

STUDIES ON THE POPULATION STRUCTURE OF HAWKSBILL
TURTLE (*Eretmochelys imbricata*) FROM INDONESIA
USING MITOCHONDRIAL DNA (mtDNA) CONTROL
REGION SEQUENCES

SUFFIAN BIN MUZAHAR

FACULTY OF MARITIME STUDIES AND MARINE SCIENCE
UNIVERSITI MALAYSIA TERENGGANU

2008

**STUDIES ON THE POPULATION STRUCTURE OF HAWKSBILL TURTLE
(*Eretmochelys imbricata*) FROM INDONESIA USING MITOCHONDRIAL DNA
(mtDNA) CONTROL REGION SEQUENCES**

By

Suffian Bin Muzahar

**Research Report submitted in partial fulfillment of
the requirements for the degree of
Bachelor of Science (Marine Biology)**

**Department of Marine Science
Faculty of Maritime Studies and Marine Science
UNIVERSITI MALAYSIA TERENGGANU
2008**

This project should be cited as:

Suffian Bin Muzahar. 2008. Studies on the Population Structure of Hawksbill Turtle (*Eretmochelys imbricata*) from Kimar, Indonesia using MtDNA Control Region Sequences. Undergraduate thesis, Bachelor of Science (Marine Biology), Faculty of Maritime Studies and Marine Science, University Malaysia Terengganu, Terengganu.

No part of this project report may be reproduced by any mechanical, photographic or electronic process, or in the form of phonographic recording, nor may it be stored in a retrieval system, transmitted, or otherwise copied for public or private news, without written permission from the author and the supervisors of the project.

1100061870



JABATAN SAINS MARIN
FAKULTI PENGAJIAN MARITIM DAN SAINS MARIN
UNIVERSITI MALAYSIA TERENGGANU

**PENGAKUAN DAN PENGESAHAN LAPORAN
PROJEK PENYELIDIKAN I DAN II**

Adalah ini diakui dan disahkan bahawa laporan penyelidikan bertajuk:

**Studies on the Population Structure of Hawksbill Turtle (*Eretmochelys imbricata*)
From Indonesia using Mitochondrial DNA (mtDNA) Control Region Sequences**

Oleh **Suffian Bin Muzahar**, No.Matrik **UK11835** telah diperiksa dan semua pembetulan yang disarankan telah dilakukan. Laporan ini dikemukakan kepada Jabatan Sains Marin sebagai memenuhi sebahagian daripada keperluan memperoleh Ijazah Sarjana Muda Sains (Biologi Marin), Fakulti Pengajian Maritim dan Sains Marin, Universiti Malaysia Terengganu.

Disahkan oleh:

.....
Penyelia Utama

Nama: Dr. Juanita Joseph

Cop Rasmi: **DR. JUANITA JOSEPH**
Pensyarah
Jabatan Sains Marin
Fakulti Pengajian Maritim dan Sains Marin
Universiti Malaysia Terengganu
(UMT)

Tarikh: 4/5/2008

.....
Ketua Jabatan Sains Marin

Nama:

Cop Rasmi:

Tarikh:

ACKNOWLEDGEMENTS

First and foremost I would like to extend my deepest gratitude to Allah s.w.t because without the blessing from Allah, this project would not be successful. Allah had also guided me throughout the whole process and provided me with faith so that I could finish all the given works in the scheduled time.

Next, I would like to express my sincere gratitude to my Supervisor, Dr. Juanita Joseph for all her guidance during the whole process of completing my project. All her guidance and constructive suggestion had helped me throughout my difficult time trying to finish up the entire project successfully.

Samples used in this project were provided from South East Asian Fisheries Development Center (SEAFDEC). For that I would like to express my sincere appreciation to all the personnel in SEAFDEC that had collected and preserved the samples, especially personnel from Indonesia. SEAFDEC had also kindly funded this project and lend their laboratory for my convenience, and for that I'm very grateful for them. Not forgetting to Puan Wahidah Mohd Arshaad, my second supervisor and also a research officer from SEAFDEC. I would like to thank her because she had helped me whenever I needed it. Without her help, I would never have the chance to complete my final year project.

I would also like to thank my friends who had supported me and helped me along the way. My course mates, housemates and my best friends had always showered me with words of encouragements and never-ending support whenever I needed them. Without them, I am nobody.

Next, I would also like to extend my love and gratitude to my family; my mom, dad and all my siblings that had supported me morally and also financially throughout the whole process. Thanks for their prayers and love, I had managed to finish up my project successfully.

