ISLAND BIOGEOGRAPHY OF LEAF BEETLES (COLEOPTERA, CHRYSOMELIDAE) ON THE WEST COAST ISLANDS OF SABAH, MALAYSIA

YEONG KAM CHENG

UMS
UNIVERSITI MALAYSIA SABAH

INSTITUTE FOR TROPICAL BIOLOGY AND CONSERVATION
UNIVERSITI MALAYSIA SABAH

2019

ISLAND BIOGEOGRAPHY OF LEAF BEETLES (COLEOPTERA, CHRYSOMELIDAE) ON THE WEST COAST ISLANDS OF SABAH, MALAYSIA

YEONG KAM CHENG

UMS
UNIVERSITI MALAYSIA SABAH

THESIS SUBMITTED IN FULFILLMENT FOR THE DEGREE OF MASTER OF SCIENCE

INSTITUTE FOR TROPICAL BIOLOGY AND CONSERVATION
UNIVERSITI MALAYSIA SABAH

2019

UNIVERSITI MALAYSIA SABAH

BORANG PENGESAHAN STATUS TESIS

JUDUL: BIOGEOGRAFI PULAU MENGENAI KUMBANG DAUN DARI PULAU-PULAU PANTAI BARAT SABAH, MALAYSIA

IJAZAH: SARJANA SAINS (EKOLOGI PROSES)

Saya YEONG KAM CHENG, Sesi 2015-2019, mengaku membenarkan tesis Sarjana Sains ini disimpan di Perpustakaan Universiti Malaysia Sabah dengan syarat-syarat kegunaan seperti berikut:-

1. Tesis ini ada 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

UMS
UNIVERSITI MALAYSIA SABAH

Disediakan Oleh,

_____ _____

YEONG KAM CHENG (Tandatangan Pustakawan)
MX1511005T

Tarikh: 28 February 2019

_____
(Dr. Liew Thor Seng)
Penyelia

DECLARATION

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

6 September 2018

.....
YEONG KAM CHENG
(MX1511005T)

UMS
UNIVERSITI MALAYSIA SABAH

CERTIFICATION

NAME	:	**YEONG KAM CHENG**
MATRIC NUMBER	:	**MX1511005T**
TITLE	:	**ISLAND BIOGEOGRAPHY OF LEAF BEETLES (COLEOPTERA, CHRYSOMELIDAE) ON THE WEST COAST ISLANDS OF SABAH, MALAYSIA**
DEGREE	:	**MASTER OF SCIENCE (ECOLOGICAL PROCESSES)**
DATE OF VIVA	:	**12 FEBRUARY 2019**

CERTIFIED BY

SIGNATURE

SUPERVISOR

Dr. LIEW THOR SENG .....

UMS
UNIVERSITI MALAYSIA SABAH

ACKNOWLEDGEMENT

First and foremost, I extend my gratitude to my supervisor, Dr. Liew Thor Seng for his guidance, encouragement and constructive advices throughout the completion of thesis. My deepest gratitude to Institute for Tropical Biology and Conservation, and Borneo Marine Research Institute, Universiti Malaysia Sabah for the helps from staffs and logistic supports.

I thank Universiti Mlaysia Sabah for providing financial support through the UMSGreat Grant (GUG0014-STWN-M-1/2016). I also would like to thank various agencies for their permission to conduct this study on the islands: Sabah Parks for Tunku Abdul Rahman Park and Pulau Tiga Marine Park (permit TS/PTD/5/4 Jld.54(112)) and Sapangar Navy Base (TLDM) for Pulau Udar Kecil (permit MWL2.100- 2/2/3- (9)). Special thanks to Sabah Park staffs: Justinus Guntabid, Muhammad Aliff bin Suhaimin, Victor Siam, Sukur B. Sukardi, Simon Limbawang and others (Sabah Parks); Dr. Zulherry Isnain (Small Island Research Unit), Assoc. Prof. Dr. Rossita Hj. Shapawi (Borneo Marine Research Institute, Universiti Malaysia Sabah); and staff of Marudu Travel Express (Pulau Dinawan) for their assistance to provide transportation and logistics throughout the fieldwork. I thank Foo She Fui, Cornelius, Simon Kuyun, Phung Kin Wah, Phung Chee Chean, Foon Junn Kitt and Chang Zi Yuan for their assistance during fieldwork and laboratory work.

