EXPLORING THE DIVERSITY OF BUTTERFLIES (LEPIDOPTERA) AT DIFFERENT ELEVATIONS IN GENTING HIGHLANDS AND THE VALIDITY OF *GRAPHIUM* SPECIES IN PENINSULAR MALAYSIA

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ABSTRACT

Butterflies play an ecological role as pollinators, prey, defoliators and herbivores. They are in abundant and diverse in many ecosystems. However, they are serves as indicators of ecosystem change and predict environmental alternation. This study was conducted to (1) compare the attractiveness of ten types of fruit bait and make recommendations on the most efficient fruit bait types for trapping butterflies in the South East Asian tropical forest; (2) investigate how butterfly diversity is related to elevation at Genting Highlands in Peninsular Malaysia; and (3) explore the phylogenetic relationships within *Graphium* and the validity of *Pathysa*.

The bait trap is one of the most common methods used for trapping butterflies in the field and particularly for fruit feeding butterflies. The present study was conducted to determine the efficiency of traps baited with ten different fruit baits. Traps were baited with either: banana (*Musa* spp), chiku (*Manikara sapota*), citrus (*Citrus aurantifolia*), dragon fruit (*Hyllocereus undatus*), guava (*Psidium guajava*), papaya (*Carica papaya*), pineapple (*Ananas comosus*), rose apple (*Syzygium malaccense*), star fruit (*Averrhoa carambola*) and watermelon (*Citrullus lanatus*) at Ulu Gombak, Selangor, Malaysia. A total of 194 Nymphalids butterflies of 28 species were recorded in our study. Banana was found to be the most attractive bait trapping a total of 14 species of Nymphalids. Based on our study, we would recommend using banana for collecting butterfly in the South-East Asia tropics.

Several previous studies indicate that butterfly diversity declines with elevation due to increasingly unfavourably environmental conditions and reduced vegetation. Here, we investigate how butterfly diversity is related to elevation at Genting Highlands in Peninsular Malaysia. A total of 2, 876 butterflies belonging to 214 species were collected from six sites of different elevation between January and December 2011. Nymphalidae (1599 individuals) was the most abundant family and *Ypthima*

*pandocus*was the most abundant species (718 individuals), followed by *Eurema hecabe* (194 individuals) and *Leptosia nina* (75 individuals). The highest diversity (118 species, H'=3.882) was seen at low elevation (480 m a.s.l.) with declining species diversity at higher sites.

The COI mtDNA barcodes for *Graphium* specimens from Museum of Zoology, University of Malaya and Jengka, Pahang was sequenced to test the utility of DNA barcoding for the identification of *Graphium* species. In addition, the sequences of 28S rRNA were used to examine, in conjunction with COI, phylogenetic relationships and investigate the validity of *Pathysa* and *Parantocopsis* as distinct genera. All species of *Graphium* possessed a distinctive cluster of DNA barcodes with the exception of the specimens originally identified as *Graphium bathycles* and *Graphium chironides* which shared DNA barcodes. Furthermore, the morphological identification of *Graphium bathycles* and *Graphium chironides* was ambiguous as the specimens overlapped for the diagnostic characters reported for each taxon. Moreover, the maximum parsimony trees of the COI and 28S rRNA showed a similar topology with *Paranticopsis* species forming a clade within a larger clade comprising the *Pathysa* species. In order for *Pathysa* to be a valid genus, at least three other clades within *Graphium* s.l. would also have to be raised as genera.

ABSTRAK

Kupu-kupu memainkan peranan penting dalam ekologi sebagai pendebunga, mangsa-pemangsa, defoliator dan herbivor.Ia boleh dijumpai dengan banyak dan kepelbagaian di ekosistem. Selain itu, ia juga merupakan penunjuk bagi perubahan ekosistem dan peramal bagi kitaran alam sekitar. Tujuan kajian ini dijalankan adalah untuk (1) menentukan kecekapan bagi sepuluh jenis buah-buahan sebagai umpan dan mengesyorkan umpan yang paling efektif bagi mensampelkan kupu-kupu di hutan tropika Asia Tenggara; (2) mengkaji bagaimana kepelbagaian kupu-kupu dihubungkaitkan dengan ketinggian di Genting Highlands, Semenanjung Malaysia; dan (3) meneroka hubungan filogenetik dalam *Graphium* dan kesahihan bagi *Pathysa*.

Perangkap berumpan merupakan satu cara yang biasa digunakan untuk mensampel kupu-kupu di kawasan kajian, terutamanya memerangkap spesies kupu-kupu pemakan-buah. Kajian ini dijalankan untuk menentukan kecekapan bagi sepuluh jenis buah-buahan sebagai umpan dalam pensampelan kupu-kupu. Setiap perangkap berumpan mengandungi sama ada: pisang (*Musa* spp), ciku (*Manikara sapota*), limau nipis (*Citrus aurantifolia*), buah naga (*Hyllocereus undatus*), jambu batu (*Psidium guajava*), betik (*Carica papaya*), nanas (*Ananas comosus*), jambu merah (*Syzygium malaccense*), belimbing besi (*Averrhoa carambola*), dan tembikai (Citrullus lanatus) di Ulu Gombak, Selangor, Malaysia. Secara keseluruhannya, sejumlah 194 ekor kupu-kupu Nymphalid yang terdiri daripada 28 spesies telah direkodkan.Pisang merupakan umpan yang paling efektif dalam mensampelkan kupu-kupu, iaitu sebanyak 14 spesies direkodkan.Melalui kajian ini, disyorkan bahawa pisang adalah digalakkan sebagai umpan bagi mensampelkan kupu-kupu di hutan tropika Asia Tenggara.

Beberapa kajian yang telah dijalankan menunjukkan kepelbagaian kupu-kupu berkurangan dengan penambahan ketinggian yang disebabkan peningkatan keadaan alam sekitar yang tidak sesuai.Kajian juga dijalankan untuk melihat bagaimana

kepelbagaian kupu-kupu dihubungkaitkan dengan ketinggian di Genting Highlands, Semenanjung Malaysia. Secara keseluruhannya, sebanyak 2,876 ekor kupu-kupu yang terdiri daripada 214 spesies telah diperolehi dari 6 kawasan kajian yang mempunyai ketinggian berlainan dari Januari hingga Disember 2011. Nymphalidae (1599 individu) merupakan famili yang paling banyak didapati dan *Ypthima pandocus* merupakan spesies kupu-kupu yang paling banyak didapati (718 individu), diikuti oleh *Eurema hecabe* (194 individu) dan *Leptosia nina* (75 individu). Nilai kepelbagaian yang paling tinggi (118 spesies, H'=3.882) diperolehi di kawasan ketinggian rendah (480m a.s.l.) dengan pengurangan kepelbagaian spesies di kawasan tinggi.

Penentuan urutan fragmen bagi gen COI mtDNA barkod telah dijalankan bagi specimen *Graphium* daripada Muzium Zoologi, Universiti Malaya bagi mengkaji utility barkod DNA dalam penentuan spesies kupu-kupu bagi spesies *Graphium*. Tambahan pula, kita juga menentukan urutan fragmen bagi 28S rRNA untuk mengenalpasti, mengabungkan penanda dengan COI, hubungan filogenetik bagi *Graphium* spesies serta mengkaji kesahihan masing-masing bagi *Pathysa* dan *Paranticopsis* sebagai satu genus tersendiri.Didapati semua spesies bagi *Graphium* tergolong dalam satu kelompok tersendiri bagi barkod DNA dengan pengecualian bagi spesimen yang dikenalpasti sebagai G. *bathycles* dan G. *chironides*, dimana kedua-dua spesies ini berkongsi barkod DNA yang sama. Bagi kajian lanjutan yang dijalankan, didapati identifikasi melalui ciri-ciri morfologi adalah serupa dan ciri-ciri diagnostik yang bertindih dilaporkan bagi setiap takson.Pokok parsimoni maksimum bagi COI dan 28S rRNA menunjukkan topologi yang serupa dengan spesies bagi *Paranticopsis* yang membentuk satu kumpulan dalam kumpulan besar yang mengandungi spesies-spesies *Pathysa*. Bagi menaik taraf *Pathysa* menjadi satu genus tersendiri, ia perlu membentuk sekurang-kurangnya tiga kelompok dalam golongan *Graphium* s.l.

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LIST OF SYMBOLS AND ABBREVIATIONS

%	Percentage
&	And
<	Less than
=	Equal
>	More than
℃	Degree Celsius
a.s.l	Above sea level
ANOVA	Analysis of Variance
BOLD	Barcode of Life Data
COI	Cytochrome c oxidase I
d	Berger-Parker index
D_{mg}	Margalef's index
DNA	Deoxyribonucleic acid
E	Shannon evenness
EF1- α	Elongation factor 1 alpha
et al.	Latin phase *et alia* (and other)
G.	Graphium
H'	Shannon-Weiner diversity index
m	Meter
PCR	Polymerase chain reaction
r	rValue
RNA	Ribonucleic acid
s.l	Sensu lato

CHAPTER 1

GENERAL INTRODUCTION

Lepidoptera is the second most diverse insect order in term of species richness, the first being Coleoptera. The known species of Lepidoptera contribute approximately 10% of the known Animal Kingdom (Dawood & Nakanishi, 2004). The total number of lepidopteran species was estimated to be in the rangeof 280,000 to 1.4 million species (Solis & Pogue, 1999), but only 160,000 species had been described (Kristensen *et al*., 2007). Lepidoptera is a major group of plant-feeding insects and lepidopteran diversity can indicate the health of plant communities (Pogue, 2009).

Butterflies (approximately 10% of Lepidoptera) are one of the best-studied insect groups and play vital roles as herbivores, pollinators and environmental quality indicators (Braby, 2000; Subahar *et al*., 2007). Butterflies can be found in most places on earth, from the barrens of subarctic zones to the jungles of the tropics to backyard plots in cities (Cassie, 2004). Given their association with plant diversity, butterflies are sensitive to the habitat disturbance and commonly used as an indicator taxon for ecological research (Kocher & William, 2000).

Bait trapping is a popular sampling method used to study the diversity of butterflies in forest canopy. Bait trapping has been used to monitor changes in species abundance over time, to compare the species composition and abundance between sites and to track the movement of individuals (Hunghes *et al.*, 1998). Fruit feeding butterflies can be sampled in traps baited with fermenting fruit (Hughes *et al*., 1998). Bait traps are easy to operate and require few person-hours allowing simultaneous sampling with standardized effort at different sites.

Analysis of altitudinal changes in species diversity of biotas can provide important information such as the aspects of the environment limit on the distribution of organisms. The biotic factor has been discussed regarding species richness at different elevation (McCoy, 1990; Rahbek, 2005).

In order to use a group of organisms as a biodiversity model, it is important that the group is taxonomically tractable. This is the case for the butterflies of Peninsular Malaysia which have a long history of taxonomic studies (Felming, 1975, Corbet& Pendlebury, 1992). However there remain a few groups with controversial or ambiguous taxonomy. *Graphium* is a genus of swallowtail butterflies known as swordtails or kite swallowtails. Some authors (Munroe, 1961; Saigusa *et al*., 1982) classified *Pathysa* as a subgenus of *Graphium*, but Igarashi (1984) raised *Pathysa* as a distinct genus because of the distinctive wing shapes and pattern of the adults. There have been no studies of the phylogenetic relationships of *Graphium* s.l. species of Peninsular Malaysia. This provides an example of the state of a butterfly taxonomy in the region, with implication for the use of butterflies in ecological studies.

The objectives of this thesis are:

1. To compare the attractiveness of ten types of fruit bait, and make recommendations on the most efficient fruit bait types for trapping butterflies in the South East Asian tropical forest.
2. To investigate how butterfly diversity related to elevation along the Genting Highlands in Peninsular Malaysia.
3. To explore the phylogenetic relationships within *Graphium* and the validity of *Pathysa*.

CHAPTER 2

LITERATURE REVIEW

2.1 Why study butterflies?

Butterflies and moths belong to the order of Lepidoptera. The scientific name of Lepidoptera was translated from Greek as wings with scales (Larsen, 1984). There are about 18,000 species of butterflies in the world (Larsen, 1991; Pierce, 1995). Peninsular Malaysia has 1,031 species of butterflies with 21 endermic species (Corbet & Pendlebury, 1992). Butterflies are informally classified into two groups, true butterflies (Papilionoidea) and skippers (Hesperiidae). Papilionoidea is composed of five families: Lycaenidae (blues), Nymphalidae (nymphs), Papilionidae (swallowtails), Pieridae (white and sulphurs), and Riodinidae, all are found in Peninsular Malaysia. Classification below the family level (subfamilies and tribes) still remains more controversial and largely unresolved. Recently molecular phylogenetics has helped progress towards a consensus regarding these groups (DeSalle *et al.*, 2005; Wilson, 2011a).

Among insects, butterflies are one of the most useful group for biodiversity studies, as they have a wide distribution of highly diverse species and they are relatively easy to record and to identify (Pogue, 2009).Butterflies are considered as the major group of plant-feeding insects, and due to this ecological role, butterfly diversity can indicate the ecological health of plant communities (Pogue, 2009).A study by Hogsden & Hutchinson (2004) showed the diversity and species richness of butterflies were positively correlated with plant species diversity, where plants communities are important to support butterflies in larval and adult stages. The destruction of host plants and human disturbances can affect the diversity of butterflies (Durairaj *et al.*, 2012).

Butterflies have been used as “flagship” species for conservation and indicate the environmental quality (Kocher & William, 2000; Schulze *et al*., 2002).The study of butterflies in different ecological condition may provide important clues for a great variation of diversity patterns in different taxa (Axmacher & Fiedler, 2008).Therefore, butterflies have been used as anindicator to provide potential insight into butterfly ecology and methods for rapid assessment of communities (Hill *et al*., 2001; Lewis & Senior, 2011).

