SPECIES RICHNESS AND GENETIC DIVERSITY OF THE *ACROPORA* FAUNA ALONG THE NORTH-WEST COAST OF SABAH

ROLANDO ROBERT

PERPUSTAKAAN
UNIVERSITI MALAYSIA SABAH

UMS
UNIVERSITI MALAYSIA SABAH

THESIS SUBMITTED IN FULFILLMENT FOR
THE DEGREE OF MASTER OF SCIENCE

BIOTECHNOLOGY RESEARCH INSTITUTE
UNIVERSITI MALAYSIA SABAH
2017

UNIVERSITI MALAYSIA SABAH

BORANG PENGESAHAN STATUS TESIS

JUDUL : **SPECIES RICHNESS AND GENETIC DIVERSITY OF THE *ACROPORA* FAUNA ALONG THE NORTH-WEST COAST OF SABAH**

IJAZAH : **MASTER OF SCIENCE (BIOTECHNOLOGY)**

Saya **ROLANDO ROBERT**, sesi **2013 – 2014**, mengaku membenarkan tesis Sarjana ini disimpan di Perpustakaan Universiti Malaysia Sabah dengan syarat-syarat kegunaan seperti berikut:

1. Tesis ini adalah hak milik Universiti Malaysia Sabah.
2. Perpustakaan Universiti Malaysia Sabah dibenarkan membuat Salinan untuk tujuan pengajian sahaja.
3. Perpustakaan dibenarkan membuat salinan tesis ini sebagai bahan pertukaran antara institusi pengajian tinggi.
4. Sila tandakan (✓):

☐ SULIT (Mengandungi maklumat yang berdarjah keselamatan atau kepentingan Malaysia seperti yang termaktub di dalam AKTA RAHSIA 1972)

☐ TERHAD (Mengandungi maklumat TERHAD yang telah ditentukan oleh organisasi/badan di mana penyelidikan dijalankan)

☑ TIDAK TERHAD

PERPUSTAKAAN
UNIVERSITI MALAYSIA SABAH
UMS
UNIVERSITI MALAYSIA SABAH

Disahkan oleh,

NURULAIN BINTI ISMAIL
LIBRARIAN
UNIVERSITI MALAYSIA SABAH

ROLANDO ROBERT
MZ1321007T

(Tandatangan Pustakawan)

Tarikh: 17 Ogos 2017

(Prof. Madya. Dr. Vijay Kumar)
Penyelia

DECLARATION

I hereby declare that the materials in this thesis are my own except for photographs, excerpts, equations, summaries, and references, which have been duly acknowledged.

17 August 2017

....

Rolando Robert

MZ1321007T

UMS
UNIVERSITI MALAYSIA SABAH

CERTIFICATION

NAME : **ROLANDO ROBERT**

MATRIC NO. : **MZ1321007T**

TITLE : **SPECIES RICHNESS AND GENETIC DIVERSITY OF THE *ACROPORA* FAUNA ALONG THE NORTH-WEST COAST OF SABAH**

DEGREE : **MASTER OF SCIENCE (BIOTECHNOLOGY)**

DATE OF VIVA : **15 JUN 2017**

CERTIFIED BY;

1. **MAIN SUPERVISOR**
   ASSOC. PROF. DR. VIJAY KUMAR

Signature

UMS
UNIVERSITI MALAYSIA SABAH

PROF. MADYA DR. VIJAY KUMAR
PROFESOR MADYA
INSTITUT PENYELIDIKAN BIOTEKNOLOGI
UNIVERSITI MALAYSIA SABAH

2. **CO-SUPERVISOR**
   ASSOC. PROF. DR. KENNETH FRANCIS RODRIGUES

ASSOC. PROF. DR. KENNETH FRANCIS RODRIGUES
Assoc. Prof.
Biotechnology Research Institute
Universiti Malaysia Sabah

ACKNOWLEDGEMENTS

Coming up with this thesis had been a huge undertaking for me and I could not have done it without the help of many, of which only a few I am able to mention here. First and foremost, I would like to thank my supervisor and mentor, Assoc. Prof. Dr. Vijay Kumar and co-supervisor, Assoc Prof. Dr. Kenneth Rodrigues who generously provided the means and the training in various aspects of becoming a research scientist — most importantly, for tolerating my tendency to procrastinate (usually by doing something else seemingly productive) and to go off on a tangent. This study was performed in conjunction with the bioprospecting works for the Discovery of the Next Generation of Fluorescent Proteins (Grant ID: GL00110), an endeavour funded by the Sabah State Government through Sabah Biodiversity Centre (SaBC) and Protein Technologies Limited (PTL); special thanks are in order for Dr. Abdul Fatah bin Amir, the Director of SaBC and Dr. Farid Khan, principal investigator of PTL UK. Assistance in the laboratory was provided by technicians Azian, Emran, Moni, and Vida as well as Dr. Chee Wei, Dexter, Fernandes, Dr. Hydayaty, Sadat, Salahauddin, Tyng, and Zarifi. Field assistants Amira, Matt, Shahniza, Suet Mun, and Firdaus participated in the sample collection while Banggi Youth Club and UMS Boathouse provided logistical support in the field. The contributions by former directors and the current (acting) director of the Biotechnology Research Institute, Assoc. Prof. Dr. Vijay, Prof. Michael Wong, and Dr. Zarinah Amin along with supporting staffs in providing a conducive environment for performing research are duly acknowledged.