Last but not least, I express my sincere appreciation to my parents who supported me financially and their continuous encouragement during my difficulties throughout the duration of this study

YEONG KAM CHENG

6 SEPTEMBER 2018

UMS
UNIVERSITI MALAYSIA SABAH

ABSTRACT

Sabah is a state of Malaysia located on the northern part of the island of Borneo. Most of the leaf beetle fauna studies from this region conducted over the past 15 years have focussed on the mainland habitats while the leaf beetle fauna from island habitats (*ca*. 500 islands) have largely been overlooked. This study looks into the leaf beetle fauna of 13 small satellite islands off the west coast of Sabah. All specimens were first sorted into morpho-species (operational taxonomic unit, OTU) before being identified to species rank where possible based on morphological characters and species names assigned when the specimens fitted the description of species in the literature. A total of 75 OTUs from 35 genera and 5 subfamilies were collected according to morphology, 12 of which were identifiable to species level. In addition, the DNA barcode for each OTU was cross checked with records in GenBank and BOLD to verify their identity. The number of species recorded was reduced from 12 species and 63 OTUs (total 75 OTUs) to 12 species and 56 OTUs (total 68 OTUs) after removal of the colour polymorphic species based on DNA barcode analyses. A total of 64 Barcode Index Numbers consisting of 101 DNA barcodes were obtained from the 12 leaf beetle species and 48 OTUs. Based on the DNA barcode analyses, it was possible to confirm several polymorphic OTUs and cryptic species. The mean intraspecific and interspecific genetic divergence were determined as 0.77 % and 16.11 %, respectively. DNA barcodes of this study show a low similarity with records in GenBank and BOLD, highlighting the lack of representation and the urgency of studying leaf beetles from this region. Besides that, the species composition pattern of leaf beetle show strong nestedness pattern and influenced by both area and isolation. Furthermore, the species richness was largely determined by the island area and their isolation distance from mainland. However, isolation might not be significant as the furthest island, Pulau Mengalum was influenced by both forest fire and El-Nino drought The study provides the first documentation of leaf beetle fauna from island habitats of Sabah and the first DNA barcoding data for leaf beetles from this part of the world, with the next steps being larger scale sampling over a wider geographical scale for a better understanding of tropical arthropod diversity and the underlying determinant factors for the high species richness.

Keywords: Chrysomelidae, ETIB, integrative taxonomy, Sabah Park, misidentification

UNIVERSITI MALAYSIA SABAH

ABSTRAK

BIOGEOGRAFI PULAU MENGENAI KUMBANG DAUN DARI PULAU-PULAU PANTAI BARAT SABAH, MALAYSIA

Sabah adalah sebuah negeri di Malaysia yang terletak di bahagian utara pulau Borneo. Kebanyakan kajian fauna kumbang daun telah dijalankan sejak 15 tahun yang lalu di rantau ini. Ini telah memberi tumpuan kepada habitat tanah besar manakala sebahagian besar fauna kumbang daun dari habitat pulau (sekitar 500 pulau) telah diabaikan. Kajian ini mengkaji fauna kumbang daun daripada 13 pulau kecil di pesisir barat Sabah. Semua spesimen telah disusun mengikut morfologi spesies dahulu (unit taksonomi operasi, OTU) sebelum dikenalpasti kepada peringkat spesies berdasarkan ciri-ciri morfologi dan nama-nama spesies yang diberikan daripada penerangan spesies dalam literatur. Sejumlah 75 OTU daripada 35 genera dan 5 subfamili dikumpulkan mengikut morfologi, 12 daripadanya dikenalpasti pada peringkat spesies. Di samping itu, kod bar DNA untuk setiap OTU telah disemak dengan rekod dalam GenBank dan BOLD untuk pengesahan identiti spesies. Bilangan spesies telah direkodkan dan mengurangkan 12 spesies serta 63 OTU (jumlah 75 OTUs) kepada 12 spesies dan 56 OTUs (jumlah 68 OTUs) selepas penyingkiran spesies polimorfik warna berdasarkan analisis kod bar DNA. Sejumlah 64 kod bar indeks yang terdiri daripada 101 kod bar DNA telah diperoleh dari 12 spesies kumbang daun dan 48 OTU. Pengesahan OTU polimorfik dan spesies kriptik adalah berdasarkan analisis kod bar DNA. Kecapahan genetik intraspesies dan interspesies telah ditentukan sebagai 0.77% dan 16.11% masing-masing. Kod bar DNA kajian ini memperlihatkan persamaan yang rendah dengan rekod dalam kedua-dua GenBank dan BOLD yang menunjukkan kekurangan maklumat kumbang daun dari rantau ini. Selain itu, corak komposisi spesies kumbang daun menunjukkan corak berkumpulan yang kuat dan dipengaruhi oleh keluasan kawasan dan pengasingan pulau. Selain itu, kekayaan spesies adalah ditentukan oleh keluasan kawasan pulau dan jarak pengasingan pulau dari tanah besar. Walau bagaimanapun, jarak pengasingan pulau menunjukkan tidak signifikan bagi pulau terjauh, Pulau Mengalum yang telah dipengaruhi oleh kebakaran hutan dan musim kemarau El-Nino. Kajian ini dokumentasi pertama fauna kumbang daun dari habitat pulau-pulau di Sabah dan maklumat kod bar DNA yang pertama untuk bidang kajian kumbang daun serta persampelan skala besar dalam skala geografi yang lebih luas untuk pemahaman yang baik mengenai kepelbagaian arthropod tropika dan faktor penentu yang mendasari kekayaan spesies.