2.2 How to sample butterflies?

Butterflies sampling with sweep net is one of the most commonly used methods for sampling butterflies. Sweep nets are generally made from strong and close woven material with light weight. The sturdy cotton is designed for thrashing and withstands vigorous movementthrough vegetation. Insects occurring in medium height vegetation can be collected with the sweep net. The best time for catching butterflies is between 0800 and 1500 on sunny days, which is the peak time of flight activity(Dawood & Nakanishi, 2004).

Previous studies on the butterfly surveys (Hogsden & Hutchinson, 2004; Clark, *et al*., 2007; Parandhaman *et al*., 2012) used the transect methods which were from the modifications based on the “Pollard walk” method described by Pollard (1977). The changes in abundance of butterflies can be assessed through this transect count method. This method involved travelling transect route at a uniform pace to record observed butterfly species within defined limits. This method was used to monitor the abundance of butterflies and hence provided useful information on the phenology and ecology of butterflies.

Bait traps arecommonly method used to lure butterflies into the trap (Austin & Riley, 1995). There are three guild types of butterflies: nectar feeding, fruit feeding anda

combination of both (Hall & Willmott, 2000; Devries & Walla, 2001). There are some butterflies that also feed primarily on non-floral foods such as rotting fruit, animal dung, carrion, or shellfish (Omura & Honda, 2003; Boggs & Dau, 2004; Molleman *et al*., 2005a; Brock & Kaufman, 2006). Vertebrate dung is a well-known lure for male butterflies, especially in the tropics countries (Rod & Ken, 1988). Butterflies are also attracted by charcoal and the remains of campfires (Larsen, 1984). Most fruit feeding butterflies are found in tropical and sub-tropical regions (Tang *et al*., 2013). Most fruit-feeding butterflies can be captured at ground level. This maybe due to the rotting fruit falling on the forest ground (Hughes *et al*., 1998).

2.3 Distribution of butterflies

The distribution of butterflies is a dynamic, ever-changing process (Opler & Krizek, 1984). All butterflies are highly specialized insects wherby each species has the ability to adapt and live in a certain type of habitat (biotope) (Higgins, 1983). The suitability of the habitat depends upon the presence of the appropriate hosts, geographic barriers, such as altitude, temperature, sunshine or shade (Opler & Krizek, 1984; , Kim, 2009).

Temperature and rainfall have been found as the factors that can affect the distribution of butterflies (Hill *et al*., 2003; Stefanescu *et al*., 2004). Larval behaviour, flight activity, and reproduction of butterflies are highly dependent upon the temperature (Andrew & Hughes, 2005; Nabeta *et al*., 2005). Moreover, heavy rainfall may decrease the lifetime of larvae and pupae due to the destruction of food sources (flowers and fruits) (Jia *et al*., 2010). The changes on local microclimates and light level were able to affect the distribution of butterflies (Niell *et al*., 2007; Parandhaman *et al*., 2012). Fermon *et al*. (2000) mentioned that larval development depends on the microclimate condition.

A vast of butterflies that can be found in the tropics during dry seasons (Larsen, 1984). The groups of butterflies commonly wouldgather on damp grounds that are saturated with certain minerals in the tropics (Rod & Ken, 1988). They ingest the dissolved salts and nitrogen compound (Larsen, 1984). Generally, the male butterflies taking up salts and other necessary nutrient at puddle edges or on wet ground (Heath, 2004).

2.4 Butterfly taxonomy – molecular phylogenetics and DNA barcoding

Identification of butterfly species is traditionally based on external morphological characteristics such as wing venation (Corbet & Pendlebury, 1992). Some butterfly species are difficult to identifyby morphological characters due to the lack of information on diagnostic characters, e.g. *Graphium bathycles* and *Graphium chironides*. This problem can be solved with DNA barcoding. DNA barcoding can be used for species identification which can rapidly sort and discover specimens (Hebert *et al*, 2003; Hebert & Gregory, 2005; Hajibabaei *et al*., 2006). It also can be a great utility in conservation biology and an efficient method to mapping the extent of a species (Stoeckle, 2003).

To date, large scale of studies which have examined the effectiveness of DNA barcoding for species discovery in Lepidopera (Hebert *et al*., 2004a; Janzen et al., 2005; Hajibabaei *et al*., 2006), birds (Hebert, 2004b), fishes (Teletchea, 2009) and spiders (Barrett & Hebert, 2005). According to Hajibabaei *et al*. (2006), tropical Lepidoptera species can be distinguished by using DNA barcodes and it has been proven that three Lepidoptera families in Costa Rica which can be effectively discriminated by the cytochrome c oxidase I (COI) DNA barcodes.

Molecular phylogenetics can be used to discover the systematic relationship among the Lepidoptera species based on the mitochondrial or/andthe DNA nuclear

genes (Braby *et al*., 2006; Kristensen *et al*., 2007; de-Silva *et al*., 2010; Sohn *et al*., 2013). Mitochondrial cytochrome oxidase subunit I (COI), elongation factor-1 alpha (EF1-α) and wingless have been used to generate molecular data to analyze phylogenetic relationships and determine genetic relationships (Silva-Brandao *et al*., 2006; Simonsen *et al*., 2010; Wilson, 2010). There are some *Graphium* species that are difficult to be distinguished by morphological characters, hence DNA sequences have been used to determine and investigate the phylogeny of *Graphium* butterflies in this study. In Taiwan, Tsao & Yeh (2008) used COI to discriminate the subspecies of swallowtail butterflies; the molecular data obtained were useful for taxon identification and evaluation for subspecies differentiation. Moreover, molecular data reveals the insight into morphology and life evolution of insects.

In a study on molecular phylogenetics of Lepidoptera with priority gene regions by Wilson (2010), it has been shown that the COI and 18S genes were the universal primer, which produced most distinct bands on the gel and it has also been successful in PCR amplification. Moreover, the highest quality sequences were also produced by the COI and 18S genes. While, Wilson (2010) concluded that DNA barcode fragment of COI is easier to be sequenced compared to other genes and had a high score for utility and high quality of sequences in all taxa.

Mitochondrial cytochrome c oxidase subunit I (COI) with approximately 650 bp regions can serve as DNA barcode for species identification (Hebert *et al*, 2003). DNA sequences provide an insight into species-level relationship, new information to understand the evolutionary and genetic relationships (Hajibabaei *et al*., 2007; Prudic *et al*., 2008).

Molecular methods have given advantages for studyies of phylogeny, systematics and the problem of morphological differentiation (Hebert & Gregory, 2005; Dinca *et al*., 2010; Wilson, 2011b). Molecular methods also can be applied in any life

history stages to solve identifieation problems (Stoeckle, 2005; Kavitha *et al*., 2013; Meiklejohn *et al*., 2013). DNA sequences store useful phylogenetic information that helps us to identify hidden species, and hence discover new species, and useful for phylogenetic reconstruction (Galtier *et al*., 2009; Wilson, 2011a). As a result, DNA sequences have been generated and publically available in the international DNA databases such as BLAST (Basic Local Alignment Search Tool), GenBank and BOLD (Barcode of Life Data system, http://www.boldsystems.org). It is useful to perform DNA based identification to identify unknown species by comparing sequences from databases in GenBank or BOLD. Therefore, the molecular method is the quickest effective to solve species problems compared to the traditional morphology taxonomic methods. Hence, it is suggests as one of the best methods in phylogenetic studies.

CHAPTER 3

WHICH IS THE BEST FRUIT BAIT TO COLLECT FRUIT FEEDING BUTTERFLIES OF THE GENUS *MYCALESIS* IN GOMBAK?

3.1 Introduction

Adult butterflies generally feed on substrates containing sugar or minerals such as nectar, rotting fruits, tree sap, carion and dung (DeVries *et al*., 1997; Hall & Willmott, 2008; and Ribeiro *et al*., 2012).According to DeVries (1987), there are two types of guilds of feeding butterflies in tropical forest: nectar-feeding or fruit-feeding. Fruit-feeding butterflies feed on the juice of rotting fruits, tree saps or rotting plant material (Molleman *et al*., 2005a) and show preferences for different fruit based on texture, chemical composition, and other variables (Corbet, 2000).

Butterfly proclivity for rotting foods have been exploited by researchers undertaking numerous ecological and biodiversity studies (eg. Barlow *et al*., 2007; Luk *et al*., 2011) with various bait trapping designs being employed (e.g. DeVries, 1979;Kingsolver & Daniel, 1979; Boggs & Jackson, 1991;Sculley & Boggs, 1996 and Hall & Willmott, 2008). Likewise, wildlife photograpers have used rotting fruit or nectar to lure butterflies to the location of choice (Krizek, 1990).

In this study, the efficiency of ten types of fruit bait has been compared and the most efficient fruit bait will be suggested for use in the South East Asia tropical forest.

3.2 Material and methods

This study was conducted in the secondary forest area of Ulu Gombak Forest Reserve (Figure 3.1) between January and April 2012. At each of ten sampling plots I installedten butterfly traps in a line (Figure 3.1; following DeVries, 1987), at least 2m apart, under canopy and each baited with a different fruit. Traps were baited with either: banana (*Musa* spp), chiku (*Manikara sapota*), citrus (*Citrus aurantifolia*), dragon fruit (*Hyllocereus undatus*), guava (*Psidium guajava*), papaya (*Carica papaya*), pineapple (*Ananas comosus*), rose apple (*Syzygium malaccense*), star fruit (*Averrhoa carambola*) and watermelon (*Citrullus lanatus*).

Each butterfly trap was checked and emptied once per day early in the morning and new bait was added. The number of individuals and the number of species trapped in each trap were recorded every day. The bait traps were installed in the forest for a minimum of ten days. All sampling was conducted during ten sunny days. Morphological identification of trapped specimens followed Corbet & Pendlebury (1992) and all preserved specimens were deposited in the Museum of Zoology, University of Malaya.

ANOVA was used to test if there were significant differences between 1) the number of individuals collected and 2) the number of species collected, at the ten different types of fruit. Similarity in species diversity of butterflies sampled at the different fruit was assessed using the Bray-Curtis similarity index (in PAST; Hammer, 1999).

3.3 Result and discussion

A total of 28 species from 16 genera were collected during the study (Table 3.1). Twenty-seven species were nymphalids and one species was a lycaenid. The majority of butterflies collected were from the Satryrinae (87%) and the rest were from the Limentidinae (10%) and the Charaxinae (3%). The highest number of individuals were collected at banana baited traps (25%) followed by papaya (19%) and watermeon (17%). The chiku and rose apple baited traps collected the fewest number of individuals. Similarily, traps baited with banana collected more species (14/28 species, 50%) compared with other fruit baits. In a study in Ghana, Bossart *et al*. (2005) also found that traps baited with banana were the most successful at collecting butterflies. The one-way ANOVA test showed that the number of individualscollected between the fruit trapswas significantly different ($F=10.07$, $p=0.000$) and that the species richness of butterflies collected was significantly different ($F=22.65$, $p=0.000$) between the fruit *traps*.

Mycalesis was the most abundant genus collected, accounting for 71% (137) of trapped individuals.*Mycalesis orseis* was the most common species (70 individuals) and was collected at all types of fruit bait during the study. I found thatbanana was the most attractive fruit bait followed by papaya with one more individual than watermelon to attract the *Mycalesis* species.Almost 60% of *Mycalesis maianeaus* and *Mycalesis orseis* were attracted by banana, papaya and watermelon (31/46 individuals; 39/70 individuals). This agrees with the study by Saikia *et al*. (2010) who found *Mycalesis* were highly attracted to traps baited with fresh banana.

Many studies have used banana in bait traps. Hughes *et al*., (1988) collected nymphalids of the subfamilies Brassolinae, Charaxinae, Morphinae, Nymphalinae and Satyrinae with banana in Neotropical Costa Rica. In South East Asia, Luk *et al.* (2011) collected 20 species with banana in Mentawai islands, Indonesia. Seven species were

collected with banana bait in tropical rainforest in Malaysian Borneo(Beck and Schulze, 2000). These were *Amathusia phidippus*, *Lexias pardalis*, *Mycalesis horsfieldi*, *Mycalesis maianeas*, *Mycalesis orseis*, *Neorina lowii* and *Zeuxidia amethystus*, showing close similarity to the species trapped at banana in the present study(*Amathuxidia amythaon*, *Charaxes durfordi*, *Prothoe franck*, *Melantis phedima*, *Mycalesis maianeas*, *Mycalesis orseis*, *Neorina lowii*).

The diversity of butterflies collected by banana, papaya and watermelon was similar (Figure 3.2). Four species were collected by all three fruits (*Dophla evelina*, *Mycalesis maianeaus*, *Mycalesis orseis* and *Neorina lowii)* and together, banana, papaya and watermelon accounted for more than 60% of the species collected. However, fifteen species of butterflies were only collected at one type of fruit.

Butterflies rely on scent to find food hidden under leaf litter in the forest (Sourakov *et al*., 2012). Sourakov *et al*., (2012) reported that volatile compounds released by rotting fruit can guide butterflies during foraging. They found that ripe banana produced the highest amounts of volatile compounds, perhaps suggesting why banana has been found to be the most effective fruit bait. Other studies also show that odor preferences may vary among butterfly genera and that species differ in their ability to find preferred food (Molleman *et al*., 2005b). Color vision also plays a role in food source detection by butterflies (Zaccardi *et al*., 2006). Monarch butterflies (Nymphalidae) have been shown to have strong innate preference for orange, yellow or red flowers (Blackiston *et al*., 2011) and the study by Omura & Honda (2005) showed that yellow color was the preference color by *Vanessa indica* (Nymphalidae) and it depends on flower's color during flower visiting. In this study, I found that most of the species were attracted to yellow fruit flesh.