Rolando Robert
17 August 2017

UMS
UNIVERSITI MALAYSIA SABAH

ABSTRACT

Staghorn corals (*Acropora* Oken, 1815) are the most abundant reef-building corals in Sabah but current knowledge on their species richness is deficient at many localities while baseline data on their genetic diversity are non-existent. In this study, 122 staghorn coral individuals were sampled from coral reefs around Pulau Tiga Park, P. Mantanani, and P. Banggi, Sabah using SCUBA, following which taxonomic assignment was conducted by examining their skeletal morphology. Afterwards, 50 (N) representative individuals were subjected to DNA barcoding using nucleotide sequences of the partial cytochrome *c* oxidase subunit-I (*COI*) and cytochrome *b* (*CYB*), and multi-locus microsatellite genotyping using 11 universal markers containing tri-, tetra-, penta-, and hexa-nucleotide repeat motifs. A total of 33 different species were identified from the three localities of which 14 occur in P. Tiga Park, 26 in P. Mantanani, and 22 in P. Banggi. Two species, *Acropora dendrum* and *A. desalwii*, are reported for the first time in Sabah. Meanwhile, the 50 individuals barcoded and genotyped represent the 33 identified species and a morphotype designated as *Acropora* sp. Substitution rate of the staghorn coral *CYB* (mean p-distance: 0.38%) was faster than the substitution rate of the *COI* (mean p-distance: 0.24%), while the value recorded for the *COI-CYB* was the average of these two rates (mean p-distance: 0.32%). Subsequently, haplotype diversity (H_d) with respect to the *CYB* sequences (number of haplotypes: 36; H_d ± S.D. = 0.91 ± 0.04) was higher than that with respect to the *COI* sequences (number of haplotypes: 25; H_d ± S.D. = 0.73 ± 0.07). The number of *COI-CYB* haplotypes was the highest at 42 (H_d ± S.D. = 0.97 ± 0.02), as more nucleotide substitutions were captured. Consequently, the phylogeny of the staghorn corals inferred using the *COI-CYB* sequences exhibited the best resolution but all the trees reconstructed shared a common multifurcating topology, which suggested a polyphyletic evolutionary pattern among the staghorn corals. The discriminatory power of the DNA barcodes currently employed were therefore inadequate for intrapopulation studies, which underlined the potential of the hypervariable microsatellite markers for such investigations. The microsatellite markers used in this study were polymorphic with number of alleles ranging from seven to 23 per locus (mean = 13.5). Estimates of genotypic diversity suggested a state of heterozygote deficit within the Sabah population as the level of observed heterozygosity (H_o = 0.39 ± 0.14) was over two-fold lower in contrast to the levels expected (H_e = 0.84 ± 0.08; Nei's H_e = 0.83 ± 0.08). The lack of genotypic diversity within the Sabah staghorn corals could signal lowered adaptive capacity of the population to extreme changes in their environment that warrants urgent conservation measures. The results presented here further add to current knowledge on the species richness of staghorn corals in Sabah and represent the first quantitative baseline data on their genetic diversity.

UMS
UNIVERSITI MALAYSIA SABAH

ABSTRACT

Staghorn corals (*Acropora* Oken, 1815) are the most abundant reef-building corals in Sabah but current knowledge on their species richness is deficient at many localities while baseline data on their genetic diversity are non-existent. In this study, 122 staghorn coral individuals were sampled from coral reefs around Pulau Tiga Park, P. Mantanani, and P. Banggi, Sabah using SCUBA, following which taxonomic assignment was conducted by examining their skeletal morphology. Afterwards, 50 (*N*) representative individuals were subjected to DNA barcoding using nucleotide sequences of the partial cytochrome *c* oxidase subunit-I (*COI*) and cytochrome *b* (*CYB*), and multi-locus microsatellite genotyping using 11 universal markers containing tri-, tetra-, penta-, and hexa-nucleotide repeat motifs. A total of 33 different species were identified from the three localities of which 14 occur in P. Tiga Park, 26 in P. Mantanani, and 22 in P. Banggi. Two species, *Acropora dendrum* and *A. desalwii*, are reported for the first time in Sabah. Meanwhile, the 50 individuals barcoded and genotyped represent the 33 identified species and a morphotype designated as *Acropora* sp. Substitution rate of the staghorn coral *CYB* (mean *p*-distance: 0.38%) was faster than the substitution rate of the *COI* (mean *p*-distance: 0.24%), while the value recorded for the *COI-CYB* was the average of these two rates (mean *p*-distance: 0.32%). Subsequently, haplotype diversity (H_d) with respect to the *CYB* sequences (number of haplotypes: 36; H_d ± S.D. = 0.91 ± 0.04) was higher than that with respect to the *COI* sequences (number of haplotypes: 25; H_d ± S.D. = 0.73 ± 0.07). The number of *COI-CYB* haplotypes was the highest at 42 (H_d ± S.D. = 0.97 ± 0.02), as more nucleotide substitutions were captured. Consequently, the phylogeny of the staghorn corals inferred using the *COI-CYB* sequences exhibited the best resolution but all the trees reconstructed shared a common multifurcating topology, which suggested a polyphyletic evolutionary pattern among the staghorn corals. The discriminatory power of the DNA barcodes currently employed were therefore inadequate for intrapopulation studies, which underlined the potential of the hypervariable microsatellite markers for such investigations. The microsatellite markers used in this study were polymorphic with number of alleles ranging from seven to 23 per locus (mean = 13.5). Estimates of genotypic diversity suggested a state of heterozygote deficit within the Sabah population as the level of observed heterozygosity (H_o = 0.39 ± 0.14) was over two-fold lower in contrast to the levels expected (H_e = 0.84 ± 0.08; Nei's H_e = 0.83 ± 0.08). The lack of genotypic diversity within the Sabah staghorn corals could signal lowered adaptive capacity of the population to extreme changes in their environment that warrants urgent conservation measures. The results presented here further add to current knowledge on the species richness of staghorn corals in Sabah and represent the first quantitative baseline data on their genetic diversity.