Kata kunci: Chrysomelidae, ETIB, taksonomi integratif, Taman Sabah, salah pengenalan

UNIVERSITI MALAYSIA SABAH

LIST OF CONTENTS

		PAGE
TITLE		i
DECLARATION		ii
CERTIFICATION		iii
ACKNOWLEDGEMENT		iv
ABSTRACT		v
ABSTRAK		vi
LIST OF CONTENTS		vii
LIST OF TABLES		x
LIST OF FIGURES		xi
LIST OF ABBREVIATIONS		xiv
LIST OF APPENDICES		xv
CHAPTER 1 INTRODUCTION		
1.1	Background of study	1
1.2	Objectives	3
CHAPTER 2 LITERATURE REVIEW		
2.1	Island Definition	4
2.1.1	Borneo and Surrounding Islands	4
2.1.2	The Significance of Island Biodiversity	6
2.1.3	Equilibrium Theory of Island Biogeography (ETIB)	7
2.1.4	Mechanism of positive species-area relationship	10
2.1.5	Limitations of ETIB	11
2.2	Chrysomelidae Latreille, 1802	12
2.2.1	Brief History on Chrysomelidae Classification	12
2.2.2	General Biology of Chrysomelidae	13
2.2.3	Means of Dispersal for Leaf Beetles	16
2.2.4	Leaf Beetle Studies in Sabah	19
2.3	DNA Barcoding	21
2.3.1	Limitations of Traditional Species Identification System	21
2.3.2	New Insight in Species Identification, DNA Barcodes	21
2.3.3	Conflicts with Traditional Species Identification System	24

UMS
UNIVERSITI MALAYSIA SABAH

2.3.4 Integrative Taxonomy 25
2.3.5 Current DNA Barcoding Progress in Malaysia 26
CHAPTER 3 METHODOLOGY
3.1 Study Design 27
3.2 Study Site 28
3.3 Leaf Beetles Sampling and Processing 29
3.4 Molecular Analysis 31
3.4.1 DNA Extraction 31
3.4.2 Polymerase Chain Reaction Amplification 31
3.4.3 Gel Electrophoresis 32
3.4.4 DNA Purification and Sequencing 33
3.5 Phylogenetic Analysis 33
3.6 Species Checklist 34
3.7 Data Analysis 35
3.7.1 Sampling completeness 36
3.7.2 Principal Components Analysis (PCA) 36
3.7.3 Mantel Test 37
3.7.4 Species Richness Determinant Factors 37
3.7.5 Species-Area Relationship (SAR) 38
CHAPTER 4 RESULTS
4.1 Chrysomelidae species richness 39
4.1.1 Species checklist 42
4.2 DNA barcoding 78
4.2.1 General Results 78
4.2.2 Sequences Analyses 78
4.2.3 Species Identification Comparison 91
4.3 Island Biogeography of Leaf Beetles 98
4.3.1 Sampling Completeness 98
4.3.2 Species Composition Pattern 102
4.3.3 Species Richness Determinant Factors 106
4.3.4 Species-Area Relationship (SAR) 107
CHAPTER 5 DISCUSSION
5.1 Species Checklist 109

