mangrove areas in Peninsular Malaysia	106
5.4	Maximum likelihood relationship between 22 sequences of <i>B. boddarti</i> representing three populations from selected mangrove areas in Peninsular Malaysia	107
5.5	Bayesian inference analysis between 22 sequences of <i>B. boddarti</i> representing three populations from selected mangrove areas in Peninsular Malaysia	108
5.6	Aligned variable sites from 22 haplotypes of <i>B. boddarti</i> from three populations in mangrove areas of Peninsular Malaysia	111

5.7	The mtDNA <i>COI</i> gene haplotype network of <i>B. boddarti</i> from three populations in Peninsular Malaysia	113
5.8	Mismatch distribution of <i>B. boddarti</i> for different geographical regions and whole population	117
6.1	The result of Agilent 2100 Analysis whereby RIN number >6.8 for every >20 µl of RNA and >50 ng/µl was strongly recommended for NGS analysis	128
6.2	Library construction and quality assessment of <i>B. boddarti</i>	129
6.3	Workflow for library preparation and sequencing of <i>B. boddarti</i>	130
6.4	The analysis workflow for data without a reference genome	131
6.5	The overview of Trinity (a) Inchworm assembly, (b) Chrysalis pools contigs and Butterfly module	132
6.6	The number of distribution for different microsatellite motifs in <i>B. boddarti</i> transcriptome	139
6.7	Distribution pattern of SSR motifs	140
6.8	The classification of genes containing microsatellite locus based on the Gene ontology (GO) annotation	142
6.9	Distribution of genes containing microsatellites loci in 32 pathways	143
6.10	Fragment analysis result of observed heterozygosity in <i>B. boddarti</i> using Izz 4-6 primer	146
6.11	Null alleles across loci generated by Microchecker	147
6.12	Population structure of three <i>B. boddarti</i> populations in Peninsular Malaysia	154

LIST OF ABBREVIATIONS

°	Degree
°C	Degree Celsius
&	And
%	Percentage
=	Equal
µm	Micrometer
-th	is a suffix which forming ordinal and fractional numbers
™	Trade mark
π	Nucleotide diversity
16S	rRNA(16S)
A	Ampere
AFLP	Amplified fragment length polymorphism
AMOVA	Molecular variance
ANOVA	One-way analysis of variance
<i>Ar</i>	Allele richness
bp	Base pair
BOLD	Barcode of Life Data System
BSP	Bayesian Skyline Plots
cytb	Cytochrome <i>b</i>
<i>COI</i>	Cytochrome <i>c</i> oxidase I gene
dbEST	Division of GenBank containing EST
DNA	Deoxyribnucleotide
dNTP	Deoxynucleotide
<i>E</i>	Evenness Index
<i>et al.</i>	And others
EPP	Extrapair paternity
EST	Expressed sequence tags
FIASCO	Fast isolation by AFLP of sequences containing repeats
<i>F_{is}</i>	The inbreeding coefficient
<i>F_s</i>	F-statistic

F_{st}	Fixation index
h	Haplotype diversity
H	Shannon–Wiener diversity index
H_e	Expected heterozygosity
HKY+G	Hasegawa-Kishino-Yano model
H_o	Observed heterozygosity
HWE	Hardy Weinberg equation
ISSR	Inter-simple Sequence Repeat
K2P	Kimura 2-Parameter
LD	Linkage disequilibrium
N/A	Not available
N/n	Total number of individuals caught
NCBI	National Center for Biotechnology Information
N_e	Effective population size
NGS	Next Generation Sequencing
NJ	Neighbour-joining
N_m	Gene flow
m	Meter
M	Molar
MCMC	Markov Chain Monte Carlo
ML	Maximum likelihood
mm	Milimeter
mM	Milimolar
MSN	Minimum Spanning Network
mtDNA	Mitochondrial DNA
P	Probability
PCR	Polymerase chain reaction
PIMA	PCR-based isolation of microsatellite arrays
RAMPO	Random-amplified microsatellite polymorphisms
RAMS	Randomly amplified microsatellites
RAPD	Random Amplified Polymorphic DNA
RFLP	Restriction Fragment Length Polymorphism

RNase	An enzyme to promote RNA breakdown
s	Second
SE	Standard error
SNP	Single nucleotide polymorphisms
SSR	Simple sequence repeat
Ta	Temperature of annealing
TrN	Tamura Nei model
UPGMA	Unweighted pair-group using arithmetic averages
V	Volt
VNTR	Variable number of tandem repeat
X	Times
xg	Earth gravitational force