Last but not least, I would like to thank Universiti Malaysia Terengganu; all lecturers, authorized personnel and everyone that had help me along the way not just during the course of completing the project but also through the whole three years I am here. Thank you all.

Suffian Bin Muzahar

March 2008

TABLE OF CONTENTS

| | PAGE |
|---|-------------|
| ACKNOWLEDGEMENTS | i |
| LIST OF TABLES | v |
| LIST OF FIGURES | vi |
| LIST OF ABBREVIATIONS | vii |
| ABSTRACT | viii |
| ABSTRAK | ix |
| | |
| CHAPTER | |
| 1.0 INTRODUCTION | 1 |
| | |
| 2.0 LITERATURE REVIEW | 5 |
| 2.1 General biology of sea turtle | 5 |
| 2.2 Biology of hawksbill turtle (<i>Eretmochelys imbricata</i>) | 7 |
| 2.3 Molecular genetic study | 11 |
| 2.3.1 Genetic markers | 11 |
| 2.3.2 Mitochondrial DNA (mtDNA) | 12 |
| 2.3.3 Polymerase Chain Reaction (PCR) | 13 |
| 2.3.4 Samples | 14 |
| | |
| 3.0 METHODOLOGY | 16 |
| 3.1 Sample collection | 16 |
| 3.2 Laboratory analysis | 18 |
| 3.2.1 Deoxyribonucleic Acid (DNA) extraction | 18 |
| 3.2.2 PCR | 18 |
| 3.2.3 Gel electrophoresis | 20 |
| 3.2.4 PCR cleanup | 20 |
| 3.2.5 Sequencing | 22 |

| | | |
|------------|--|----|
| 3.3 | Statistical analysis | 22 |
| 4.0 | RESULTS | 24 |
| 4.1 | Laboratory analysis | 24 |
| 4.1.1 | DNA extraction | 24 |
| 4.1.2 | PCR | 26 |
| 4.1.3 | Sequencing | 27 |
| 4.2 | Data analysis | 28 |
| 4.2.1 | Haplotypic diversity of the mitochondrial control region | 28 |
| 4.2.2 | Phylogenetic relationship | 31 |
| 4.2.3 | Population structure | 33 |
| 5.0 | DISCUSSION | 34 |
| 5.1 | DNA extraction | 34 |
| 5.2 | mtDNA amplification | 34 |
| 5.3 | Haplotype diversity | 35 |
| 5.4 | Phylogenetic relationship | 36 |
| 5.5 | Population structure | 37 |
| 5.6 | Conservation relevance | 38 |
| 6.0 | CONCLUSION | 40 |
| | REFERENCES | 42 |
| | APPENDIX A | 48 |
| | CURRICULUM VITAE | 50 |

LIST OF TABLES

| TABLE | | PAGE |
|-------|---|------|
| 1 | Polymorphic sites in the 380 base pair sequence, defining 4 haplotypes recorded for the hawksbill turtle samples in Kimar, Belitung, Indonesia. | 28 |
| 2 | Haplotype frequencies for hawksbill turtle nesting beaches in Southeast Asia. Data for Malaysia were from Joseph (2006). | 29 |
| 3 | Haplotype (h) and nucleotide (π) diversities for hawksbill turtle samples from this study (Indonesia) and from Joseph, 2006 (Malaysia). | 30 |
| 4 | Pairwise tests for genetic differentiation among hawksbill turtle samples from Southeast Asia | 33 |

LIST OF FIGURES

| FIGURE | | PAGE |
|--------|---|------|
| 1 | Adult hawksbill turtle | 7 |
| 2 | Characteristics of hawksbill turtle (Pritchard and Mortimer, 1999) | 8 |
| 3 | Map of sampling location. | 17 |
| 4 | Schematic diagram of CTAB protocol (modified from Bruford <i>et al.</i> 1992). | 19 |
| 5 | Step by step diagram of commercial PCR purification kit (Favorgen). | 21 |
| 6 | Successful DNA extraction using CTAB protocol (Bruford <i>et al.</i> 1992). | 24 |
| 7 | Successful DNA extraction using CTAB protocol (Bruford <i>et al.</i> 1992). | 25 |
| 8 | PCR products of Hawksbill Turtle amplified by PCR 5 and 6. | 26 |
| 9 | PCR products of Hawksbill Turtle amplified by PCR 5 and 6. | 27 |
| 10 | Neighbour-joining tree of Kimura 2-parameter distance for the Southeast Asia hawksbill turtles based on 16 mtDNA control region sequences, rooted using four hawksbill turtle haplotypes from the Caribbean (Bass <i>et al.</i> 1996) as an outgroup. | 32 |