UMS
UNIVERSITI MALAYSIA SABAH

5.2 DNA Barcoding 110
5.3 Island Biogeography of Leaf Beetles 114
5.3.1 Species composition pattern 114
5.3.2 Species richness determinant factors 115
CHAPTER 6 CONCLUSION 117
REFERENCES 120
APPENDICES 149

UMS
UNIVERSITI MALAYSIA SABAH

LIST OF TABLES

		PAGE
Table 2.1	Number of leaf beetles species recorded in Sabah.	20
Table 3.1	Coordinate, area (km^2), distance from the nearest mainland (km), and number of plots for the 13 west coast islands of Sabah.	30
Table 4.1	Number of genera and OTUs of each subfamily recorded from west coast islands of Sabah.	40
Table 4.2	Number of subfamilies, genera and species recorded on each sampled Sabah west coast island.	41
Table 4.3	Barcode Index Number (BINs) of each sequenced taxa with the BORNEENSIS accession number	80
Table 4.4	Max, mean intraspecific distance for each species and their distance to the nearest neighbour.	83
Table 4.5	Top-hit search results from NCBI GenBank and Barcode of Life Data System (BOLD)	92
Table 4.6	Summary of BLAST and BOLD top-hit results based on pairwise identity percentage (%)	94
Table 4.7	Observed species richness (S_{obs}), abundance-based coverage estimator species richness (S_{ACE}), and sampling completeness of leaf beetles from 13 west coast islands of Sabah.	98
Table 4.8	The principal component axes (PC) of the Principal Components Analysis (PCA).	102
Table 4.9	Eigenvectors of leaf beetles species for first three PCA axes.	103
Table 4.10	Model selection results, ranked by change in Akaike's Information Criterion (ΔAICc) adjusted for small sample sizes and Akaike weights (w_i) used to evaluate influences on leaf beetle species richness in 13 west coast islands of Sabah.	107
Table 4.11	Linear model of island area and isolation effect on leaf beetle species richness.	107

LIST OF FIGURES

		PAGE
Figure 2.1	The mathematical graph of equilibrium theory of island biogeography. The graph was plotted with rate of change in species richness against the number of species present. The intercept between the red lines and blue lines represent the number of species present at equilibrium state.	9
Figure 3.1	The 13 Sabah west coast islands selected and sampled in this study. (A) Sabah overview map with the location of each island group (highlighted in red and selected in box); (B) Sapangar bay island group; (C) Tunku Abdul Rahman Park; (D) Pulau Dinawan and Pulau Mantukod; (E) Pulau Tiga; (F) Pulau Mengalum. Pulau = Island.	28
Figure 4.1	Dorsal and ventral habitus of leaf beetle species. A *Altica aenea;* B *Aphthona* sp. 1; C *Argopistes* sp. 1; D *Argopistes* sp. 2; E *Erystus villicus*; F *Hemipyxis* sp.; G *Hyphasis* sp.; H *Lanka* sp.	45
Figure 4.2	Dorsal and ventral habitus of leaf beetle species. A *Schenklingia* sp.; B *Aulacophora* sp.; C *Strobiderus* sp.; D *Hoplosaenidea malayensis*; E *Hoplosaenidea* sp. 1; F *Hoplosaenidea* sp. 2; G *Hoplosaenidea* sp. 3; H *Hoplosaenidea* sp. 4.	48
Figure 4.3	Dorsal and ventral habitus of leaf beetle species. A–B *Hoplosaenidea* sp. 5; C *Hoplosaenidea variabilis*; D *Metrioidea grandis*; E *Monolepta* sp. 1; F *Monolepta* sp. 2; G *Monolepta* sp. 3; H *Monolepta* sp. 4.	52
Figure 4.4	Dorsal and ventral habitus of leaf beetle species. A–D *Monolepta* sp. 5; E *Monolepta* sp. 6; F *Monolepta* sp. 7; G *Monolepta* sp. 8; H *Monolepta* sp. 9.	55
Figure 4.5	Dorsal and ventral habitus of leaf beetle species. A *Monolepta* sp. 10; B *Monolepta* sp. 11; C *Monolepta* sp. 12; D *Monolepta* sp. 13; E – F *Monolepta* sp. 14; G *Monolepta* sp. 15; H *Monolepta* sp. 16.	58
Figure 4.6	Dorsal and ventral habitus of leaf beetle species. A *Monolepta* sp. 17; B *Monolepta* sp. 18; C *Ochralea nigripes*; D *Clitena* sp.; E *Sumatrasia* sp.; F *Dercetina* sp.; G-H genus indet. nr. *Monolepta*.	61