My study found that banana was the most effective fruit bait to trap *Mycalsesis orseis* in the study site. The average numbers of species per trapcompared against ten types of fruit baithave been shown in Figure 3.3. The average number of species per trap indicated that banana attracted the highest average number of species per trap (three species) compared to other fruit bait. Figure 3.4 shows the changes in species composition among ten types of fruit bait in this study. I found that the banana obtained the highestspecies composition of butterflies, while chiku obtained the lowest species composition of butterflies. Banana baited traps can also be used to access the vertical habitats of fruit feeding butterflies (Tangah *et al.*, 2004). I would recommend the use of traps baited with banana or with a combination of fruit (banana, papaya and watermelon) to collect fruit feeding butterflies in Peninsular Malaysia.

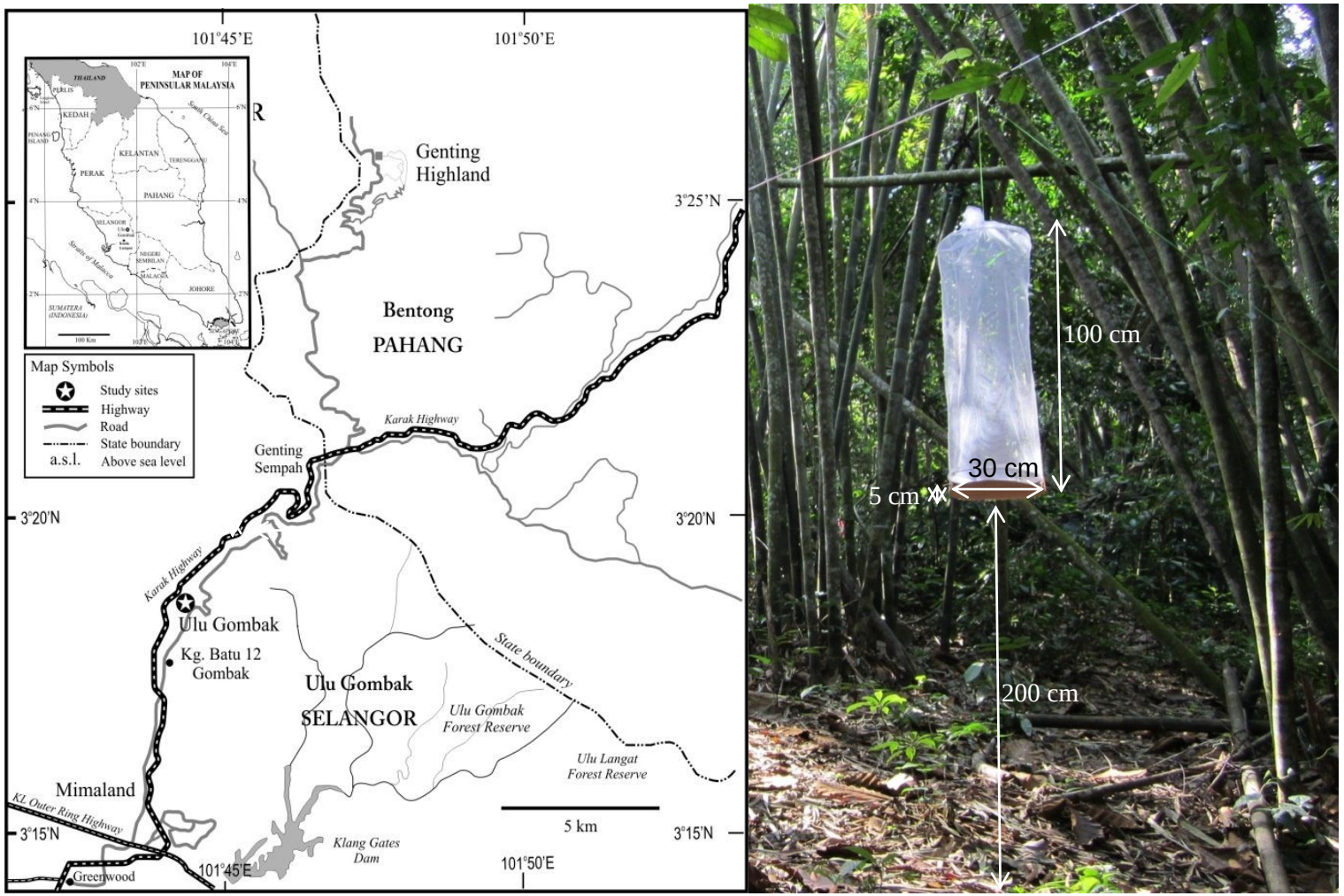

Figure 3.1 A butterfly bait trap in position at Ulu Gombak, Selangor

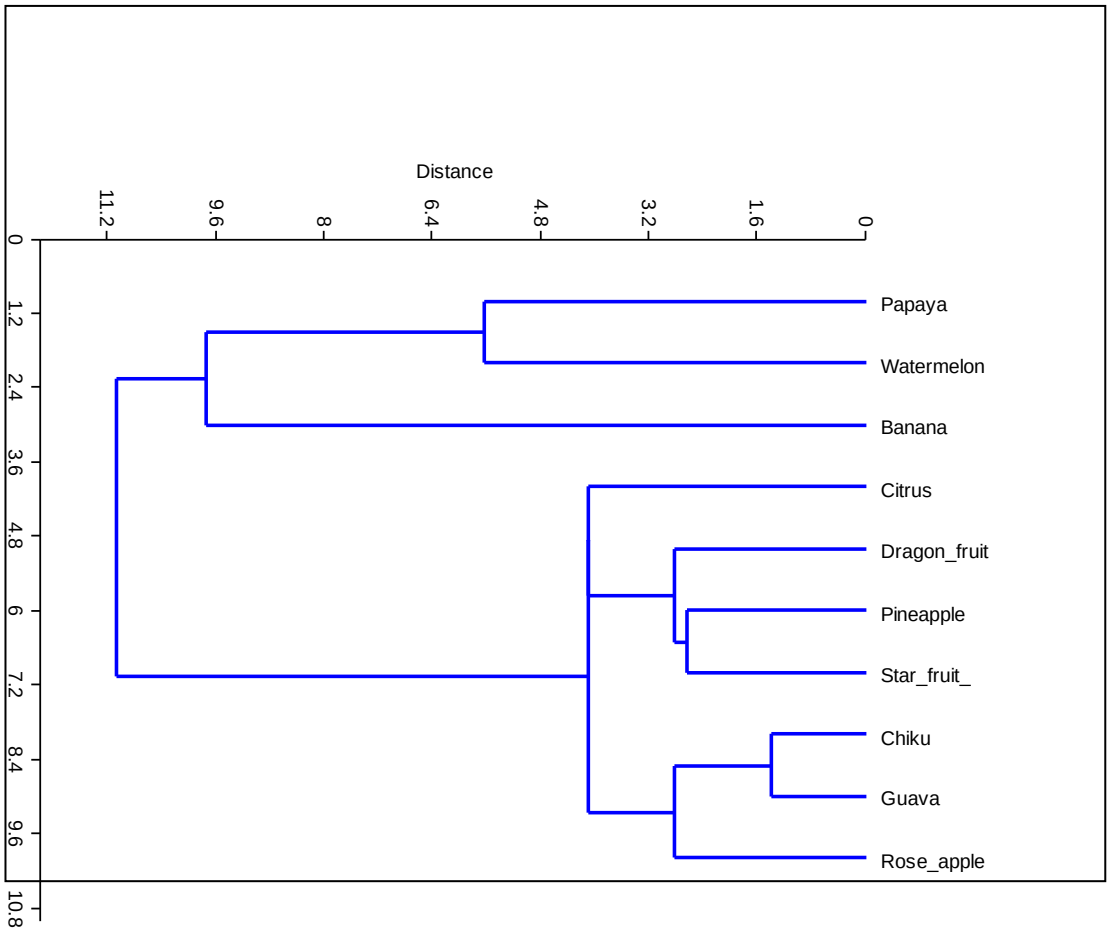

Figure 3.2 Dendrogram showing degree of similarity in butterfly species richness among ten types of fruit bait based on a cluster analysis of Bray-Curtis similarity indices.

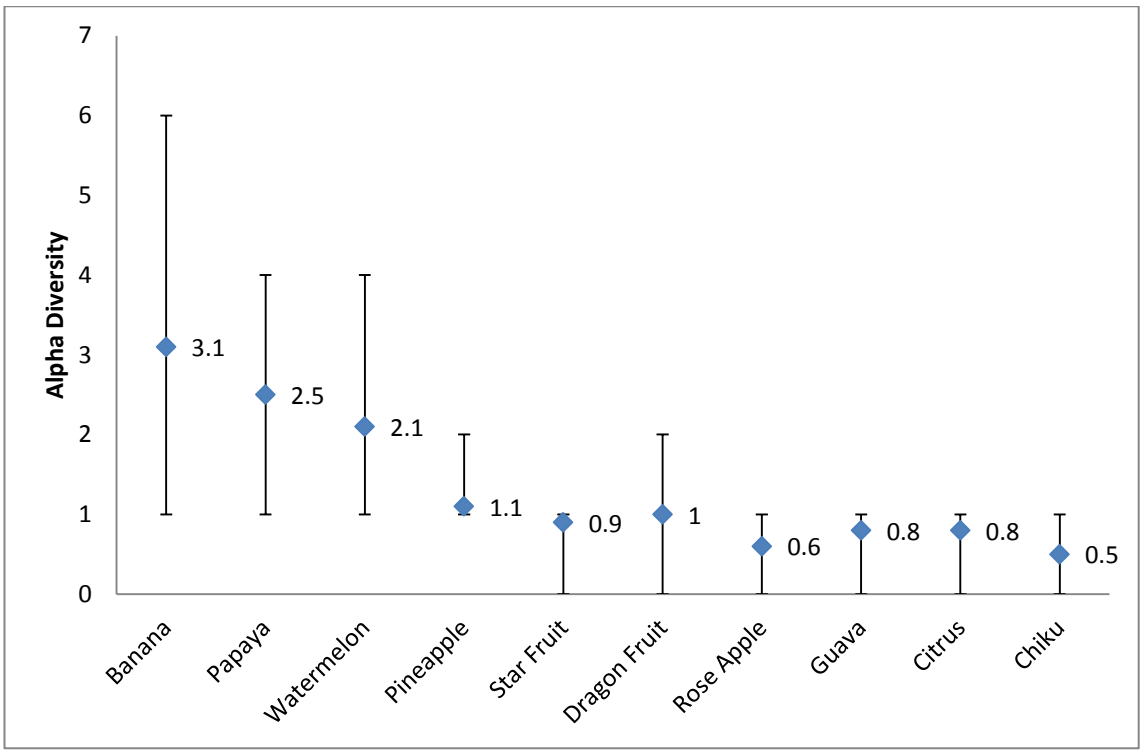

Figure 3.3 The range for number of species per trap against ten types of fruit bait.

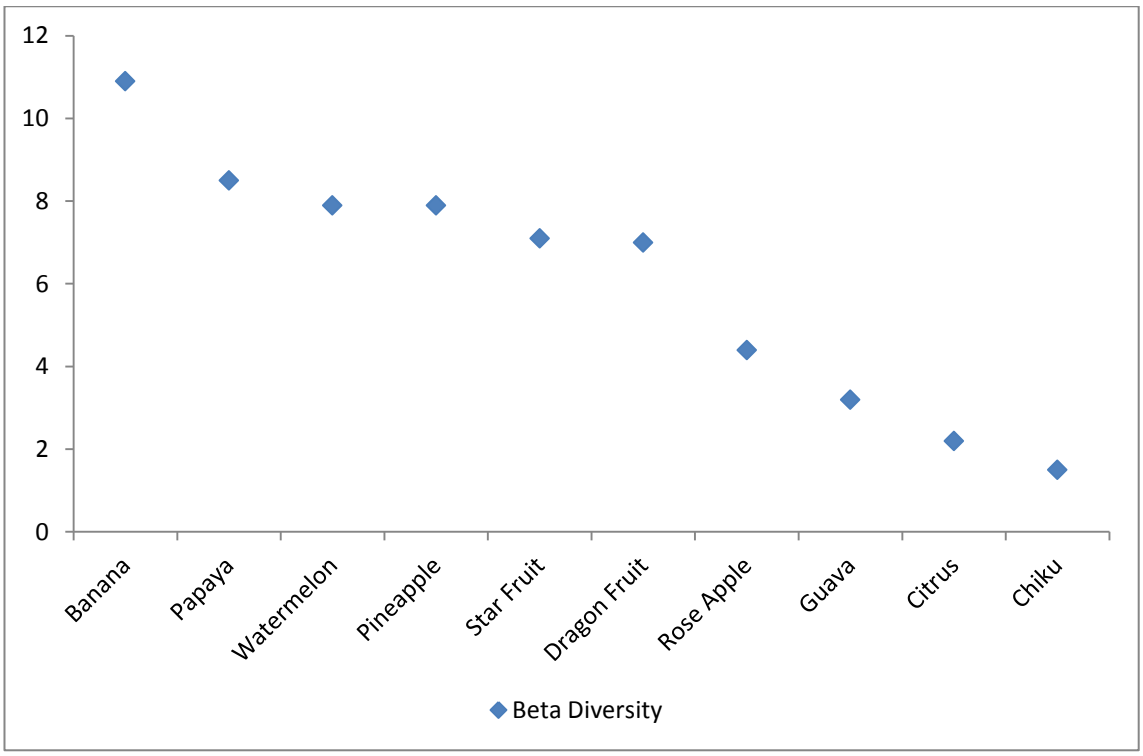

Figure 3.4 The changes in species composition against ten types of fruit bait.