UMS
UNIVERSITI MALAYSIA SABAH

ABSTRAK

KEKAYAAN SPESIS DAN KEPELBAGAIAN GENETIK FAUNA ACROPORA DI SEPANJANG PANTAI BARAT LAUT SABAH

Karangan Staghorn *(Acropora Oken, 1815) adalah komponen utama terumbu karang di Sabah namun maklumat mengenai kekayaan species kumpulan tersebut adalah masih kurang di kebanyakkan tempat manakala kadar kepelbagaian genetik karang* staghorn *di Sabah belum lagi dikaji. Dalam kajian ini, 122 individu karang* staghorn *telah disampel melalui kerja-kerja penyelaman di kawasan terumbu karang di sekitar Taman Pulau Tiga, P. Mantanani, dan P. Banggi. Spesis sampel-sampel tersebut telah dikenalpasti melalui diagnosis morfologi. Jujukan nukleotida sitokrom c oksidase subunit-I (COI) dan sitokrom b (CYB) telah dipilih sebagai lokus-lokus* DNA barcoding *manakala sebelas penanda mikrosatelit universal yang mengandungi motif berulang tri-, tetra-, penta-, dan heksa-nukleotida telah digunakan untuk pengenotipan karang* staghorn *tersebut. Sebanyak 33 spesis telah dikenalpasti di ketiga-tiga lokaliti tersebut di mana 14 ditemui di Taman P. Tiga, 26 di P. Mantanani, dan 22 di P. Banggi. Dua spesis, Acropora dendrum dan A. desalwii adalah dilaporkan di Sabah buat pertama kalinya. Aplikasi* DNA barcoding *dan pengenotipan mikrosatelit melibatkan sejumlah 50 individu mewakili 33 spesis yang telah dikenalpasti dan satu morfotip dilabel sebagai Acropora sp. Jujukan nukleotida CYB (purata jarak berkadar: 0.38%) karang* staghorn *tersebut bermutasi dengan kadar lebih cepat bebanding dengan COI (purata jarak berkadar: 0.24), manakala kadar bagi jujukan berangkai COI-CYB menunjukkan nilai perantara (0.32%). Sehubungan dengan itu, kepelbagaian haplotip* (H_d) *berdasarkan jujukan CYB (bilangan haplotip: 36;* $H_d = 0.91 \pm 0.04$) *adalah lebih tinggi berbanding kepelbagaian haplotip berdasarkan jujukan COI (bilangan haplotip: 25;* $H_d = 0.73 \pm 0.07$). *Bilangan haplotip COI-CYB adalah paling tinggi (42;* $H_d \pm S.D. = 0.97 \pm 0.02$) *kerana ia merangkumi mutasi di keseluruhan jujukan COI dan CYB setiap individu. Sehubungan dengan itu, anggaran filogeni karang* staghorn *Sabah berdasarkan jujukan COI-CYB mempamerkan resolusi lebih tinggi namun semua filogeni dalam kajian ini mempamerkan corak polifiletik. Sebaliknya, penanda mikrosatelit yang digunakan dalam kajian ini adalah polimorfik, terdiri daripada tujuh ke 23 alel bagi setiap lokus (min = 13.5). Analisis genotip karangan* staghorn *dalam kajian in menunjukkan terdapatnya defisit heterozigot dalam populasi Sabah, di mana tahap heterozigot diperhatikan* ($H_o = 0.39 \pm 0.14$) *adalah dua kali ganda lebih rendah berbanding dengan tahap yang dijangka* ($H_e = 0,84 \pm 0,08$; H_e *Nei* $= 0.83 \pm 0.08$). *Kekurangan kepelbagaian genotip dalam kalangan karangan* staghorn *di Sabah mungkin menandakan bahawa kapasiti populasi tersebut untuk mengadaptasi dalam keadaan persekitaran yang melampau adalah rendah — justeru, langkah-langkah pemuliharaan perlu segera diambil. Hasil kajian yang dibentangkan di sini memapankan lagi pengetahuan semasa mengenai kekayaan spesies karang* staghorn *di Sabah dan ianya mewakili data asas kuantitatif pertama bagi kepelbagaian genetik karangan tersebut.*