UNIVERSITI MALAYSIA SABAH

Figure 4.7	Dorsal and ventral habitus of leaf beetle species. A genus indet. nr. *Monolepta*; B *Scelodonta* sp.; C *Colasposoma auripenne*; D *Aulacia* sp.; E *Colaspoides* sp. 1; F *Colaspoides tuberculata*; G *Basilepta* sp. 1; H *Basilepta* sp. 2.	65
Figure 4.8	Dorsal and ventral habitus of leaf beetle species. A *Basilepta* sp. 3; B *Basilepta* sp. 4; C *Basilepta* sp. 5; D *Basilepta* sp. 6; E – H *Nodina* sp.	68
Figure 4.9	Dorsal and ventral habitus of leaf beetle species. A *Nodina* sp.; B *Pagria* sp.; C *Rhyparida* sp. 1; D *Rhyparida* sp. 2; E *Cleorina malayana*; F *Brontispa longissima*; G *Gonophora* sp.; H *Dactylispa* sp. 1.	73
Figure 4.10	Dorsal and ventral habitus of leaf beetle species. A *Dactylispa* sp. 2; B *Notosacantha* sp. 1; C *Notosacantha* sp. 2; D Hispines sp.; E *Plagiodera* sp.; F *Phola sedecimpustulata*; G *Lema* sp.	76
Figure 4.11	An overview of the present project workbench in Barcoding of Life Data System (BOLD) platform (dx.doi.org/10.5883/DS-BCHRY18).	79
Figure 4.12	NJ – tree. The orange box represents the position of the magnified neighbour joining tree. Violet colour indicates the overlook cryptic species.	87
Figure 4.13	NJ – tree. The orange box represents the position of the magnified neighbour joining tree. Blue and yellow colour indicate the species with phenotypic variation.	88
Figure 4.14	NJ – tree. The orange box represents the position of the magnified neighbour joining tree. Red and green colour indicates the species with phenotypic variation.	89
Figure 4.15	Bayesian Inference tree using COI mitochondrial gene. Outgroup indicates by the black colour branch.	90
Figure 4.16	Sample completeness curve for each of the 13 sampled islands. For each of the graph, the solid dot represents the reference sample (observed), the solid line segment represents the rarefaction and the dotted line segment represents the extrapolation up to a maximum sample size of 600. The shaded area indicates the 95 % confidence interval with 100 bootstrap replications.	99
Figure 4.17	Sample-size-based rarefaction and extrapolation (R/E) curve for each of the 13 studied islands. For each of the	100

graph, the solid dot represents the reference sample (observed), the solid line segment represents the rarefaction and the dotted line segment represents the extrapolation up to a maximum sample size of 1000. The shaded area indicates the 95 % confidence interval with 100 bootstrap replications.

Figure 4.18 Coverage-based rarefaction and extrapolation (R/E) curves for species richness comparison of the 13 sampled islands. For each of the graph, the solid dot represents the reference sample (observed), the solid line segment represents the rarefaction and the dotted line segment represents the extrapolation up to a maximum sample size of 1000. The shaded area indicates the 95 % confidence interval with 100 bootstrap replications. 101

Figure 4.19 Principal component analysis ordination plot of the 13 sampled west coast islands of Sabah using species composition incident data. 105

Figure 4.20 The relationship between species composition dissimilarity and their spatial distance of the sampled Sabah west coast islands. 106

Figure 4.21 Linear regression of log-transformed species richness (log S_{ACE}) against log-transformed island area (log Area) for leaf beetle species from west coast islands of Sabah ($\log S = 0.80 + 0.22 \log A$). Each point represents an island and the shaded area shows 95 % confidence interval. 108

UNIVERSITI MALAYSIA SABAH

LIST OF ABBREVIATIONS

%	-	Percent
AICc	-	Akaike's Information Criterion corrected for small sample
BI	-	Bayesian Inference
BOLD	-	Barcoding of Life Data System
COI	-	Cytochrome c oxidase subunit I
DNA	-	Deoxyribonucleic Acid
ETIB	-	Equilibrium Theory of Island Biogeography
et al.	-	and others; Latin phrase *et alia*
Ka	-	Thousand years ago
LM	-	Linear Model
NJ	-	Neighbour Joining
PCA	-	Principal Component Analysis
PCR	-	Polymerase Chain Reaction
PFM	-	Power function model
sp.	-	Species
Taq	-	*Thermus aquaticus*
TBE	-	Tris Borate-EDTA