Table 3.1 List of butterflies trapped in bait traps baited with ten different fruit types.

	Banana	**Chiku**	**Citrus**	**Dragon Fruit**	**Guava**	**Papaya**	**Pineapple**	**Starfruit**	**Rose apple**	**Watermelon**	**Total**
Amathuxidia amythaon	2	0	0	1	0	1	1	1	1	0	7
Charaxes durnfordi	0	0	0	0	0	0	0	**1**	0	0	1
Discophora timora	0	0	0	0	0	0	0	1	0	1	2
Dophla evelina	1	0	0	1	0	1	1	0	0	1	5
Erites angularis	0	0	0	0	0	**1**	0	0	0	0	1
Erites argentina	0	0	0	0	0	0	0	0	**1**	0	1
Erites medura	**1**	0	0	0	0	0	0	0	0	0	1
Euthalia ipona	0	0	0	0	0	0	0	0	**1**	0	1
Faunis canens	0	0	0	0	0	**1**	0	0	0	0	1
Lexias cyanipardus	**1**	0	0	0	0	0	0	0	0	0	1
Melanitis leda	0	0	0	0	0	0	0	0	0	**1**	1
Melanitis phedima	3	0	0	1	0	0	1	1	0	0	6
Mycalesis intermedia	0	0	0	2	0	4	1	1	0	1	9
Mycalesis janardana	0	0	0	0	0	2	0	0	0	0	2
Mycalesis maianeaus	9	4	0	2	4	10	2	1	2	12	46
Mycalesis mineus	7	0	1	0	1	0	0	0	0	0	9
Mycalesis orseis	14	3	6	5	3	12	6	6	2	13	70
Mycalesis persus	0	0	0	0	**1**	0	0	0	0	0	1
Neorina lowii	1	0	0	0	0	2	0	0	0	1	4
Paralaxita damajanti	0	0	0	0	0	0	**1**	0	0	0	1
Prothoe franck	3	0	0	0	0	1	0	0	0	0	4
Ragadia makuta	2	0	3	0	0	0	0	0	0	1	6
Tanaecia aruna	0	0	0	1	0	0	1	0	0	1	3
Tanaecia godarti	0	0	0	**1**	0	0	0	0	0	0	1
Tanaecia pelea	2	0	0	0	0	2	2	1	0	0	7
Taneacia iapis	**1**	0	0	0	0	0	0	0	0	0	1
Zeuxidia aurelius	**1**	0	0	0	0	0	0	0	0	0	1
Zeuxidia doubledayi	0	0	0	0	0	0	0	0	0	**1**	1
Total number of individuals	**48**	**7**	**10**	**14**	**9**	**37**	**16**	**13**	**7**	**33**	**194**

CHAPTER 4

DIVERSITY PATTERNS OF BUTTERFLY COMMUNITIES (LEPIDOPTERA) AT DIFFERENT ELEVATIONS IN GENTING HIGHLANDS, PENINSULAR MALAYSIA

4.1 Introduction

Butterflies are a group of specialized day-flying Lepidoptera whose ecological role in an ecosystem is not only as herbivores, but also as pollinators, seed dispersers and prey (Corbet & Pendlebury, 1992; Nelson, 2007; Sreekumar & Balakrishnan, 2001). They are among the most easily recognizable of all animals through their wings, which are colourful and opaque, with a characteristic shape. Butterflies can be found in most places on earth, from the barrens of subarctic zones, to the jungle streams of the tropics, to backyard plots in the most densely populated human cities. Consequently, changes in butterfly diversity have been used to monitor and to provide an early warning of environmental change (Sreekumar & Balakrishnan, 2001), including habitat disturbance (Spitzer *et al*., 1997).

Habitat disturbance can cause some changes in light levels, vegetation composition, temperature and humidity (Sparrow *et al*., 1994; Sawchik *et al*. 2005).Survival of butterflies can be treated with the loss of vegetation, which offer particular structural elements and suitable microclimates for sun basking, mating, breeding and nectaring (Dover *et al*., 1997; Wiklind, 1984). Vegetation is known to vary with elevation in the tropics (Lien & Yuan, 2003).

Studying the effect of elevation on species diversity can provide predictive information on the distribution of organisms in the environment, biogeographical patterns and factors influencing the structure of communities (Sanchez-Rodriguez & Baz, 1995). Several studies indicate that butterfly diversity is influenced by the

elevation (Kumar *et al*., 2009; Pyrcz *et al*., 2009; Sreekumar & Balakrishnan, 2001; Subahar *et al*., 2007). Lawton *et al*. (1987) suggested that the observed decline in insect diversity with elevation is caused by reduced habitat area, reduced resource diversity, increasingly unfavourable environmental condition (lower temperature, lower humidity, strong wind) and reduced primary productivity at higher elevations. Likewise, changes in vegetation structure and lower plant diversity have been linked to the declination of insect diversity at higher elevation (Cavieres *et al*., 2000;Brehm*et al*., 2003; Pyrcz, 2009).

In this study, i investigated how butterfly diversity is relates to the elevation at the Genting Highlands, Peninsular Malaysia. To my knowledge there have been no comprehensive inventories or systematic comparative studies on the butterfly diversity at different elevations in Peninsular Malaysia. An inventory of butterfly diversity at six sites has been presented, and this inventory provides a useful benchmark for future monitoring of environmental changes in these sites.

4.2 Materials and Methods

Butterflies were surveyed at six sites of Genting Highlands where located in the Titiwangsa Mountains, Peninsular Malaysia(Figure 4.1; Table 4.1). The Titiwangsa Mountains are known as the backbone of Peninsular Malaysiaand it starts from the north of the Southern Thailand, across the border of Malaysia, and then to the valley of Negeri Sembilan, and then it ends in the south near Jelebu, Negeri Sembilan. Gunung Korbun is the highest mountain in the Titiwangsa range with an elevation of 2,183 m a.s.l.The six sampling sites were separated by intervals of approximately 300m of elevation.

Butterfly sampling was conducted using the transect method modified from the Pollard Walk described by Pollard (1977). Sampling was carried out in two

rounds,whereby the first round was conducted between January and June 2011, and the second round was conducted between July and December 2011. Five transects of 100m x 10m were established in each site.All butterflies occurred with 2.5 meter to the left, right or in front were captured by sweep net. Each transect was surveyed five times, twice in each round. All of the butterflies were sampled by two collectorswalking in a constant pace along each transect with a sweep net. The sampling duration for each transect was between 45 and 60 minutes. The butterfly sampling took place from 0900 to 1200, and 1400 to 1600, which corresponds to the peak activity period for butterflies.Walks were abandoned during cloudy and rainy conditions and repeated on the next sunny day. Temperature and humidity at each site were measured using a data logger.

The butterflies were sacrificed in the field, pinned, oven dried, and identified (without dissection) using Corbet & Pendlebury (1992). Samples from selected specimens were sent for DNA barcode analysis at the Biodiversity Institute of Ontario, Canada. Several identifications were corrected on the basis of the DNA barcode sequences. All collected specimens were deposited at the Museum of Zoology, University of Malaya (UMKL).

Four indices were used to assess diversity at each site; the Shannon-Weiner diversity index (H'), the Shannon evenness (E), Margalef's index (Dmg), and the Berger-Parker index (d) were calculated in PAST (Hammer, 1999).

$$H' = -\sum\left(\frac{\mathrm{n}i}{\mathrm{N}}\right)\ln\left(\frac{\mathrm{n}i}{\mathrm{N}}\right)$$

N = number of individuals sampled, n*i* = number of individuals belonging to the *i*th species.

$$\mathrm{E} = \frac{\mathrm{H}'}{\ln \mathrm{S}}$$

S = number of species

$$D_{mg} = \frac{S - 1}{\ln(N)}$$

$$d = \frac{N_{max}}{N}$$

N_{max}=the number of individual of the most dominant species.

Spearman rank correlation was used to test for any association between the indices and elevation. Similarity between sites was determined by using the Jaccard's similarity indices calculated in PAST (Hammer, 1999).

4.3 Results

A total of 2, 876 individuals from 214 species (Appendix 2), representing all the butterfly families, were collected.The most abundant species in the Genting Highlands was *Ypthima pandocus* (Nymphalidae) (n=718), followed by *Eurema hecabe* (Pieridae) (n= 194) and *Jamides pura* (Lycaenidae) (n= 62).

S2 (480m a.s.l.) yielded the highest number of species (n=118) and S6 (1,700m a.s.l.) yielded the lowest number of species (n=34).The highest diversity (H' = 3.882) was recorded at S2 (480m a.s.l.) whereas the lowest diversity (H' = 2.642) was recorded at S6 (1,750m a.s.l.).

The similarity of study sites was divided neatly based on elevation, with the first split between the high elevation sites and the mid/low elevation sites. The next split was between the low elevation sites and the mid elevation sites (Figure 4.2).

All of the sites had an uneven distribution due to dominant species (Figure 4.2). This dominance effect was most pronounced at S5, and the dominant species was *Ypthima pandocus* (d = 0.3374; 192 individuals).

The Spearman rank correlation showed that elevation was significantly correlated with diversity ($p<0.01$), except when measured as species richness ($p=0.015$) and evenness ($p=0.154$). Diversity (all measures) was not significantly correlated with

temperature ($p>0.01$). The strongest correlation was seen between humidity and diversity ($p<0.01$), for all indices except evenness ($p=0.201$). All of the environmental parameters were highly correlated with each other ($p<0.01$).

4.4 Discussion

The total number of species collected in this study (214) constituted about 21% of the total 1031 species of butterflies found in Peninsular Malaysia (Corbet & Pendlebury, 1992). Nymphalidae was the most common butterfly family with the highest number of species (n=86) and individuals (n=1599) encountered. *Ypthima pandocus* was the most abundant species and was observed at all sites in high numbers. The domination of *Ypthima pandocus*at all the study sites isprobably related to its food plant (Gramineae) which was also very common across the range.

All six sampling sites had an uneven distribution of species with a dominant species occurring at each site. This species was the ubiquitous *Ypthima pandocus*. Other common species were *Euploea tulliolus*, *Euploea radamanthus* and *Jamides pura*– which frequent in open places. In this study, three genera (*Mycalesis*, *Ypthima*, *Neptis*) and most of the hesperiids were often found in the shrub and bushy plant areas. *Eurema* and *Ypthima* were frequently found in the evergreen, semi-evergreen and open forest. I observed that some species were associated with particular sites, for example, four species of lycaenid (*Udara camenae*, *Udara toxopeusi*, *Udara placidula* and *Udara selma*) were found in high abundance at the high elevation, high humidity and low temperature sites. *Ideopsis vulgaris* was confined to the mid elevation. The five genera (*Eurema*, *Catopsilia*, *Appias*, *Delias*, and *Euploea*) were commonly found at the open plain area, demonstrating a 'light-loving' proclivity. Mud-puddling is a well-known behaviour among some tropical butterfly groups (Beck *et al*., 1999). During my

sampling days, Ihave observed *Cyrestis*, *Jamides*, *Delias* and *Appias* seeking moisture on the forest floor.

Species richness showed an increase with elevation between the first and second lowest site, 480m a.s.l., after which richness declined. Janzen (1973) and Janzen *et al*. (1976) concluded that the species richness in the tropics is high at middle elevation rather than at low elevation,with the higher net accumulation of photosynthate at mid elevation providing a larger resource base for herbivourous insects (also see Viejo *et al*., 1989; Wang *et al*., 2012). Other authors suggested that, the decrease in diversity with increasing elevation may be caused by the harshness of environmental condition and area reduction (Lawton *et al*., 1987). I found that butterfly diversity (although not species richness) was significantly negatively correlated with elevation along the Titiwangsa Mountains. This is in general agreement with other studies which showed that community structure, abundance and diversity of insects were negatively correlated with elevation (MacArthur, 1972; McCoy, 1990; Rahbek, 1995). Lien & Yuan (2003) reported that butterflydiversity, species richness and species abundance in the low elevation habitats were higher than in high elevation habitats, the butterfliy diversitydiffers significantly in high and low elevation sites.

My findings showed that humidity, but not temperature, had a significant correlation with butterfly diversity along the Titiwangsa Mountains. This is surprising because temperature is one of the key environmental factors affecting most Lepidoptera with direct effects on larval behaviour (Broersma *et al*., 1976). Also, temperature is one of the important environmental factors to influence butterfly behaviour, development, survival and reproduction (Andrew & Hughes, 2005). Additionally, butterfly flight is highly constrained by temperature, and they can spend more time in flight at high ambient temperature (Heinrich, 1986). Previous studies have demonstrated a significant

correlation between temperature and diversity (Pollard, 1988; Roy *et al*., 2001; Turner *et al*., 1987).