UMS
UNIVERSITI MALAYSIA SABAH

LIST OF CONTENTS

Page

TITLE i

DECLARATION ii

CERTIFICATION iii

ACKNOWLEDGEMENTS iv

ABSTRACT v

ABSTRAK vi

LIST OF CONTENTS vii

LIST OF TABLES xi

LIST OF FIGURES xiii

LIST OF SYMBOLS xxi

LIST OF ABBREVIATIONS xxii

LIST OF APPENDICES xxiii

CHAPTER 1: INTRODUCTION 1

1.1 Background 1

1.2 Problem Statement and Rationale 3

1.3 Research Questions and Hypotheses 4

1.4 Aim and Objectives 5

CHAPTER 2: LITERATURE REVIEW 6

2.1 Taxonomy of the Genus *Acropora* 6

    2.1.1 Taxonomically Informative Morphological Characters 7

    2.1.2 Species Richness of Staghorn Corals in Sabah 11

    2.1.3 The Pitfalls of Morphological Taxonomy 17

2.2 The Significance of Molecular Genetics Tools to Study Biodiversity 18

2.3 The Scope of DNA Barcoding 19

UMS
UNIVERSITI MALAYSIA SABAH

Page

2.3.1 General Criteria for the Selection of DNA Barcoding Regions 20

2.3.2 Challenges in DNA Barcoding of Staghorn Corals 21

2.3.3 Successes in Applying DNA Barcoding to Corals: Case Studies 24

a. Inclusion of the Genus *Alveopora* into the Family Acroporidae 24

b. Recognition of Subspecies *Isopora* as a Distinct Genus 25

c. Delimitation of *Acropora prolifera* as a Hybrid Entity 25

2.4 Microsatellite Markers for Population Genetics 27

2.4.1 Microsatellite Markers for Staghorn Corals 28

2.4.2 Potential Issues with Genotyping using Transferable Microsatellite Markers 29

2.4.3 Microsatellite Genotyping of Staghorn Corals 31

2.5 Summary of the Review 32

CHAPTER 3: METHODOLOGY 33

3.1 Collection and Preparation of Samples 33

3.2 Identification of Species and Taxonomic Conventions Observed 37

3.3 Isolation of Genomic DNA and Agarose Gel Electrophoresis 37

3.4 DNA Barcoding of the Staghorn Corals 38

3.4.1 Amplification and Molecular Cloning of the PCR Amplicons 39

3.4.2 Sequencing of the Cloned Inserts 42

3.4.3 Bioinformatics Analyses of the Nucleotide Sequences 42

3.5 Microsatellite Genotyping of the Staghorn Corals 44

3.5.1 Amplification of the Microsatellite Loci and Fragment Analysis 45

3.5.2 Analysis of Data 46

CHAPTER 4: RESULTS 49

4.1 Species Identified and Richness at the Three Localities 49

UMS
UNIVERSITI MALAYSIA SABAH

4.1.1 Updates on the Sabah Species-Richness Records 81

4.2 Genomic DNA and Agarose Gel Electrophoresis 84

4.3 DNA Barcoding of the Staghorn Corals 84

4.3.1 Comparative Sequence Analyses 88

4.3.2 Haplotype Diversity of the DNA Barcoding Loci 93

4.3.3 Phylogenetic Reconstructions 101

4.4 Microsatellite Genotyping of the Staghorn Corals 105

4.4.1 Scoring of Electromorphs and Assignment of Alleles 106

4.4.2 Allelic Polymorphism and Relative Frequency 106

4.4.3 Measures of Heterozygosity and Genotype Frequency 109

4.4.4 Genetic Identity and Distance and Phylogenetic Inference 110

CHAPTER 5: DISCUSSION 115

5.1 Due Considerations in the Sampling Procedure 115

5.2 First Records on the Staghorn Coral Richness at the Three Coral Reef Localities 116

5.2.1 Species Richness Pattern of the Staghorn Corals in Sabah 117

5.2.2 Inference on the Staghorn Coral Richness of Sabah in relation to the Richness Reported Elsewhere 119

5.3 Fitness of *COI* and *CYB* as DNA Barcodes 120

5.3.1 Haplotype Sharing between Congeneric Species of Staghorn Corals in Sabah and Possible Causative Mechanisms 122

5.3.2 Estimating the Phylogeny of the Staghorn Corals of Sabah based on the Slowly Evolving *COI* and *CYB* 124

5.4 Cross-Species Transferability of the Universal Microsatellite Markers for Staghorn Coral 125

5.4.1 Observed Polymorphism of the Microsatellite Electromorphs and Potential Sources of Scoring Errors 127

5.4.2 Size Distribution of the Electromorphs and Predictions on their Mutational Patterns 128

UMS UNIVERSITI MALAYSIA SABAH

Page

5.4.3 Genotypic Diversity of the Sabah Staghorn Corals 129

5.4.4 Phylogeny of the Sabah Staghorn Corals Inferred using the Microsatellite Genotypes 130

CHAPTER 6: CONCLUDING REMARKS 131

6.1 Conclusion 131

6.2 Recommendations for Future Studies 132

REFERENCES 134

APPENDICES 153

UMS
UNIVERSITI MALAYSIA SABAH

LIST OF TABLES

		Page
Table 2.1	Staghorn coral species of Sabah reported in literature and their geographical distribution	12
Table 3.1	Details of the coral reef sites surveyed and the sample size	35
Table 3.2	Details of the DNA barcoding primers	39
Table 3.3	Sequences of the primers used to amplify the microsatellite loci, the repeat motif, and the expected size	45
Table 4.1	Checklist of staghorn coral morphospecies collected from P. Tiga Park, P. Mantanani, and P. Banggi	50
Table 4.2	Localities and voucher accession numbers of the DNA barcoded individuals	85
Table 4.3	Genbank accession numbers of the *COI* and *CYB* nucleotide sequences deposited into the GenBank repository	89
Table 4.4	Polymorphic nucleotide positions defining the 25 haplotypes of the *COI* ($N = 50$). The enumerated positions are relative to the sequences in Figure 4.6. Full stops indicate similarities to the Hap_01 sequence and the position marked with the red rectangle is a parsimony-informative site.	95
Table 4.4b	Haplotype diversity among the staghorn corals based on the *COI* sequences	96
Table 4.5a	Polymorphic nucleotide positions defining the 36 haplotypes of the *CYB* ($N = 50$). Enumerated positions are relative to the sequences in Figure 4.7. Full stops indicate similarities to the Hap_01 sequence and the positions marked with the blue rectangles are parsimony-informative sites.	97
Table 4.5b	Haplotype diversity among the staghorn corals based on the *CYB* sequences	99
Table 4.6	Haplotype diversity among the staghorn corals based on the concatenated *COI–CYB* sequences	100
Table 4.7	Number of alleles scored for each loci and their size distribution	107
Table 4.8	Observed and expected heterozygosity and Nei's (1978) expected heterozygosity	109