CHAPTER 1

INTRODUCTION

1.1 Background of the study

Integrative identification of fish larvae

Mangrove forest is one of the vegetation in Malaysia which harbours variety kind of plants and animals. Throughout this study, focus on fish larvae identification and the population genetic studies of a mangrove resident fish the Blue-spotted mudskipper, *Boleophthalmus boddarti* will be the main highlights. The selection of specimen for this study was based on their importance in reflecting the current mangrove ecosystem status in general and on their own importance towards the ecosystem, in particular. Selection of mangrove ecosystem as sampling sites was based on the ample amount of mangrove forest available in Malaysia (505,382 ha) which automatically ranked as the second largest surface area covered with mangrove forest among Asian countries (Sandilyan and Kathiresan, 2014). About 99,180 ha of mangrove area was distributed in Peninsular Malaysia (Tan, 2007), and the rest of them were in Sabah and Sarawak. Mangrove is a productive area used by aquatic animals, especially fish.

Three hypotheses to explain on high abundance of fish in mangrove areas have been proposed (Laegdsgaard and Johnson, 2001; Wang *et al.*, 2009) with first hypothesis was on the predator refuge hypothesis, where the presence of mangrove pneumatophores and prop roots provide hiding place for the juvenile and fish larvae who migrated into vegetated mangrove areas especially at the areas where the trees was surrounded by water. The second hypothesis was on feeding where the aquatic organisms was more attracted to an area with ample amount of food available in the mangrove due to the presence of benthic fauna (Chong *et al.*, 1990; Laegdsgaard and Johnson, 2001); the third hypothesis was stressing on the huge spread of mangrove areas along the coastline offering wide space for the mangrove migrant to reside (Hajisamae *et al.*, 2006).

The newly hatched fish eggs and fish larvae tend to reside in a habitat that provide high food sources, low predation rate and stable ocean conditions for growth (Frietas and Muelbert, 2004). They also generally reside in shallow waters as larger predators will usually avoid such environments (Ara *et al.*, 2011a). With the notion of ample fish larvae in mangrove areas, it is important for the stakeholders to properly recognize the exact species acquiring the

habitat. This is to ensure the wellbeing of fish larvae is intact despite any anthropogenic activities around the habitat.

However the morphological identification of fish larvae can be a huge challenge with the minimal amount of taxonomic key available and the rapid development of larva to juvenile stage. Other than that, it also requires considerable skills and taxonomic expertise. Traditionally, larvae identification has always used morphological characters such as body shape, pigmentation, meristic count and measurements. Nevertheless, the presence of rare and cryptic species which are morphologically similar but genetically distinct has limited the ability for traditional method such as morphology in the identification process (Ko *et al.*, 2013). Furthermore, the different levels of expertise and capabilities among fish larvae taxonomist has make it as a dependent variable in fish larvae morphological identification process. Due to these concerns, adoption of molecular method in identification of fish larvae has been integrated in this study.

A molecular method called DNA barcoding has shed a new light in identification process of animal across many taxa. The utilization of the mitochondrial DNA especially the cytochrome c oxidase I (COI) gene as a marker in the identification process was found fitting as they are present in almost all eukaryotes and have higher evolution rates than nuclear genes hence confirms it is best to discriminate between closely related taxa (Hebert *et al.*, 2003). It was not only help in resolving animal classification but also plays an important role in identification of fish larvae as the early stage of fish are mostly unknown and the fish larvae of different fish are usually quite similar (Leis and Carson-Ewat, 2000) especially with the available records for public viewing are 135,627 fish barcode sequences, representing 10,757 fish species in the Barcode of Life Data System (BOLD). This platform provides an independent means of testing the validity of existing taxonomic systems, revealing cases of inappropriate synonymy or overlooked taxa (Krishna *et al.*, 2012). However, the introduction of DNA barcoding by Hebert *et al.* (2003) was with the intention to help solve species identification conflicts using mitochondrial genes without any means to undermine the work of taxonomist as claimed by Ebach and de Carvalho (2010).

The Blue-spotted mudskipper, *Boleophthalmus boddarti* as claimed earlier, a resident fish in mangrove ecosystem were also amphibious where they are well adapted to survive outside water. The ability of them to not only swim, but also walk and skip (Ravi, 2005) might offer interesting information regarding their genetic relationship in between population both at inter- and intrapopulation level. The relationship among and within population of marine organism was worth to be studied as they was found to have high dispersal potential due to the lack of barriers in the ocean hence enable the fish larvae to disperse long distance via ocean currents (Tenggardjaja *et al.*, 2016).