Table 4.1 Ecological description of sampling sites

Location of Study Site	Code	Date of Samplings	Geographical Information	Elevation (m a.s.l)*	Elevation Category	Mean Temperature (℃)*	Mean Humidity (%)*
Gombak, Selangor	S1	22-24/3/2011 29-30/3/2011 21-23/9/2011 27-28/9/2011	3°19' 27.10"N 101°45'09.94"E	200	Low	23.83	42.93
Kampung Janda Baik, Pahang	S2	28/2/2011 1-4/3/2011 26-28/10/2011 30-31/10/2011	3°20'12.81"N 101°51'17.64"E	480	Low	25.504	57.66
Bukit Tinggi, Pahang	S3	19-23/4/2011 2-4/11/2011 9-10/11/2011	3°23'17.81"N 101°50'30.83"E	890	Medium	24.90	67.83
Awana, Pahang	S4	30-31/5/2011 1,3,6/6/2011 3-5/12/2011 8-9/12/2011	3°24'09.87"N 101°46'54.61"E	1,064	Medium	21.83	73.67
Chin Swee Cave Temple, Pahang	S5	2,5,7,16-17/6/2011 10-12/12/2011 18-19 December 2011	3°24'50.16"N 101°47'13.59"E	1,490	High	20.12	85.40
Bukit Cincin, Pahang	S6	24-28/5/2011 26-28/11/2011 1-2/12/201	3°26'21.39"N 101°46'59.36"E	1,750	High	19.24	93.26

*These variable were highly correlated (P<0.01)

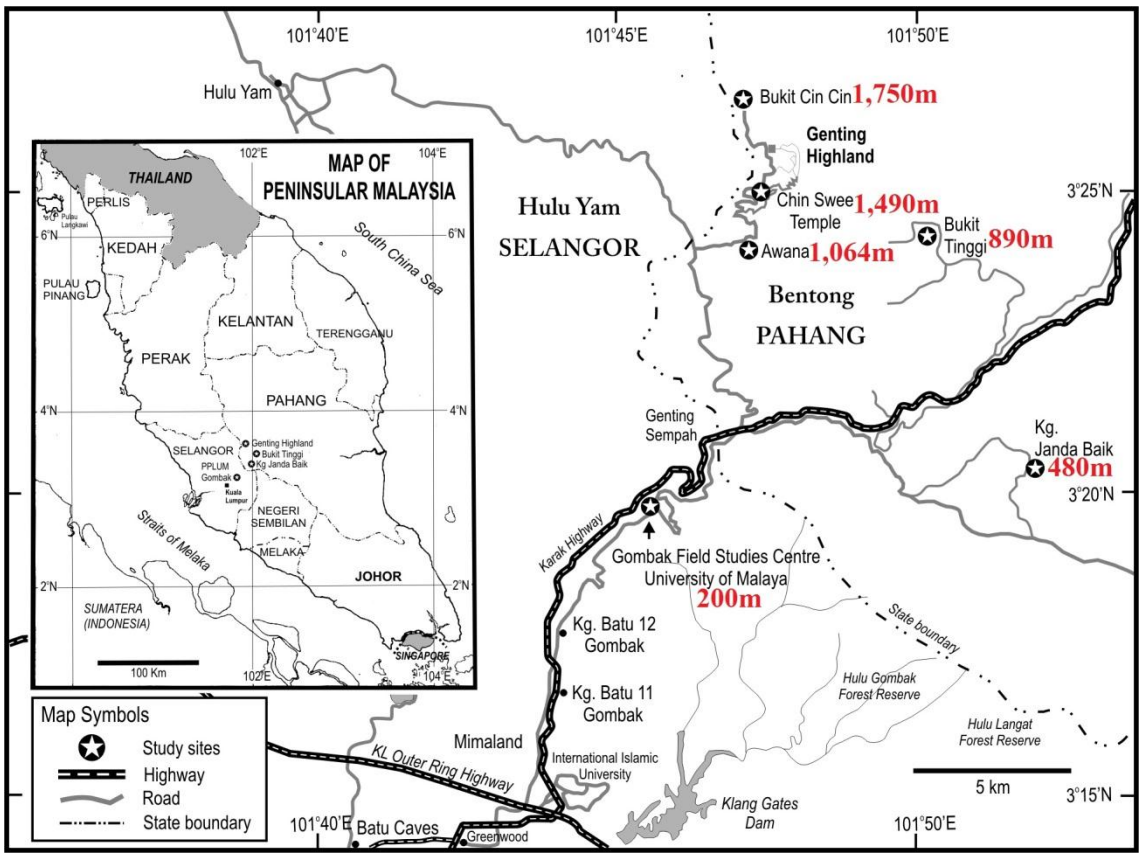

Figure 4.1 Location of sampling sites (above sea level): Gombak (S1), Janda Baik (S2), Bukit Tinggi (S3), Awana Genting (S4), Chin Swee Cave Temple (S5), Bukit Cincin (S6).

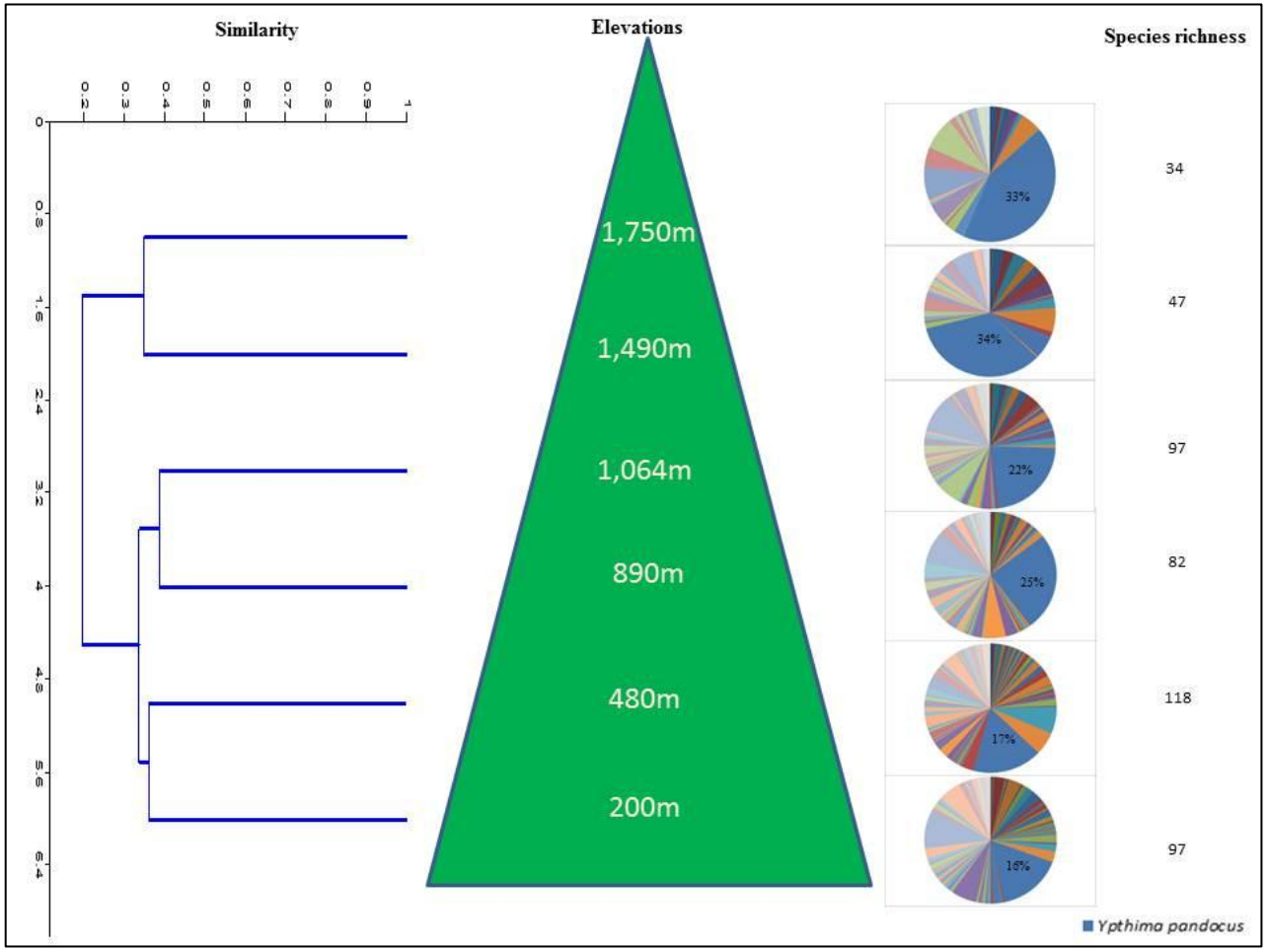

Figure 4.1 The Jaccard similarity clustering graph of butterfly species and composition of butterflies at different elevations in the Genting Highlands.The legend shows the most dominant species.

CHAPTER 5

RESOLVING THE IDENTITIES OF THE *GRAPHIUM* (LEPIDOPTERA: PAPILIONIDAE) BUTTERFLIES OF PENINSULAR MALAYSIA

5.1 Introduction

Graphium s.l.is a genus of swallowtail butterflies (Papilionidae) commonly known as the swordtails or kite swallowtails. In peninsular Malaysia, *Graphium* species are frequently found at puddles and along riverbanks, with one of the most common and widespread species being *Graphium sarpedon luctatius*, colloquially known as the common bluebottle or blue triangle. Other well-known species include *Graphium agamemnon agamemnon*, *Graphium antiphates itamputi*, and *Graphium doson evemonides*. The colourful appearance and unique shape of *Graphium* wings have made the species popular with collectors and butterfly watchers, and moreover, specimens can often be found in frames for sale as souvenirs in most of the tourist places in Malaysia. Despite their popularity the taxonomy of *Graphium* species is unresolved and inconsistently applied.

Butterfly enthusiasts may be familiar with the five species of *Graphium*, one species of *Pathysa* and two species of *Paranticopsis*, featured in the introduction to Malaysian butterflies by Yong (1983). Nine species of *Graphium* and seven species of *Pathysa* were listed in "The Butterflies of the Malay Peninsula" checklist (Corbet & Pendelbury, 1992) which was consistent with Igarashi's (1984) and Miller's (1987) treatment of *Graphium* s.s and *Pathysa* as sister genera. Previously authors had classified *Pathysa* as a subgenus of *Graphium* (Munroe, 1961; Saigusa *et al*., 1977) with three: *Graphium, Pathysa* and *Arisbe* (Munroe, 1961); or four: *Graphium, Pathysa, Arisbe, andPazala* (Hancock, 1983), other subgenera. More recently *Pathysa* had again been subsumed under *Graphium* (Makita *et al*., 2003). The monophyly of *Graphium* s.l.

with regards to the closely related genus *Lamproptera* has also been questioned (Igarashi, 1984; Makita *et al*., 2003).

Morphological identification of *Graphium* species is difficult, even for experts, requiring the examination of subtle differences in the banding, spotting and colour patterns on the wings (Corbet & Pendelbury, 1992). In swallowtails such differences are often complicated by age, population and seasonal variations (e.g. Collins & Morris, 1985; Scott, 1986; Larsen, 1991). In Peninsular Malaysia, *Graphium bathycles bathycloides* and *Graphium chironidesmalayanum* are particularly hard to distinguish from each other (Corbet & Pendelbury, 1992) and have variously been treated as subspecies of the same or different species under various names (Elliot, 1982; Collins & Morris, 1985).

In this study, I sequenced the COI mtDNA barcode (Hebert *et al*. , 2003) for 35 individuals belonging to 11 species of *Graphium* s.l. native to Peninsular Malaysia (Corbet & Pendelbury, 1992) to test the utility of DNA barcoding for the identification of *Graphium* species. In addition, I sequenced the 28S rRNA gene of 12 specimens and in combination with the DNA barcodes used this data to examine the phylogenetic relationships of these species, and then investigated the validity of *Pathysa* and *Paranticopsis* as distinct genera or as subgenera of *Graphium*.

5.2 Materials and Methods

Based on availability and the age of the specimens, a few representatives from the Museum of Zoology, University of Malaya (UMKL) collection of *Graphium* were selected for DNA sequencing (Table 1). COI mtDNA barcodes were generated at UMKL or the Canadian Centre for DNA Barcoding using standard protocols (Wilson, 2012). 28S rRNA sequences were generated at UMKL from the same DNA extracts using the primers: 28SD2-F (5'-AGAGAGAGTTCAAGAGTACGTG-3') and 28SD2-

R (5'-TTGGTCCGTGTTTCAAGACGGG-3') (Belshaw and Quicke, 1997).Details of the specimens and DNA sequences (including GenBank accession numbers) are available on BOLD (Ratnasingham and Hebert, 2007) in the Public Dataset: DS-GRAPH.

Our newly assembled dataset was combined with 13 sequences of COI and 14 sequences of 28s rRNA for *Graphium* species,found in Peninsular Malaysia, publicly available on GenBank or BOLD. *Lampropterameges virescens* was also included (from UMKL collection and GenBank) along with *Papilio* outgroups (from GenBank) (Table 1). Maximum parsimony phylogenetic analyses were conducted in MEGA 5.0 (Tamura et al., 2011) using default settings and were tested for 'reliability' using 1000 bootstrap replicates.

5.3 Results and Discussion

5.3.1 *Graphium bathycles bathycloides*and *Graphium chironides malayanum*

There are two *Graphium* species in Peninsular Malaysia that are particularly hard to distinguish based on morphology – *G. bathycles bathycloides* and *G. chironides malayanum* (Eliot, 1982). Globally *G. bathycles* is considered to comprise two subspecies, with the nominate subspecies found in Java, and *G. bathycles bathycloides* having a wider distribution, being found throughout Southeast Asia. *Graphium chironides*, originally described as *G. chiron*, a name still used by some authors (Collins & Morris, 1985), has a more confused taxonomic history. Subsumed as a subspecies of *G. bathycles* by Rothschild (1895), the taxon was more recently raised as a distinct species by Saigusa*et al*. (1977) with a second subspecies, *G. chironides malayanus*, described for Peninsular Malaysia (Eliot 1982).