UMS UNIVERSITI MALAYSIA SABAH

LIST OF TABLES

		Page
Table 2.1	Staghorn coral species of Sabah reported in literature and their geographical distribution	12
Table 3.1	Details of the coral reef sites surveyed and the sample size	35
Table 3.2	Details of the DNA barcoding primers	39
Table 3.3	Sequences of the primers used to amplify the microsatellite loci, the repeat motif, and the expected size	45
Table 4.1	Checklist of staghorn coral morphospecies collected from P. Tiga Park, P. Mantanani, and P. Banggi	50
Table 4.2	Localities and voucher accession numbers of the DNA barcoded individuals	85
Table 4.3	Genbank accession numbers of the *COI* and *CYB* nucleotide sequences deposited into the GenBank repository	89
Table 4.4	Polymorphic nucleotide positions defining the 25 haplotypes of the *COI* ($N = 50$). The enumerated positions are relative to the sequences in Figure 4.6. Full stops indicate similarities to the Hap_01 sequence and the position marked with the red rectangle is a parsimony-informative site.	95
Table 4.4b	Haplotype diversity among the staghorn corals based on the *COI* sequences	96
Table 4.5a	Polymorphic nucleotide positions defining the 36 haplotypes of the *CYB* ($N = 50$). Enumerated positions are relative to the sequences in Figure 4.7. Full stops indicate similarities to the Hap_01 sequence and the positions marked with the blue rectangles are parsimony-informative sites.	97
Table 4.5b	Haplotype diversity among the staghorn corals based on the *CYB* sequences	99
Table 4.6	Haplotype diversity among the staghorn corals based on the concatenated *COI–CYB* sequences	100
Table 4.7	Number of alleles scored for each loci and their size distribution	107
Table 4.8	Observed and expected heterozygosity and Nei's (1978) expected heterozygosity	109

UMS UNIVERSITI MALAYSIA SABAH

Page

Table 5.1 Species groups of the 33 species of staghorn corals *sensu* Wallace (1999). The groups are arranged from the basal clade (Humilis group) to the most recent clade (Echinata group) 125

UMS
UNIVERSITI MALAYSIA SABAH

LIST OF FIGURES

Page

Figure 2.1 Character states of the coralla which are categorised as: (A) arborescent or tree-like; (B) caespitose or bushy; (C) digitate or finger-like; (D) plate; (E) arboresent-table; (F) corymbose, which resembles a trimmed hedge; (G) hispidose or bottlebrush-like; (H) tabular 8

Figure 2.2 Character states of the radial corallites: (A) tubular, round openings; (B–C) tubular, oblique openings; (D) conical; (E) rounded tubular; (F) appressed tubular; (G–H) tubular, dimidiate openings; (I) nariform, elongate openings; (J) nariform, round openings; (K) appressed tubular; (L) immersed; (M–N) labellate, straight lip; (O–P) labellate, rounded lip; (Q–R) cochleariform 9

Figure 2.3 Categories and grades of the coenosteum development: from (A – B) costate; (C – F) costate with increasing degrees of spinule development; (G) reticulate; (H – J) simple spnules; (K) forked spinules; (L – Q) increasingly elaborated spinules; to (R) meandroid, elaborated spinules 10

Figure 3.1 Localities where samples were collected (marked with red closed circles) 34

Figure 3.2 (A) the researcher on the right and a field assistant during sample collection (photo credit: M. Yusuf B.); (B) schematic of the sampling strategy employed in the sample collection 36

Figure 4.1 *Acropora acuminata* (A) fragment from an arborescent-table colony which comprised of horizontal branches and upwardly curving vertical branches; (B) radial corallites were of two sizes: long, tubular with nariform opening interspersed with sub-immersed radial corallites; (C) an arborescent-table colony in P. Banggi; vouchers acc. nos.: BRI 02.RP008, BRI 02.SB003, BRI 03.MK014 51

Figure 4.2 *Acropora aspera* (A) fragment of a colony with many branchlets emerging from the main branches that gives it a sprawling outline; (B) portion of a branch showing well-spaced radial corallites of mixed size, all of which possess thickened outer walls and underdeveloped inner walls; (C) a juvenile colony in P. Banggi; voucher acc. nos.: BRI 02.SB010, BRI 03.MB006, BRI 03.MB018, BRI 03.SD030 52

UMS UNIVERSITI MALAYSIA SABAH

Page

Figure 4.3 *Acropora carduus* (A) fragment of a colony exhibiting a sprawling hispidose form; (B) radial corallites are single sized: appressed tubular with sparse distribution along the branches; (C) a sprawling hispidose colony in P. Banggi; voucher acc. nos.: BRI 03.MK018, BRI 03.MK062 53

Figure 4.4 *Acropora cerealis* (A) fragment of a colony showing a corymbose outline; (B) branches were slender and the radial corallites were tubular with upwardly extending outer walls which give the species a scaly appearance; (C) a corymbose colony in P. Tiga Park; voucher acc. no.: BRI 01.LL006 54

Figure 4.5 *Acropora clathrata* (A) a thin fragment of a plate colony with highly fused horizontal branches where vertical branchlets would develop at the centre of a colony; (B) radial corallites were appressed tubular and of uniform size and become extended near the margins of a colony; (C) a side-attached plate colony in P. Tiga Park; voucher acc. nos.: BRI 01.MR010, BRI 01.MR014, BRI 01.MR016 55

Figure 4.6 *Acropora dendrum* (A) fragments from a corymbose colony; (B) radial corallites of uniform size, with several having extended outer walls with cochleariform openings; (C) the colony sampled in P. Mantanani with predominantly brown colouration and blue tips; voucher acc no.: BRI 02.RP012 56