UMS
UNIVERSITI MALAYSIA SABAH

LIST OF APPENDICES

		Page
Appendix A	Number of individuals for each leaf beetle subfamily, genus and species from each island (Total and subtotal are bolded)	149
Appendix B	Generic diagnosis of genera	154
Appendix C	Distribution of sequence divergence (K2P distance/uncorrected p-distance)	167
Appendix D	R Script	173

UMS
UNIVERSITI MALAYSIA SABAH

CHAPTER 1

INTRODUCTION

1.1 Background of the study

Islands have gained a lot attentions for its high research potential, where it simplified the complexity of the natural world that enable theories development and testing (Whittaker, 1998). Earlier research conducted by Darwin (1859) and Wallace (1869) have provided a strong foundation knowledge for the development of the latter theory, for example, the *Equilibrium Theory of Island Biogeography* (ETIB) by MacArthur and Wilson (1963, 1967). This theory postulate that the island biodiversity is in the dynamic equilibrium between isolation-dependent immigration and area-dependent extinction (MacArthur and Wilson, 1963, 1967) The species richness on the island is determined by the immigration rate and extinction rate, which is negatively correlated to the isolation distance from the source area (species-isolation relationship) and island size (species-area relationship), respectively. Since then, thousands of studies have been conducted, yet, the underlying determinant factors of the species richness and species composition patterns on island habitat is still under investigation.

Borneo is the third largest islands in the world that located in the most geographically complex tropical region in the world, the Malay Archipelago. Besides that, Borneo also one of the well-recognised mega biodiverse area with high habitat destruction rate (Mittermeier *et al.*, 2011; Myers *et al.*, 2000). During the formation of Borneo, thousands of island were gradually isolated from the Borneo mainland. At the present day, there are 45 islands with different size and isolation are found at

the west coast of Sabah (JUPEM, 2005). The flora and fauna diversity on these islands are rarely investigated and our knowledge on the biodiversity on these islands are still primitive. However, researchers are currently in a race against time to documents these island biodiversity to the high deforestation rates in the region (Sodhi *et al.*, 2010).

Chrysomelidae Latreille, 1802 is one of the most diverse beetle families, with 35,000 - 60,000 species around the world (Jolivet, 2015; Splipnski *et al.*, 2011). The study of leaf beetle fauna in Borneo started in the 19th century, with the first valid species described by Suffrian (1854). Since then, research on Bornean leaf beetle has increased significantly, where the majority research focuses on the mainland habitat and description of new species based on morphological characters (Borowiec *et al.*, 2013; Takizawa, 2017a; Takizawa and Mohamedsaid, 2015). Although Sabah has the highest number of islands in Malaysia, leaf beetle species diversity on these islands has been little explored and the underlying factors for the leaf beetle species richness on the island habitats remain unknown.

The majority of the recent taxonomic works are based on morphological characteristics (Medvedev and Romantsov, 2017a; Mohamedsaid, 2006b; Moseyko, 2012) and few studies have utilised the molecular approaches to infer the phylogeny of leaf beetles (Crampton-Platt *et al.*, 2015; Kishimoto-Yamada *et al.*, 2013). Using this conventional taxonomic approach alone is challenging because sexual dimorphism and colour pattern variants or phenotypic polymorphism are common, especially variables within the subfamily Galerucinae (Chaboo, 2007; Crownson, 1981; Gómez-Zurita *et al.*, 2016; Prado, 2013). Consequently, DNA barcoding has been added to the taxonomist's toolkit in order to complement the species identifications that are based on morphological characters (Gómez-Zurita *et al.*, 2016; Hebert *et al.*, 2003a; Pentinsaari *et al.*, 2014). To date, there is no DNA barcode sequences of the Bornean leaf beetle was registered in the Barcoding of Life Data System (BOLD) (BOLD, 2018).

For all the reasons stated above, the present study aimed to (1) document the leaf beetles species from 13 West Coast islands of Sabah to obtain baseline knowledge of leaf beetle diversity on the island habitats; (2) generate DNA barcodes

for known and newly described Bornean leaf beetle species to serves as supplementary information for the morphological identification and building up of the DNA barcodes reference database to enable rapid species identification; and (3) investigate the biogeography pattern of the leaf beetle and the possible underlying determinant factors for the species composition pattern and species richness on the west coast islands of Sabah.