My originalspecimens have been assigned to both species names (by MSA) in UMKL. The identical or near identical DNA barcode sequences (1bp difference)

motivated me to examine the morphology of these specimens in further detail, alongside images of the type specimens provided by the Natural History Museum, London. I examined the UMKL specimens and the photographs of the types for the diagnostic characters described in Corbet & Pendlebury (1992) and in the description of *G. chironides malayanum* (Eliot, 1982) (Figure 5.1). The determination of many character states proved to be ambiguous. For example, for character D (Table 5.2), the postdiscal markings in spaces 1b and 4 are 'richer orange' for *G. bathycles bathycloides*, but 'orange-yellow' for *G. chironidesmalayanum*. I found it difficult to objectively determine either of these character states to the specimens (Figure 5.1) given the subjective nature of colour-based characters and the tendency for colours to fade on specimens. For character B, *G. bathyclesbathycloides* has 'little more than a dot' in the space 5 on the upperside forewing, while *G. chironides malayanum* has a spot 2-2.25mm wide. I found a marking around 1mm wide in all our specimens, more substantial than a dot but too small for *G. chironides malayanum* (Figure 5.1). I found that the simplest character to score objectively was character G (Table 5.2) "a long window in the central part of space 1b" on the underside hindwing. Rothschild (1895) describes this character as being absent (usually) in *G. bathyclesbathycloides* and of variable length in *G. chironides*. I observed both character states amongst the specimens, however, the 'window', when present, was considerably less substantial than that exhibited by the *G. chironides malayanum* holotype (Figure 5.1). Ultimately, morphological diagnosis could not be achieved unambiguously as the specimens overlapped for the diagnostic characters reported for each taxon (Table 5.2).

The GenBank COI sequence for *G. chironides chironides* from China (HM246463) was the closest match to my DNA barcodes for this group of specimens but was distinct (2.91% Kimura-2-parameter distance). I had requested an image of this specimen from the sequence authors, but didn't receive any response. Given that *G.*

chironides chironides (such as found in China) is morphologically and molecularly distinct from *G. bathycles* it seems to represent a 'good' species, however, the status of subspecies *G. chironides malayanum* from Peninsular Malaysia remains in doubt. My observation of overlapping morphological character states supports the treatment of *G. bathycles bathycloides* and *G. chironides malayanum* as a single taxon. In the absence of further evidence, the author subsequently assigned all my specimens to *G. bathycles bathycloides*, the more common taxon in Peninsular Malaysia (Corbet and Pendelbury, 1992).

5.3.2 DNA barcoding of *Graphium*species from Peninsular Malaysia

Besides the case discussed above, all species of *Graphium* possessed a distinctive cluster of DNA barcodes (Figure 5.2). Excluding short sequences (307bp) which gave spurious distances, the DNA barcodes showed low intraspecific divergences, maximum 0.77% K2P distance, whereas the minimum K2P distance between sister-species was 2.82%. Interestingly*G. aristeus hermocrates* showed deep divergence (4.13% Kimura-2-parameter distance) from other *G. aristeus* on BOLD from Australia and Papua New Guinea, most likely *G. aristeus parmatus,* suggesting multiple species may currently be residing under this name.

5.3.3 Phylogenetic relationships within *Graphium*and the validity of*Pathysa*and*Paranticopsis*

The species of *Paranticopsis* (Yong, 1983) formed a clade in my phylogenetic analysis of COI. This clade was nested within a larger clade containing the *Pathysa* species (sensu Corbet & Pendelbury, 1992). The species of *Pathysa* are morphologically distinct with a long, slender tapering tail on the hindwing. The *Pathysa* clade was also recovered in the phylogenetic analysis of 28S rRNA with *G. macareus*, *G. delessertii*, *G. ramaceus* and *G. agetes*,exhibiting the same genotype. I recovered two genotypes from *G. agetes hermocrates*, with the second being shared with *G. antiphates*. *G. sarpedon* was sister to the *Pathysa* clade on the COI phylogeny and *G. aristeus* was sister to *Pathysa* + *G. sarpedon*. COI has been shown to have reliable phylogenetic signal at the genus level in Lepidoptera (Wilson, 2010) and this is further supported by the fact that the COI and 28S rRNA maximum parsimony trees were highly congruent. Both recovered *Graphium* s.l. as monophyletic in agreement with recent studies (Makita *et al*., 2003). The noticeable difference was the placement of *G. agamemnon* as sister to the '*eurypylus*-group' on the 28S rRNA phylogeny (Figure 5.3), however, there was a small number of parsimony informative characters for the 28S rRNA dataset. Both gene trees recovered *Pathysa* as monophyletic, however, in order for it to be a valid genus, at least three other clades within *Graphium* s.l. would also have to be raised as genera.

Table 5.1 List of specimens analyzed in this study.

Species	Locality	BOLD Process ID or GenBank Accession No	
		COI	28S
Graphium evemon eventus	Pahang, Malaysia	UMKC006-13	UMKC006-13
Graphium evemon eventus	Negeri Sembilan, Malaysia	BOPM019-12	
Graphium evemon	Thailand	KHCBT513-11	
Graphium evemon eventus	Perak, Malaysia		AB059693
Graphium eurypylus mecisteus	Pahang, Malaysia	UMKC005-13	UMKC005-12
Graphium eurypylus	Myanmar		AB059700
Graphium doson evemonides	Pahang, Malaysia	UMKC004-13	UMKC004-13
Graphium doson evemonides	Johor, Malaysia	BOPM018-12	
Graphium doson evemonides	Negeri Sembilan, Malaysia	BOPM120-12	
Graphium doson	India	EU792483	
Graphium doson	Thailand	KHCGE417-11	
Graphium doson	Taiwan		AB059697
Graphium sarpedon luctatius	Pahang, Malaysia	UMKC011-13	UMKC011-13
Graphium sarpedon luctatius	Negeri Sembilan, Malaysia	BOPM002-12	
Graphium sarpedon luctatius	Negeri Sembilan, Malaysia	BOPM003-12	
Graphium sarpedon nipponus	Korea	GU372548	
Graphium sarpedon	China	HM246464	
Graphium sarpedon	Thailand	KHCBT569-11	
Graphium sarpedon	Japan		AB060636
Graphium bathycles bathycloides	Pahang, Malaysia	UMKC002-13	UMKC002-13
Graphium bathycles bathycloides	Negeri Sembilan, Malaysia	BOPM016-12	
Graphium bathycles bathycloides	Negeri Sembilan, Malaysia	BOPM118-12	
Graphium chironides	China	HM246463	
Graphium chiron	Myanmar		AB059698
Graphium agamemnon agamemnon	Pahang, Malaysia	UMKC001-13	UMKC001-13
Graphium agamemnon	Unknown	AF170874	
Graphium agamemnon agamemnon	Johor, Malaysia	BOPM014-12	
Graphium agamemnon	China	HM246466	
Graphium agamemnon	Borneo, Malaysia		AB059707
Graphium arycles arycles	Negeri Sembilan, Malaysia	BOPM021-12	
Graphium bathycles bathycloides	Pahang, Malaysia	UMKC003-13	UMKC003-12
Graphium bathycles bathycloides	Negeri Sembilan, Malaysia	BOPM017-12	
Graphium bathycles bathycloides	Negeri Sembilan, Malaysia	BOPM119-12	
Graphium bathycles	Myanmar		AB059699
Graphium antiphates itamputi	Pahang, Malaysia	UMKC007-13	UMKC007-13
Graphium antiphates itamputi	Negeri Sembilan, Malaysia	BOPM024-12	
Graphium antiphates itamputi	Negeri Sembilan, Malaysia	BOPM025-12	
Graphium antiphates itamputi	Negeri Sembilan, Malaysia	BOPM114-12	
Graphium antiphates	Thailand	KHCBT505-11	
Graphium antiphates	Borneo, Malaysia		AB059691
Graphium delessertii delessertii	Pahang, Malaysia	UMKC008-13	UMKC008-13
Graphium delessertii delessertii	Negeri Sembilan, Malaysia	BOPM008-12	
Graphium delessertii delessertii	Negeri Sembilan, Malaysia	BOPM009-12	
Graphium delessertii delessertii	Negeri Sembilan, Malaysia	BOPM010-12	
Graphium ramaceus pendleburyi	Pahang, Malaysia	UMKC009-13	UMKC009-13
Graphium ramaceus pendleburyi	Selangor, Malaysia	BOPM004-12	
Graphium ramaceus pendleburyi	Selangor, Malaysia	BOPM005-12	
Graphium ramaceus pendleburyi	Malaysia	BOPM006-12	
Graphium macareus	Borneo, Malaysia		AB05987
Graphium aristeus hermocrates	Kedah, Malaysia	BOPM504-12	
Graphium agetes iponus	Kenaboi FR, Malaysia	UMKC010-13	UMKC010-13
Graphium agetes iponus	Negeri Sembilan, Malaysia	BOPM022-12	
Graphium agetes iponus	Negeri Sembilan, Malaysia	BOPM023-12	

Table 5.1 (Continued) List of specimens analyzed in this study.

Species	Locality	BOLD Process ID or GenBank Accession No	
		COI	28S
Graphium agetes	Malaysia		AB059685
Lamproptera meges	China	GQ268354	
Lamproptera meges	Perak, Malaysia		AB059735
Lampraptera meges virescens	Negeri Sembilan, Malaysia	BOPM117-12	
Lampraptera meges virescens	Negeri Sembilan, Malaysia	BOPM007-12	
Lamproptera curius curius	Kuala Lumpur, Malaysia	BOPM386-12	
Lamproptera curius	Myanmar		AB059736
Papilio rutulus	Unknown	AY954560	
Papilio rutulus	Unknown		AY954530
Papilio troilus	U.S.A	GU090089	
Papilio troilus	Unknown		AF423920.1

Table 5.2 Comparison of the diagnostic characters for the specimens of *Graphium bathycles bathycloides* and *Graphium chironides malayanum*.

	Specimen Number*							
Character	**BMNH(E) #149669**	**BMNH(E) #149397**	**UMKC002-13**	**UMKC003-13**	**BOPM016-12**	**BOPM017-12**	**BOPM118-12**	**BOPM119-12**
Forewing								
A Pale blue discal markings: Broader (1), Narrower (2)	1	0	1	0	1	0	1	1
B Spot in space 5: 2-2.5mm wide (1), little more than a dot (2)	?	?	1	1	1	0	1	1
Hindwing underside								
C Basal and sub-basal spots in space 8: Faintly yellowish (1), Pale blue (0)	1	0	1	1	1	1	1	0
D Postdiscal markings in spaces 1b-4: Orange-yellow, narrow (1), Richer orange, wider (0)	1	0	0	1	1	1	0	1
E Spot at base of space 3: Prominenet pale blue spot (1), absent or vestigial (0)	1	0	0	0	1	0	1	0
F Space 5: No additional stria present (1), Additional stria present (0)	?	?	?	?	?	?	?	?
G Black lines overlying veins 1b and 2: Well separated leaving a long window in the central part of space 1b (1), More or less coalesced (0)	1	0	1	1	0	0	1	0

*****These are the types from BMNH (BMNH(E)#149669 is *G. chironides malayanum*, BMNH(E)#149397 is *G. bathycles bathycloides*) and the BOLD process IDs.

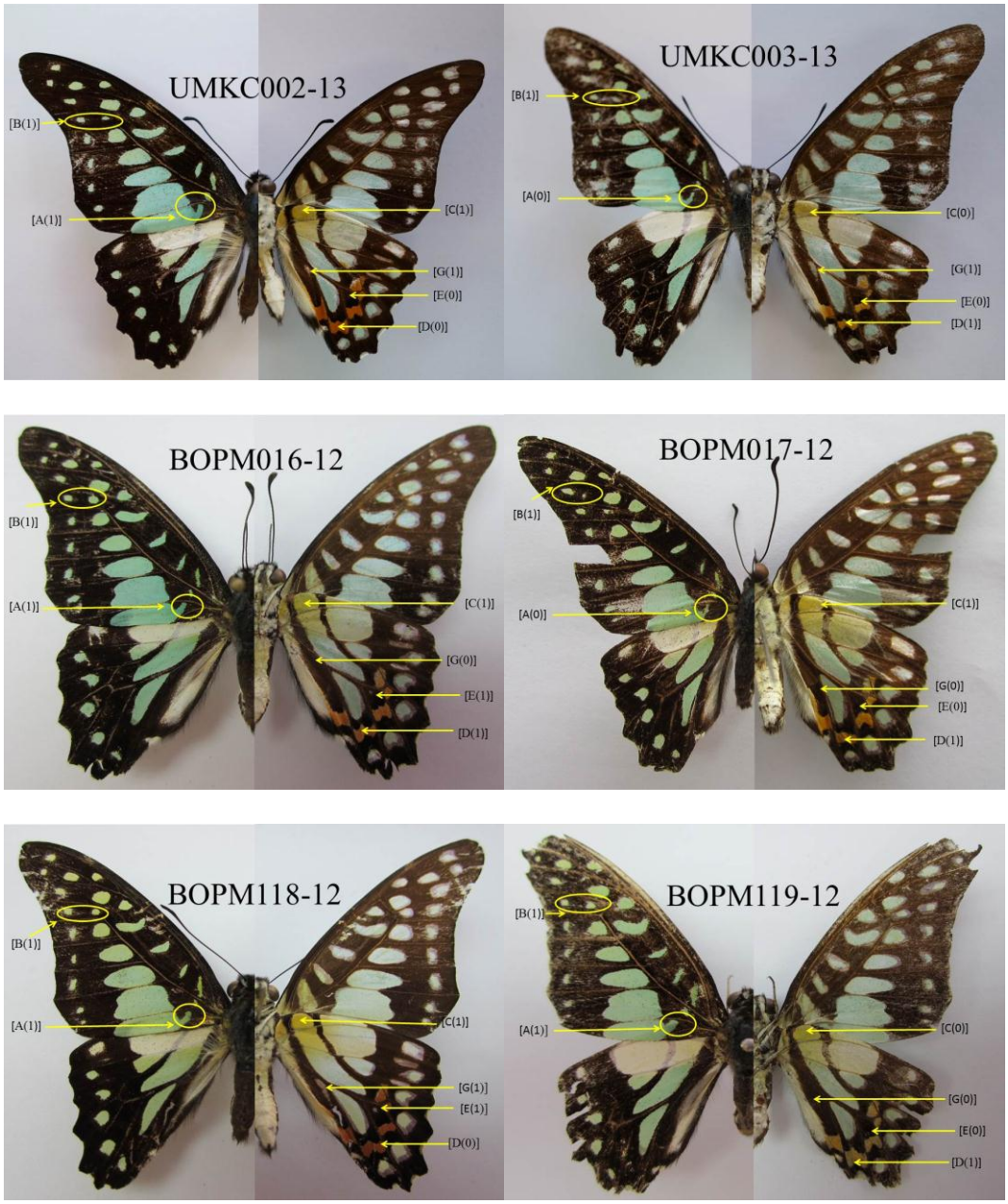

Figure 5.1 Specimens in UMKL examined for the diagnostic characters for *Graphium bathycles bathycloides* and *Graphium chironides malayanum* listed in Corbet & Pendlebury (1992).