LIST OF ABBREVIATIONS

| | |
|-------------------|---------------------------|
| bp | Base pair |
| DNA | Deoxyribonucleic Acid |
| MgCl ₂ | Magnesium Chloride |
| ml | Milliliter |
| mm | Millimeter |
| mM | Millimolar |
| mtDNA | Mitochondrial DNA |
| nm | Nanometer |
| °C | Degree celcius |
| PCR | Polymerase Chain Reaction |
| STIP | Sabah Turtle Island Park |
| μl | Microliter |
| V | Volt |

ABSTRACT

This is a collaboration of University Malaysia Terengganu (UMT) with South East Asian Fisheries Development Center (SEAFDEC). Genetic diversity of hawksbill turtle (*Eretmochelys imbricata*, Linnaeus, 1970) population from Kimar, Belitung, Indonesia was analyzed using mitochondrial DNA (mtDNA) control region sequences. All samples were amplified using Polymerase Chain Reaction (PCR) method and were electrophoresed using agarose gel. The banding patterns of the amplified products were then visualized using UV light and purified using PCR purification kit before sending all the samples for sequencing. The sequenced data were analyzed using CHROMAS 2.33, ESEE3, Arlequin 3.11 and MEGA 3.0. Other samples from previous study done (Joseph, 2006) were also analyzed along with this study. Pairwise F_{ST} tests were conducted to obtain the population structure. Besides that, neighbour-joining tree of Kimura 2-parameter was also constructed to express the phylogenetic relationship between population. Extra haplotypes from the Caribbean based on the study by Bass *et al.* (1996) were obtained from Genbank to be rooted to the tree for comparison. Haplotype diversity (h) of the Kimar population was 0.60 and nucleotide diversity of 0.0096. This suggests that there is still hope to conserve the hawksbill turtles in Kimar despite of reported 88% population decline. Pairwise tests showed that nesting population from Kimar, Indonesia was genetically distinct compare to other nesting populations from Malaysia. This result was also supported by Neighbour-joining tree of Kimura 2-parameter.

**KAJIAN STRUKTUR POPULASI PENYU KARAH (*Eretmochelys imbricata*)
DARI KIMAR, INDONESIA MENGGUNAKAN KAEDAH KAWASAN
KAWALAN URUTAN DNA MITOKONDRIA (MTDNA)**

ABSTRAK

Kajian ini merupakan hasil kerjasama bersama Pusat Perkembangan Perikanan Asia Tenggara (SEAFDEC). Kepelbagaian genetik populasi penyu karah (*Eretmochelys imbricata*, Linnaeus, 1970) dari Kimar, Belitung, Indonesia telah dikaji menggunakan kawasan kawalan urutan DNA mitokondria (mtDNA). Sampel – sampel DNA diamplifikasi menggunakan teknik “Polymerase Chain Reaction” (PCR) dan seterusnya dielektroforesiskan menggunakan gel Agarose. Corak jalur yang terhasil dari produk amplifikasi kemudiannya dilihat menggunakan cahaya sinar UV dan dicuci dengan kit cucian PCR sebelum dihantar untuk disusun. Data yang telah disusun seterusnya dianalisis menggunakan pelbagai jenis aplikasi. Sampel – sampel dari kajian terdahulu (Joseph, 2006) juga digunakan sebagai perbandingan. Ujian “Pairwise F_{ST} ” dijalankan untuk melihat struktur populasi penyu karah. Selain itu “neighbour-joining tree” juga dibina berdasarkan parameter Kimura-2 untuk menunjukkan hubungan filogeni di antara populasi. Haplotip tambahan dari Caribbean berdasarkan kajian oleh Bass *et al.* (1996) juga diambil dari Genbank untuk digunakan sebagai asas kepada perbandingan populasi tersebut. Kepelbagaian haplotip (h) untuk populasi dari Kimar adalah 0.06 dan kepelbagaian nukleotid adalah 0.0096. Ini menunjukkan bahawa masih terdapat harapan untuk menyelamatkan populasi tersebut biarpun terdapat penurunan populasi sebanyak 88% dilaporkan. Ujian “Pairwise F_{ST} ” menunjukkan populasi dari Kimar, Indonesia adalah berbeza dari segi genetik berbanding populasi dari Malaysia.