1.2 Objectives

1. To document the leaf beetle fauna on the selected islands along the Sabah West Coast,
2. To generate DNA barcodes from leaf beetle species through DNA barcoding,
3. To discover the biogeography pattern of the leaf beetle and the underlying determinant factors of the biogeography pattern.

UMS
UNIVERSITI MALAYSIA SABAH

CHAPTER 2

LITERATURE REVIEW

2.1 Island Definition

The definition of islands vastly varies, depending on the purpose of the study. For instance, islands can be divided into two major types, such as those within the sea and those within land masses (Haila, 1990). Examples of the islands within the sea are the oceanic islands and continental shelf islands, which generally defined as "a piece of land surrounding by water" while those islands within land masses are the habitat islands and non-marine islands (Haila, 1990; Whittaker and Fernández-Palacios, 2007). This study focuses on those islands within the sea and employed the definition as a piece of naturally formed land that stays above water during high tide where the species can survive, but completely surrounded by the seawater that acts as a dispersal barrier for the species (Convention on the Law of the Sea, 1982; Diamond, 1975; Millennium Ecosystem Assessment, 2003).

2.1.1 Borneo and Surrounding Islands

Although islands only make up 5.3 % of Earth's land area, islands constitute over one-third of the world's mega biodiversity areas and harbour disproportionately high species endemism and diversity which are not represented and different from their relatives elsewhere (Berry, 1983; Tershy *et al.*, 2015). Yet, the number of critically threatened and extinct species was also disproportionately high on the island compared to the same land area on the mainland (Kier *et al.*, 2009; Myers *et al.*, 2000). For example, Borneo Island, one of the mega biodiversity hotspot located within the important Indo-Malayan ecoregion are currently experiencing intensive

habitat destruction caused by anthropogenic activities (Fisher *et al.*, 2011; Myers *et al.*, 2000; Olson *et al.*, 2001; Sodhi *et al.*, 2010).

Geologically, Borneo was located on the Sunda Shelf, previously formed the continental core of the region together with Peninsular Malaysia, Thailand, Sumatra and Java that known as Sundaland, connecting Borneo to the Thai-Malay Peninsula by the mid Cretaceous (Arora *et al.*, 2010; Hall *et al.*, 2008). This connection together with Pleistocene sea-level changes facilitated species dispersal and faunal interchange among the Asian Mainland and Borneo Island (Arora *et al.*, 2010; Bird *et al.*, 2005; Lohman *et al.*, 2011; Voris, 2000). Other than climatic fluctuation, habitat expansion, contraction and diversification also lead to the high diversity of Bornean flora and fauna (Hortal *et al.*, 2009). Nevertheless, the high biotic diversity and endemism of Borneo are suggested to be driven by a combination of specialized ecological niche, refugia formation and a long period of isolation (Arora *et al.*, 2010).

At the present day, Borneo is relatively stable with little seismicity and a small number of non-active Quartenary volcanoes (Hall *et al.*, 2008; Khalil *et al.*, 2018; Wang *et al.*, 2017). The northwest coastline of Borneo stretches from Tanjong Datu at Lundu of Sarawak to Tanjong Simpang Mengayau at Kudat of Sabah, consists Sarawak, Brunei and west coast of Sabah (National Geospatial-Intelligence Agency, 2005). Although Sarawak and Brunei have the longest Borneo northwest coastline, majority islands (45 islands) were found on the Sabah West Coast (JUPEM, 2005). In addition, Sabah also owns the most number of islands (*ca*. 500 islands) among the Malaysian states, in which majority of these islands are continental islands and with a small number of volcanic and oceanic islands (Clennell, 1991; JUPEM, 2005; Mohamed *et al.*, 2016; Wood *et al.*, 1992).