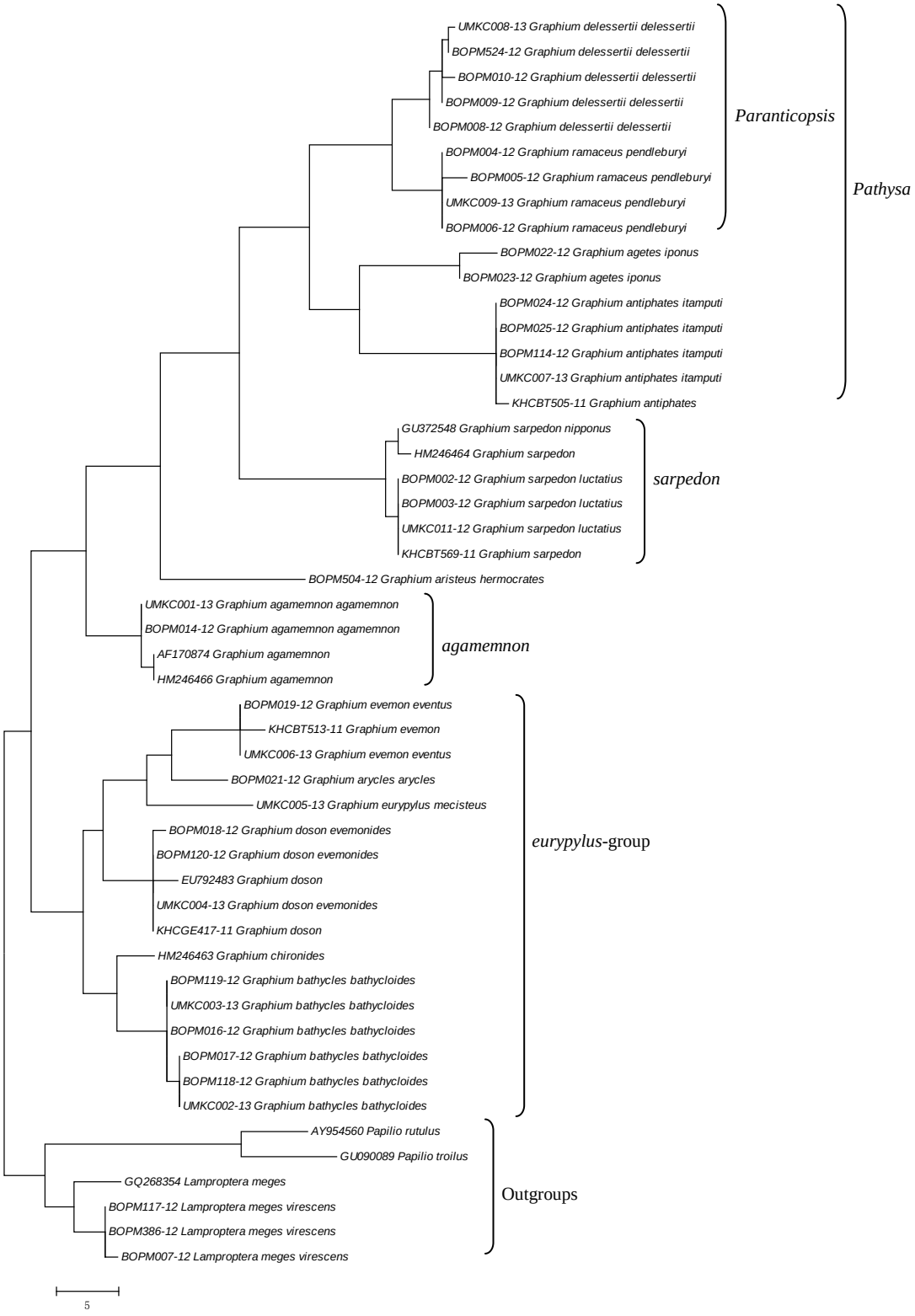

Figure 5.2 Maximum parsimony phenogram for COI barcodes for *Graphium* species found in Peninsular Malaysia.

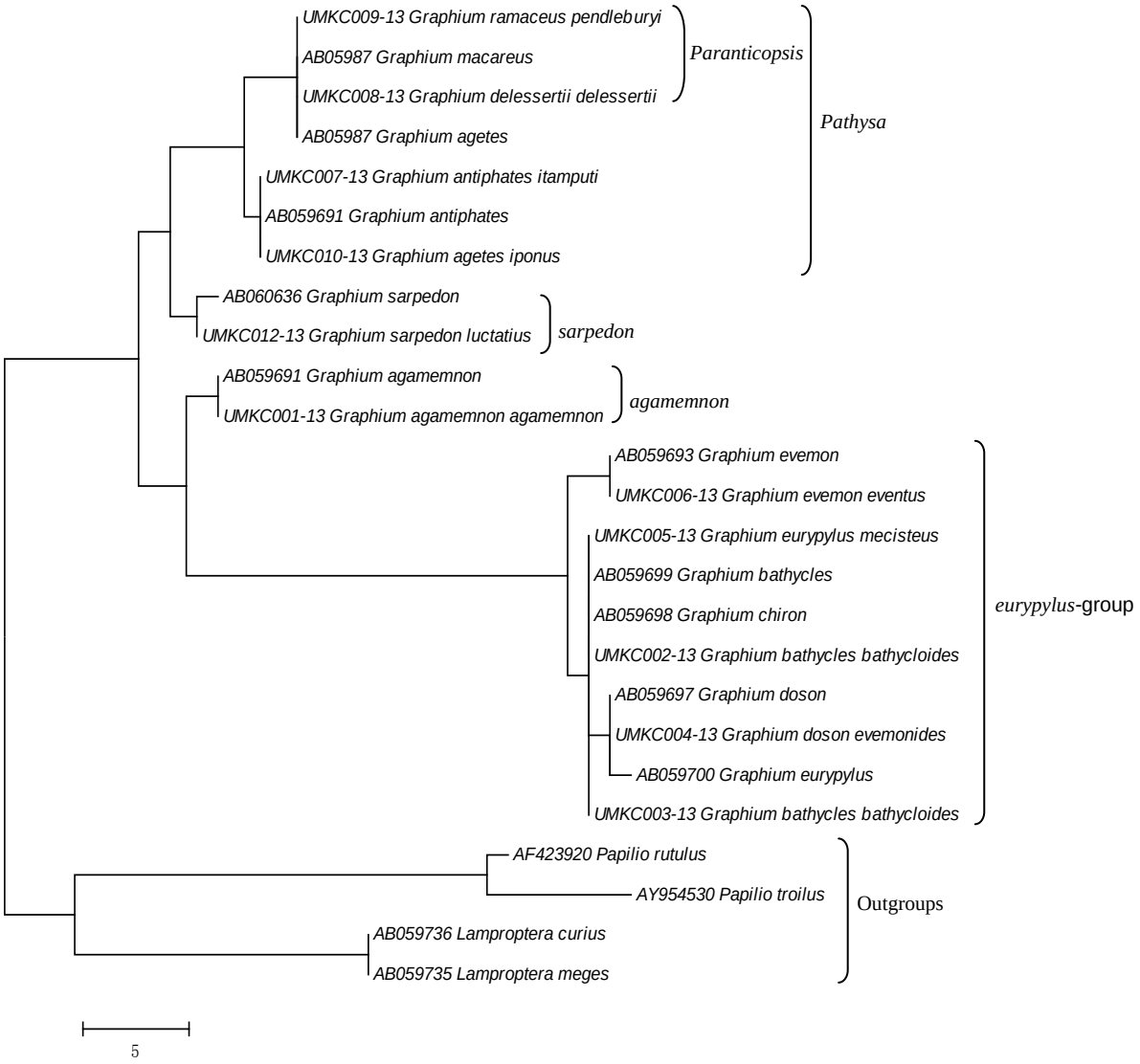

Figure 5.3 Maximum parsimony phenogram for *Graphium* species found in Peninsular Malaysia based on 28S rRNA sequences.

CHAPTER 6

GENERAL DISCUSSION

Butterflies are one of the most taxonomically studied groups of insects. They play on ecological role as pollinators, prey, defoliators and herbivores. They are abundance and diverse in many ecosystems. They serve as indicators of ecosystem change and predict environmental alternation. Butterflies are exposed to environmental influence and are sensitive to the habitat condition. They are quick to react to changes, warning about environmental damage and aiding in the interpretation of ecological condition.

The bait trap is one of the most common methods used for trapping butterflies in the field particularly for fruit feeding butterflies. The bait traps method provides an easy way to monitoring butterflies in forest. They are easier to operate and less labor intensive compared with manual sweep net capture. In chapter 3, my study provides the data on the effectiveness of ten types of fruit baits in attracting butterfly in forest reserve of Ulu Gombak, Malaysia. Fruit bait traps were baited with ten types of fruit bait, and analysis was conducted to determine the effectiveness on the catch of butterfly at the secondary forest area of Ulu Gombak Forest Reserve. A total of 194 Nymphalids butterflies from 28 species were recorded in this study. *Mycalesis orseis* was the most abundance butterfly species trapped in the fruit bait trap, with 70 individuals. Banana was found to be the most attractive bait trapping a total of 14 species of Nymphalids.

The species composition along different elevations can be used to indicate the changes of community structure with biotic and abiotic environmental pressure. There has been no comprehensive study to investigate the elevational patterns of diversity of butterflies in Malaysia. Therefore, my study in chapter 4 has documented the general

pattern of butterfly diversity at six different elevations in the Genting Highlands, Peninsular Malaysia.

Butterflies were sampled at six different elevations in the Genting Highlands to assess the effect of elevation on their distribution and diversity. A total of 2, 876 butterflies belonging to 214 species were collected from six sites of different elevation between January and December 2011. Nymphalidae (1599 individuals) was the most abundant family, *Ypthima pandocus* was the most abundant species (718 individuals), followed by *Eurema hecabe* (194 individuals) and *Leptosia nina* (75 individuals).The results show that the butterfly diversity was different between each elevation. The highest diversity (118 species, H'=3.882) was seen at low elevation (480 m a.s.l.) with declining species diversity at higher sites.

My study revealed that the elevation, temperature and humidity were found to be correlated with the butterfly diversity. Previous studies elsewhere have reported the environmental factors that influence butterfly diversity and distribution (Schwartz-Tzachor *et al*., 2008; Barua *et al*., 2010; Jia *et al*., 2010; Ribeiro & Freitas, 2010). The study by Nakashizuka, 1991; Lien & Yuan, 2003; Axmacher & Fiedler, 2008; Whitlaker, 2010 found that the changing diversity along elevations due to the climate, productivity, habitat heterogeneity and mass effect at different elevational of study sites. Previous study by Lawton *et al*. (1987) also concluded that species richness declines with elevation due to reduced habitat area, resource diversity, unfavorable environments, and reduced primary productivity at high elevation.

Temperature was positively correlated with the species richness of butterflies along elevation at the Genting Highlands in my study. The population of butterflies was decreased at high elevation due to the biological activity of butterflies, which depend on local or regional adaption (Moya-Larano, 2010). Axmacher & Fiedler (2008) indicated that climatic condition is one of the factors to influence the diversity at higher elevation

with cooler climate. Moreover, temperature may affect thelarval behavior, flight activity, foraging and courtship behavior (Boonvanno *et al*., 2000; Nabeta *et al*., 2005; Barua *et al*., 2010). Temperature and precipitation play an important role in defining the differences in habitat preference, adults activity, and adaptive of butterflies (Brakefield & Larsen, 1984; Jia *et al*., 2010).

My study indicated that the abundant species of *Ypthima pandocus*in all elevations was due to diverse of food plant comminities at six elevations. The high number of species *Ypthima pandocus* can be explained by the availability of suitable host plants at sampling sites. Hogsden & Hutchinson (2004) showed butterfly species richness and diversity was positively correlated with plant species diversity. Butterfly species richness in this study were more related to the availability of host plant for larvae and adults. Butterflies response to environmental changes before larval host plant changes (Nelson, 2007).