Figure 4.7 *Acropora desalwii* (A) fragment of a small corymbose colony; (B) radial corallites were appressed tubular and are graded, becoming larger moving away from the tip of the branches and secondary branchlets occur more frequently at the margin of branches, which is characteristic of the species (Wallace, 1999); voucher acc nos.: BRI 01.LL013B, BRI 02.SB002 57

Figure 4.8 *Acropora divaricata* (A) the branches of usually taper to a point and diverge frequently (Y-furcating), a feature makes this species appear distinctive in the field; (B) radial corallites of uniform size: tubular with dimidiate openings; (C) a caespito-corymbose colony sampled in P. Banggi; voucher acc nos.: BRI 02.RP006, BRI 02.RP017, BRI 02.RP024, BRI 02.RS004, BRI 02.RS019, BRI 03.MB005, BRI 03.MK059, BRI 03.SD003, BRI 03.SD029 58

Figure 4.9 *Acropora elseyi* (A) fragment of a hispidose colony with a hispidose outline; (B) radial corallites were appressed tubular with round to oval openings, closely arranged around the branches but do not touch; voucher acc nos.: BRI 01.MR008, BRI 02.SB016, BRI 03.MK030, BRI 03.SD025 59

UMS
UNIVERSITI MALAYSIA SABAH

Page

Figure 4.10 *Acropora florida* (A) colonies of this species comprise of a sturdy, main branch from which short, secondary branches were given off at regular intervals; (B) radial corallites were appressed tubular with undeveloped inner walls; (C) a hispidose colony in P. Tiga Park; voucher acc nos.: BRI 01.LL023, BRI 01.MR001, BRI 01.UR014, BRI 03.SD022 60

Figure 4.11 *Acropora gemmifera* (A) fragment of a corymbose colony with digitate branches; (B) radial corallites were either of uniform size or interspersed with sub-immersed forms; (C) a corymbose colony in P. Banggi; voucher acc nos.: BRI 02.RP007, BRI RP021B, BRI 03.SD024, BRI 03.SD027 61

Figure 4.12 *Acropora glauca* (A) fragment of a corymbose colony with terete branches; (B) portion of branches showing the rounded appressed radial corallites with large openings; (C) a colony sampled in P. Tiga Park; voucher acc nos.: BRI 03.UR009, BRI 03.UR018 62

Figure 4.13 *Acropora horrida* (A) fragment of a colony with irregular caespitose outline; (B) radial corallites were tubular and exsert (directed away from the branch) and the tips were fragile and crumbly when touched; voucher acc nos.: BRI 03.MK065, BRI 03.SD020 63

Figure 4.14 *Acropora hyacinthus* (A) fragment of a colony exhibiting the plate corallum morphology in which the colonies comprised of highly fused horizontal branches from which vertical branchlets were given off; (B) radial corallites were labellate and arranged in a rossette pattern around the vertical branchlets; (C) a live colony in P. Banggi showing how the species would usually form large tables and even forming tiers stacked vertically like the one photographed here; voucher acc nos.: BRI 02.RP004, BRI 03.MB002 64

Figure 4.15 *Acropora indonesia* (A) fragment of an arborescent-table colony; (B) radial corlallites are nariform with conspicuously elongated outer walls; voucher acc nos.: BRI 01.LL015, BRI 02.RS001, BRI 03.MK056, BRI 03.MK061, BRI 03.SD023 65

Figure 4.16 *Acropora lokani* (A) fragment of a caespito-corymbose colony; (B) radial corallites were appressed tubular with round to oval opening, scattered on the branches; voucher acc nos.: BRI 02.SB008, BRI 03.SD047, BRI 03.SD050, BRI 03.SD053 65

Page

Figure 4.17 *Acropora loripes* (A) fragment of a corymbose colony — specimens examined here were predominantly corymbose although more diverse corallum forms have been reported elsewhere (e.g., Wallace, 1999); (B) radial corallites were appressed tubular with narrow opening and thickened outer walls; (C) a corymbose colony in P. Banggi; voucher acc nos.: BRI 01.LL010, BRI 01.MR013, BRI 02.RP003 66

Figure 4.18 *Acropora microclados* (A) fragment of a plate colony; (B) radial corallites were nariform with most corallites having elongated outer walls; (C) a live colony in P. Tiga Park sampled with hammer and chisel; voucher acc nos.: BRI 01.LL005; BRI 01.LL013, BRI 02.RS011, BRI 02.RS016 67

Figure 4.19 *Acropora microphthalma* (A) fragment of a colony showing an irregular caespitose outline; (B) radial corallites of the specimens were tubular and exsert; (C) a colony sampled in P. Banggi; voucher acc nos.: BRI 02.RP016, BRI 03.MB006, BRI03.MB020, BRI 03.SD016 68

Figure 4.20 *Acropora muricata* (A) fragment of a small colony showing an irregular caespitose outline; (B) radial corallites were nariform or tubular with thick outer walls; (C) live colonies in P. Banggi forming a large thicket; voucher acc nos.: BRI 02.RP014, BRI 02.RP015, BRI 02.SB019, BRI 03.MB003, BRI 03.MB012, BRI 03.MK008, BRI 03.MK009 69

Figure 4.21 *Acropora nana* (A) fragment of a small corymbose colony showing the stalked branches borne on an encrusting base, which is characteristic of the species (Wallace, 1999); (B) branches were slender and terete, with neatly arranged appressed tubular radials. The radials corallites were of uniform size; voucher acc nos.: BRI 01.MR003, BRI 02.RP011, BRI 02.RP021, BRI 02.RS015 70