Both nuclear marker and mitochondrial marker were applied for this assessment as more information is expected to be seen from two kinds of markers. Both markers were actually functionally interdependent as most mitochondrial proteins are encoded by nuclear genes. However, due to maternal inheritance, the effective population size for mitochondrial genes is approximately one-quarter that for nuclear genes. Mitochondrial genes are expected to detect lower mutation rates and selection pressure meanwhile higher divergences will be seen between populations as compared to nuclear genes (Castro *et al.*, 1998).

1.2 Problem statement

The reduction in mangrove forest could have a significant impact on the levels of diversity of mangrove fish residents since the mangrove ecosystem act as an important nursery ground to many aquatic organism especially fish larvae. The acknowledgement of their existence will be better when they are identified to species level as proper conduct can be alerted to stakeholders. However, the lack of fish larvae identification keys and the possibility of changes in characteristics due to preservation such as pigmentation have limited the ability to identify the fish larvae to species level. Therefore, the limitation in morphological-based identification systems and the dwindling pool of taxonomist signal the need for a new approach to taxon recognition, for instance DNA-based identification. The mitochondrial gene of the cytochrome c oxidase subunit I (*COI*) which was maternally inherited and possessed by all livings in the world have made identification of fish larvae to species level possible. Through DNA barcoding system based on the sequence diversity and robust barcoding data available, it was found as an effective tool in differentiating animal taxa and especially in solving rare and cryptic species.

The population genetic study of a mudskipper fish is able to reflect population mixing and connectivity among locations. Thus by assessing the genetic diversity, genetic polymorphism and demographic history through mitochondrial and microsatellite markers will help uncover the evolutionary process of *Boleophthalmus boddarti* such as selection, local adaptation and genetic drift. The lack of specific microsatellite marker for *B. boddarti* justifies the selection of the species for new set of markers to be developed. At the same time the findings will also reflect on the health of their habitat as the Blue-spotted mudskipper is known as mangrove resident fish and ecosystem having high tolerance to pollutants made them suitable to act as bio-indicator to the mangrove.

1.3 Significant of the study

Applying the comparative method coupled with the molecular method in identifying fish larvae opens up a possibility to solve the unsolved cases of the unknown identity of an organism. The taxonomic keys helps determine the identity of a larva specimen based on its morphological features and with the utilization of the mitochondrial DNA especially the cytochrome c oxidase I (COI) gene allows scientists to further confirms the identity of not only a fully formed organism but also organisms at any stage of development including the egg, which was previously was inadequate in some cases, through the conventional method alone. At the same time, determining patterns and distribution of genetic variation within a species is a key step in understanding the evolutionary legacy. However, until the advent of molecular genetic markers, genetic structure was difficult, if not impossible, to be determined in natural populations. The discovery of fish larvae species in the mangrove areas and the characterization of genetic structure of a fish population provides key information useful in managing genetic stock; conservation efforts, estimation of sustainable harvest levels, proper management to avoid a population crash that would be detrimental to the harvesting industry and most importantly to help maintain population genetic diversity.

1.4 Objectives

1. To identify fish larvae using morphological characters and to quantify the diversity and distribution of fish larvae.
2. To apply DNA Barcoding method for fish larvae identification and phylogenetic comparison with their adult congeners
3. To develop and characterize microsatellite primers for Blue-spotted mudskipper, *Boleophthalmus boddarti*.
4. To examine patterns of genetic variation and population connectivity among Blue-spotted mudskipper using microsatellite markers developed from objective 3 and based on mitochondrial marker.

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LIST OF PUBLICATIONS

Journal Paper

Azmir, I. A., Esa, Y., Nurul Amin, S.M., Md Yasin, I.S., Md Yusuf, F.Z. (2017). Identification of Larval Fish in Mangrove Areas of Malaysia Using Morphology and DNA Barcoding Methods. *Journal of Applied Ichthyology*., 33:998-1006.

Conferences and Workshop participation

1. Participated in a Larval Fish Identification Workshop at Marine Science Center (COMAS), Universti Putra Malaysia, Teluk Kemang Port Dickson Negeri Sembilan on April 2015.
2. Participated in a poster presentation International Agricultural Congress 2016 (IAC) on 4th – 6th October 2016 in Bangi, Selangor. Poster title: Larval Fish: Identified Through Morphology and DNA Barcoding Methods.



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