Nonetheless, islands on the Sabah West Coast are mainly continental fragment islands, which is an island that located on the continental shelf and connected to the mainland during periods of significantly lower sea levels (Whittaker, 1998). For instance, Gaya Island and the surrounding small islands are underlain by interbedded sandstone and sedimentary rocks of the West Crocker Formation (Aris *et al.*, 2009; Mohamed *et al.*, 2016; Thomas *et al.*, 1976). Other examples of small islands composed of rocks of the West Crocker Formation are Manukan Island, Sapi

Island, Sepanggar Island, Tiga Island, Udar Besar Island, and Peduk Island. Whereas for the most remote island from the mainland, Mengalum Island is formed mainly by flat beach strands with some localized hills (Thomas *et al.*, 1976) and deep to shallow marine Neogene sediments (Idris and Kok, 1991). Out of the 45 West Coast islands, Tiga Island is the most unique island that was covered by the mud volcanic soil that comprised of mixture incorporated with mud and shale (Lin *et al.*, 2010).

2.1.2 The Significance of Island Biodiversity

In general, island biodiversity was characterised with species poverty, where islands have fewer species per unit area than the mainland, yet rich in forms found nowhere (Whittaker, 1998; Whittaker and Fernández-Palacios, 2007). Besides that, island biota also considered to be "disharmonic", the non-random representation of biota compared to the mainland as a consequence of differential dispersal and colonization abilities (Gillespie, 2007; Gillespie and Roderick, 2002; Whittaker, 1998; Whittaker and Fernández-Palacios, 2007). Species with higher dispersal ability across the seawater will have a higher portion of representatives on the island than those with low dispersal ability. In addition, the island also attributes for their high endemism compared to same area size on the mainland, which may be promoted through the paleoendemism and neoendemism (Cronk and Percy, 2009; Gillespie and Roderick, 2002; Kier *et al.*, 2009). Moreover, species on the island habitat might experience the reduced dispersal capability, innovations, size changes, and reproductive changes.

The importance of island not just ascribed to their highly threatened endemic species diversity and potential source for mainland species diversity (Jønsson and Holt, 2015), but also as the theories nurturing ground to improve our understanding of the ecology and evolution of communities in general. Several paradigm theories and discoveries were inspired by the island habitats, such as *On the Origin of Species* by Darwin, *Island Life* by Wallace, and *Equilibrium Theory of Island Biogeography* (Darwin, 1859; MacArthur and Wilson, 1967; Wallace, 1880). Therefore, islands are used as study model, as they are differ from each other in fundamental ways, which is discrete, varied entities, internally quantifiable, with multiple replicates available globally and serve as natural real-world laboratories with simplified systems (Gillespie, 2007). For instance, theories that were originally developed for islands are

now applied to a huge array of disciplines and used as the model systems for the field of evolution and speciation, conservation biology, human-land interactions, and ecosystem structure and functioning (Gillespie and Roderick, 2002; Vitousek, 2002). The use of islands in evolution and speciation studies enhance our understanding on the fundamental evolutionary concern

2.1.3 Equilibrium Theory of Island Biogeography (ETIB)

The uniqueness of the islands biodiversity have attracts many researchers in attempt to solve the underlying principle for the biogeography pattern and high level of endemism. Before 1960s, most of the biogeography research has been taxonomic and descriptive in origin, and dominated by historical orientated (MacArthur and Wilson, 1967; Whittaker, 1998). Many theories were developed as an endeavor to explain the center of origin, such as the Paradisical mountain proposed by Carl Linnaeus and how does the current distribution of living organisms formed (Browne, 1983; Nelson, 1978). Although there are some quantitative theories in biogeography (MacArthur and Wilson, 1967) prior to 1960s, Brown and Lomolino (1998) mentioned that was not a propitious time for injecting mathematical theory and ecological concepts. Nonetheless, MacArthur and Wilson introduced a new chapter to the field of biogeography in their 1963 seminal paper by proposing the new theory, "*The Theory of Island Biogeography*", which later become the new paradigm in biogeography study until present (MacArthur and Wilson, 1963). This theory combined the effects of isolation and island area on rates of immigration, speciation and extinction, where the latter part are vital elements in balancing the number of species in dynamic equilibrium on the island (**See Figure 2.1**) (Borregaard *et al.*, 2016; Walter, 2004; Whittaker, 1998). The curves of immigration rate slowly decrease as the most of the habitats are filled up while the curves of extinction increase gradually due to high inter-competition between species on the island. The intercept between the rate of immigration and extinction indicated the species richness at the state of dynamic equilibrium (Number of species present A-D in **Figure 2.1**). There are some basic principles underlying for in this theory, which are species-area relationship and species isolation relationship (MacArthur and Wilson, 1967; Whittaker, 1998).

i. Species-area relationship