Figure 4.22 *Acropora nasuta* (A) fragment of a predominantly corymbose colony; (B) radial corallites were closely arranged but not touching; graded, becoming larger moving away from the tips and the outline was nariform with oval to dimidiate openings; (C) a colony in P. Banggi; voucher acc nos.: BRI 02.RP020, BRI 02.RS020, BRI 03.MK052, BRI 03.MK060, BRI 03.SD009 71

Figure 4.23 *Acropora polystoma* (A) fragment of a predominantly corymbose colony; (B) radial corallites were tubular with dimidiate openings and the outer walls were well-developed and exsert; voucher acc nos.: BRI 02.RS008, BRI 02.RS022 72

UMS
UNIVERSITI MALAYSIA SABAH

Page

Figure 4.24 *Acropora robusta* (A) fragment of an arborescent colony — all colonies sampled were arborescent although the arborescent-table form had been reported to be more predominant elsewhere (Wallace, 1999; Wallace *et al.*, 2012a); (B) radial corallites were tubular with dimidiate openings; (C) a colony in P. Tiga Park; voucher acc nos.: BRI 01.LL008, BRI 01.MR026, BRI 01.UR010, BRI 03.MB014 73

Figure 4.25 *Acropora sarmentosa* (A) fragment of a hispidose colony with similar outline as *Acropora florida* (see Figure 4.10); (B) radial corallites were appressed tubular instead of labellate as in *A. florida*; (C) a colony in P. Banggi; voucher acc nos.: BRI 03.MB001, BRI 03.SD008, BRI 03.SD015 74

Figure 4.26 *Acropora secale* (A) fragment of a corymbose colony; (B) radial corallites were tubular with varying lengths and extended at wide angles; (B) a colony sampled from P. Banggi from which the voucher photographed here was sampled (indicated by the white arrow); voucher acc nos.: BRI 02.RS005, BRI 02.RS012, BRI 02.SB004, BRI 02.SB011, BRI 03.MK011, BRI 03.SD056 75

Figure 4.27 *Acropora selago* (A) fragment of an irregular corymbose colony; (B) branches were terete with cochleariform radial corallites; voucher acc nos.: BRI 02.RP019, BRI 02.RS024B 76

Figure 4.28 *Acropora solitaryensis* (A) fragment of a colony showing an arborescent table corallum outline; (B) radial corallites were nariform with oval to nariform openings; (C) a colony sampled in P. Banggi; voucher acc nos.: BRI 03.MK016, BRI 03.SD011, BRI 03.SD054, BRI 03.SD055 77

Figure 4.29 *Acropora spicifera* (A) fragment of a plate colony; (B) radial corallites were labellate and the outer walls are extended at wide angles (> 45°), giving the colony a scaly appearance; voucher acc no.: BRI 02.RP010 78

Figure 4.30 *Acropora stoddarti* (A) fragment of an arborescent table colony; (B) radial corallites were nariform; voucher acc nos.: BRI 02.SB001, BRI 02.SB014, BRI 03.SD006 78

Figure 4.31 *Acropora subglabra* (A) fragment of a hispidose colony; (B) radial corallites were appressed tubular; voucher acc no.: BRI 03.MK010 79

Figure 4.32 *Acropora valida* (A) fragment of a corymbose colony; (B) radial corallites were nariform; voucher acc nos.: BRI 02.RP005, BRI 02.RS002 79

UMS UNIVERSITI MALAYSIA SABAH

Page

Figure 4.33 *Acropora willisae* (A) fragment of a caespito-corymbose colony; (B) radial corallites were appressed tubular and scattered on the branches; voucher acc no.: BRI 02.SB009 80

Figure 4.34 *Acropora* sp. (A) broken fragments of a colony exhibiting an irregular caespitose corallum outline; (B) portion of a branch showing appressed tubular to tubular radial corallites.; voucher acc no.: BRI 02.SB007 80

Figure 4.35 Species richness of seven coral reef localities in Sabah as reported in this study (numbers in blue open circles) and in previous surveys (numbers in red open circles) 83

Figure 4.36 Genomic DNA of staghorn coral samples separated on 1.0% (w/v) agarose gel. Lanes S_{01} through S_{10} are gDNA samples of various staghorn corals flanked with M_1: Lambda DNA/*Hin*dIII Marker and M_2: GeneRuler^{TM} 1 kb DNA Ladder (Fermentas). 84

Figure 4.37 The amplicons for *COI* (A) and *CYB* (B) separated on 1.2% (w/v) agarose gel. Lanes S_{01} through S_{10} are amplicons of various samples, Lane M is the 100 bp Ladder (Fermentas) and Lane C is the negative control. 86

Figure 4.38 Plates showing the transformed *Escherichia coli* carrying plasmids carrying inserts after 16 h incubation (A) and 20 sub-plated colonies (B) to be screened 87

Figure 4.39 The amplicons from colony PCR of separated on 1.2% (w/v) agarose gel. Lanes S_{01} to S_{10} are amplification products of 10 clones, in which eight contained the *COI* insert while two (S_{03} and S_{05}) clones did not. Lane M is the O'GeneRuler^{TM} 100 bp Plus DNA Ladder (Fermentas). 87

Figure 4.40 Purified plasmid separated on 1.0% (w/v) agarose gel showing the characteristic three-banded pattern in which the top-most bands represent nicked or relaxed circles, the band immediately below it being linearised plasmids, while the smallest fragments constitute supercoiled plasmid. Lane M is the GeneRuler^{TM} 1 kb DNA Ladder (Fermentas). Lanes S_{01} to S_{10} are purified plasmid DNA of five cloning reactions which comprised of two replicates each. 88

Figure 4.41 An example of the pairwise comparison between the *COI* nucleotide sequences (5' – 3') of *A.aspera*_03.MB016 and *A.carduus*_03.MK018. Vertical lines indicate similarities while highlighted sites are variable sites between the pairwise sequences. 91

UMS UNIVERSITI MALAYSIA SABAH

Page

Figure 4.42 An example of the pairwise comparison between the *CYB* nucleotide sequences (5′ – 3′) of *A.florida*_01.LL003 and *A.valida*_02.SB006. Vertical lines indicate similarities while highlighted sites are variable sites between the pairwise sequences. 92

Figure 4.43 Bar graphs comparing the distributions of the intraspecific variations among pairwise *COI–CYB* sequences of conspecific staghorn corals (in 14 species, 30 individuals) and the interspecific divergence among pairwise *COI–CYB* sequences of congeneric species (in 34 species, 50 individuals). The range of values of the interspecific divergence and intraspecific variations overlap (both metrics similarly peak within 0.21 – 0.30%) although the former is distributed within a larger range (0.00 – 1.30%) than the latter (0.00 – 0.70%). 93

Figure 4.44 Phylogeny of the COI of staghorn corals from Sabah reconstructed using the ML method. Scale bar represents 0.1% genetic distance as calculated using discrete Gamma distribution model and percentage values of 10,000 bootstrap replicates are shown above the branches. 102

Figure 4.45 Phylogeny of the *CYB* of staghorn corals from Sabah reconstructed using the ML method. Scale bar represents 1.0% genetic distance as calculated using discrete Gamma distribution model and percentage values of 10,000 bootstrap replicates are shown above the branches. 103

Figure 4.46 Phylogeny of the *COI-CYB* of staghorn corals from Sabah reconstructed using the ML method. Scale bar represents 0.1% genetic distance as calculated using the discrete Gamma distribution model and percentage values of 10,000 bootstrap replicates are shown above the branches. 104

Figure 4.47 Amplified fragments of Locus 12406m3 separated on 1.0% (w/v) agarose gel. Lanes S_{01} to S_{20} comprised of 20 different individuals and Lane M is the 100 bp Ladder (Fermentas). 105

Figure 4.48 QIAxcel gel image of amplified Locus 12406m3. The molecular weight-size marker is in Lane M and Lanes S_{01} through S_{11} are fragments of various staghorn corals. 105

Figure 4.49 Examples of electropherogram plots for Locus 10366m5 (A) a homozygote, genotype CC, and (B) a heterozygote, genotype CE. Peaks at the 15 bp and 1,000 bp marks are of the alignment marker. Relative fluorescence unit of 0.5 was set as the minimum threshold and thus peaks below it (RFU < 0.5 in 4.49A) were not scored. 106

UMS
UNIVERSITI MALAYSIA SABAH

Page

Figure 4.50 Line graphs illustrating the patterns of allelic diversity (richness and relative abundance) across the 11 loci 108

Figure 4.51 Bar chart illustrating the proportion of genotypes across the microsatellite loci. The Sabah staghorn coral population appeared to be depauperate of heterozygotes. 110

Figure 4.52 Dendrogram of 11 microsatellite loci of 50 staghorn corals individuals constructed using the UPGMA method based on Nei's (1978) pairwise genetic distance. The scale represents 0.8 unit distance. 112

Figure 4.53 Two *Acropora clathrata* specimens, (A) BRI 01.MR010 and (B) BRI 01.MR014 from MidReef, P. Tiga Park that were expected to be siblings but phylogenies based on the DNA barcode sequences and microsatellite genotypes did not reflect the predicted kinship between these conspecific individuals 113

Figure 4.54 (A) condensed *COI-CYB* ML tree and (B) microsatellite genotype dendrogram of the Sabah staghorn corals in which each individual is marked with symbols denoting their site and locality: "▲" for MidReef, "■" for Larai-Larai Reef, and "●" for UKM Reef, Pulau Tiga Park; "▲" for Rocky Shore Reef, "■" for Sandy Bar Reef, and "●" for Roxy Point Reef, Pulau Mantanani; "▲" for Maliangin Kecil Reef, "■" for Maliangin Besar Reef, and "●" for Serunding Reef, Pulau Banggi 114

Figure 5.1 A multi-species assemblage of staghorn corals on a reef flat approximately 4 m deep in Serunding Reef, P. Banggi, North of Sabah 119

UMS
UNIVERSITI MALAYSIA SABAH

LIST OF SYMBOLS

π	Measure of the mean (± S.D.) of nucleotide diversity within a set of DNA sequences (Nei and Miller, 1990)
D	Genetic distance between a set of pairwise microsatellite genotypes (Nei, 1972)
$\widehat{D}$	Unbiased measure of genetic distance between a set of pairwise microsatellite genotypes (Nei, 1978)
H_d	Measure of mean (± S.D.) diversity within a population of haplotypes (Nei and Tajima, 1981)
h_e	Uncorrected measure of expected heterozygosity within a microsatellite locus (Levene, 1949)
$\hat{h}_e$	Unbiased measure of expected heterozygosity within a microsatellite locus (Nei, 1978)
H_e	Uncorrected measure of mean (± S.D.) expected heterozygosity within a set of microsatellite loci (Levene, 1949)
$\widehat{H}_e$	Unbiased measure of mean (± S.D.) expected heterozygosity within a set of microsatellite loci (Nei, 1978)
h_o	Observed heterozygosity in a microsatellite locus (Levene, 1949)
H_o	Mean (± S.D.) observed heterozygosity within a set of microsatellite loci (Levene, 1949)
I	Uncorrected measure of genetic identity between a set of pairwise microsatellite genotypes (Nei, 1972)
$\hat{I}$	Unbiased measure of genetic identity between a set of pairwise microsatellite genotypes (Nei, 1978)

UMS
UNIVERSITI MALAYSIA SABAH