Graphium butterflies are famous in Peninsular Malaysia for their colourful wings, yet their taxonomy remains unresolved. The popular guides to Malaysian butterflies, place the species in one, two or three genera. Identification of species using obscure morphological characters can be difficult, especially for the closely related taxa *Graphium bathycles* and *Graphium chironides*. My study found that all species of *Graphium* possessed a distinctive cluster of DNA barcodes with the exception of the specimens originally identified as *Graphium bathycles bathycloides* and *Graphium chironides malayanum* which shared DNA barcodes. On further examination, I also found that the morphological identification was ambiguous, as the specimens overlapped for the diagnostic characters reported for each taxon.

In this present study, I found that the diagnostic character of *Graphium bathycles bathycloides* and *Graphium chironides malayanum* were overlapping and could not be achieved unambiguously (Table 5.2). In the absence of further evidence, I

assigned all of my specimens to *Graphium bathycles bathycloides*, which is a more common taxon in Peninsular Malaysia (Corbert & Pendlebury, 1992). Therefore, the status of subspecies *Graphium chironides malayanum* from Peninsular Malaysia still remains in doubt.

Additionally, I sequenced 28S rRNA to examine, in conjunction with COI, the phylogenetic relationships of these species, and investigate the validity of *Pathysa* and *Paranticopsis* as distinct genera.The morphological character of *Pathysa* and *Paranticopsis* were distinct, butthe COI and 28S rRNA maximum parsimony trees showed a similar topology with *Paranticopsis* species forming a clade within a larger clade comprising the *Pathysa* species. Hence, the present data concluded that *Pathysa* was monophyletic with *Graphium* s.l.In order for *Pathysa* to be a valid genus, at least three other clades within *Graphium* s.l. would also have to be raised as genera.

Overall, the use of fruit as bait can enhance the collecting of fruit feeding butterfly in field and less time consuming for butterfly trapping. The diversity study provides a baseline for future ecological studies and conservation programs in the Titiwangsa Mountain. In addition, the results of the phylogenetic relationship for *Graphium* species can help to explain and update the current evolutionary status of *Graphium* in Peninsular Malaysia.

CHAPTER 7

SUMMARY

1. Banana was found to be the most effectivefruit bait to trap butterflies. Butterfly species from Satyrinae were most attracted to fruit bait, especially butterfly from the genus of *Mycalesis*.
2. My studydemonstrated that the diversity pattern of butterflies werean increase until mid-elevation and then a decrease to high elevation along the Genting Highlands.
3. My study found that all species of *Graphium* possess a distinctive cluster of DNA barcodes with the exception of the specimens originally identified as *Graphium bathycles* and *Graphium chironides*, which shared DNA barcodes.
4. On further examination, I found that the morphological identification was ambiguous as the specimens overlapped for the diagnostic morphological characters reported for each taxon. Consequently, the status of *Graphium chironides malayanum* remains in doubt.
5. The COI and 28S rRNA maximum parsimony trees showed a similar topology with *Paranticopsis* species, forming a clade within a larger clade comprising the *Pathysa* species. However, in order for *Pathysa* to be a valid genus, at least three other clades within *Graphium* s.l. would also have to be raised as genera.

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APPENDIX

Appendix 1 The number and genera of fruit feeding butterflies were recorded from 10 types of fruit bait

Bait	Number of individuals	Average number of individuals / Per trap	Number of species	Number of genera
Banana	48	4.8	14	11
Chiku	7	0.7	2	1
Citrus	10	1.0	3	2
Dragon fruit	14	1.4	8	5
Guava	9	0.9	4	1
Papaya	37	3.7	11	8
Pineapple	16	1.6	9	7
Rose apple	7	0.7	5	4
Star fruit	13	1.3	8	6
Watermelon	33	3.3	10	8

Appendix 2 The number of individuals of each species collected at six different sites in the Titiwangsa Mountains

Family	Species	Number of Individual Collected						
		S1	S2	S3	S4	S5	S6	Total (n)
Nymphalidae	*Amathuxidia amythaon*	0	1	0	0	0	0	1
	Amathusia phidippus	0	0	0	3	2	0	5
	Athyma kanwa	0	0	0	1	0	0	1
	Athyma nefte	0	5	0	1	0	0	6
	Athyma pravara	0	0	0	1	0	0	1
	Athyma selenophora	0	1	0	0	0	0	1
	Cethosia biblis	0	0	0	1	0	0	1
	Cethosia penthesilea	0	0	1	0	0	0	1
	Chersonesia intermedia	1	0	0	1	0	0	2
	Chersonesia rahria	1	0	0	0	0	0	1
	Cirrochroa Malaya	0	0	0	10	3	0	13
	Coelites epiminthia	1	0	0	0	0	0	1
	Cyrestis maenalis	1	1	1	9	16	6	34
	Cyrestis nivea	10	0	3	2	11	2	28
	Danaus melanippus	1	2	6	1	0	0	10
	Elymnias nesaea	0	1	0	0	0	0	1
	Elymnias penaga	0	5	5	0	0	0	10
	Erites argentina	1	0	0	0	0	0	1
	Euploea camaralzeman	2	0	0	0	1	0	3
	Euploea doubledayi	0	0	0	0	0	1	1
	Euploea eunice	0	0	1	1	0	0	2
	Euploea midamus	0	0	0	1	2	0	3
	Euploea mulciber	0	1	0	7	19	2	29
	Euploea radamanthus	13	5	4	13	15	0	50

Appendix 2 (Continued) The number of individuals of each species collected at six different sites in the Titiwangsa Mountains

Family	Species	Number of Individual Collected						
		S1	S2	S3	S4	S5	S6	Total (n)
	Euploea tulliolus	1	3	2	13	10	4	33
	Euthalia kanda	0	1	0	0	0	0	1
	Euthalia mahadeva	0	1	0	0	0	0	1
	Euthalia monina	1	1	0	0	0	0	2
	Hypolimnas bolina	0	1	0	0	0	0	1
	Faunis canens	0	2	0	0	0	0	2
	Idea leuconoe	0	0	0	2	0	0	2
	Ideopsis gaura	0	0	4	23	18	0	45
	Ideopsis similis	5	0	0	0	0	0	5
	Ideopsis vulgaris	0	2	0	0	0	0	2
	Junonia almana	7	3	3	0	0	0	13
	Junonia atlites	0	3	0	0	0	0	3
	Junonia hedonia	10	4	1	0	0	0	15
	Junonia iphita	4	0	0	1	0	0	5
	Junonia orithya	0	3	0	0	0	0	3
	Kaniska canace	0	0	0	0	18	6	24
	Lasippa helidore	2	0	0	0	0	0	2
	Lasippa tiga	0	1	0	0	0	0	1
	Lethe Europa	0	1	0	0	0	0	1
	Melanitis leda	5	0	0	0	0	0	5
	Melanitis zitenius	2	0	0	0	0	0	2
	Moduza procris	1	2	1	0	0	0	4
	Mycalesis cisala	0	1	0	0	0	0	1
	Mycalesis fusca	0	2	1	1	0	0	4
	Mycalesis horsfieldi	6	1	0	2	0	0	9

Appendix 2 (Continued) The number of individuals of each species collected at six different sites in the Titiwangsa Mountains

Family	Species	Number of Individual Collected						
		S1	S2	S3	S4	S5	S6	Total (n)
	Mycalesis janardana	0	6	0	0	0	0	6
	Mycalesis intermedia	3	6	1	0	0	0	10
	Mycalesis maianeas	0	2	0	0	0	0	2
	Mycalesis mineus	0	3	0	1	0	0	4
	Mycalesis orseis	2	10	3	0	0	0	15
	Mycalesis perseoides	0	8	0	0	0	0	8
	Mycalesis perseus	2	10	0	0	0	0	12
	Neorina lowii	1	0	0	0	0	0	1
	Neptis clinia	0	0	0	1	0	0	1
	Neptis duryodana	2	0	0	0	0	0	2
	Neptis hylas	1	14	3	3	2	0	23
	Neptis omeroda	1	0	0	0	0	0	1
	Neptis soma	0	1	0	0	0	0	1
	Orsotriaena medus	1	6	0	0	0	0	7
	Parantica aspasia	1	5	2	6	3	2	19
	Parantica melaneus	0	0	0	2	15	3	20
	Parantica sita	0	0	0	12	35	16	63
	Phalanta alcippe	0	0	0	1	0	0	1
	Polyura athamas	0	1	0	0	0	0	1
	Polyura hebe	1	0	0	0	0	0	1
	Pontopria hordonia	0	0	0	1	0	0	1
	Prothoe franck	1	0	0	0	0	0	1
	Ragadia crisilda	0	1	0	0	0	0	1
	Ragadia makuta	1	0	0	0	0	0	1
	Sumalia daraxa	0	1	1	3	6	0	11

Appendix 2 (Continued) The number of individuals of each species collected at six different sites in the Titiwangsa Mountains

Family	Species	Number of Individual Collected						
		S1	S2	S3	S4	S5	S6	Total (n)
	Symbrenthia hypatia	1	0	0	0	0	0	1
	Tanaecia flora	1	5	2	2	3	0	13
	Terinos terpander	1	1	0	1	1	1	5
	Tirumala septentrionis	1	0	0	0	0	0	1
	Vagrans egista	0	0	0	12	30	26	68
	Xanthotaenia busiris	0	1	0	0	0	0	1
	Ypthima baldus	7	10	3	2	0	0	22
	Ypthima fasciata	2	2	3	13	0	0	20
	Ypthima horsfieldii	7	40	3	13	0	0	63
	Ypthima huebneri	11	34	7	5	2	0	59
	Ypthima pandocus	64	102	105	151	192	104	718
	Ypthima savara	2	18	0	3	0	0	23
Lycaenidae	*Abisara geza*	0	1	0	0	0	0	1
	Acytolepis puspa	0	2	1	0	0	0	3
	Allotinus leogoron	1	1	0	0	0	0	2
	Allotinus subviolacecis	0	2	2	0	0	0	4
	Anthene emolus	8	0	1	1	0	4	14
	Anthene licates	1	0	0	0	0	0	1
	Arhopala aida	0	0	2	0	0	0	2
	Arhopala avatha	0	0	0	2	0	0	2
	Arhopala azinis	0	2	0	1	0	0	3
	Arhopala elopura	0	1	0	0	0	0	1
	Arhopala norda	0	1	0	0	0	0	1
	Caleta elna	1	1	0	0	0	0	2
	Caleta roxus	0	1	0	0	0	0	1

Appendix 2 (Continued) The number of individuals of each species collected at six different sites in the Titiwangsa Mountains

Family	Species	Number of Individual Collected						
		S1	S2	S3	S4	S5	S6	Total (n)
	Catochrysops strabo	1	2	0	0	0	0	3
	Celastrina lavendularis	1	0	2	2	0	1	6
	Celatoxin marginata	0	0	0	1	0	0	1
	Chliaria othona	0	0	0	1	0	0	1
	Curetis bulis	0	0	1	0	0	0	1
	Curetis tagalica	1	0	1	0	0	0	2
	Discolampa ethion	0	0	1	0	0	0	1
	Drupadia ravindra	1	0	0	0	0	0	1
	Heliophorus ila	0	0	0	1	0	0	1
	Hypolycaena merguia	0	1	0	0	0	0	1
	Ionolyce helicon	0	1	2	2	0	0	5
	Iraota distanti	0	1	2	0	0	0	3
	Jamides alecto	1	9	13	15	0	0	38
	Jamides caeruleus	2	0	0	3	0	0	5
	Jamides celeno	2	13	23	6	0	0	44
	Jamides elpis	3	0	0	0	0	7	10
	Jamides ferrari	1	0	0	0	0	0	1
	Jamides malaccanus	2	1	2	15	8	0	28
	Jamides pura	25	13	10	11	3	0	62
	Janides virgulatus	1	0	1	1	0	0	3
	Miletus nymphis	0	1	0	0	0	0	1
	Lampides boeticus	0	3	1	0	0	0	4
	Logania malayica	0	1	0	0	0	0	1
	Monodontides musina	0	0	2	1	1	7	11
	Nacaduba angusta	0	0	1	0	0	0	1

Appendix 2 (Continued) The number of individuals of each species collected at six different sites in the Titiwangsa Mountains

Family	Species	Number of Individual Collected						
		S1	S2	S3	S4	S5	S6	Total (n)
	Nacaduba berenice	0	0	0	1	0	0	1
	Nacaduba kurava	2	1	0	0	0	0	3
	Petrelaea dana	4	0	0	0	0	0	4
	Pithecops corvus	0	9	1	0	0	2	12
	Poritia philota	0	0	1	0	0	0	1
	Prosotas aluta	0	1	0	0	0	0	1
	Prosotas bhutea	4	0	1	2	0	0	7
	Prosotas dubiosa	2	0	0	0	0	0	2
	Rapala airbus	0	1	0	0	0	0	1
	Rapala nissa	0	0	1	0	0	0	1
	Udara camenae	1	3	4	42	0	2	52
	Udara coalita	0	0	0	0	0	15	15
	Udara cyma	0	0	0	0	0	2	2
	Udara dilecta	0	0	5	0	0	2	7
	Udara placidula	0	0	8	9	5	25	47
	Udara selma	0	0	4	0	0	15	19
	Udara toxopeusi	0	0	4	8	8	25	45
	Una usta	2	2	0	1	0	0	5
	Zeltus amasa	2	2	2	4	0	0	10
	Zizina otis	3	17	4	2	0	0	26
Pieridae	*Appias albino*	3	0	0	0	0	0	3
	Appias cardena	0	0	0	4	20	5	29
	Appias indra	0	0	0	2	0	3	5
	Appias lalassis	0	0	0	4	8	2	14
	Appias lyncida	3	6	9	1	4	0	23

Appendix 2 (Continued) The number of individuals of each species collected at six different sites in the Titiwangsa Mountains

Family	Species	Number of Individual Collected						
		S1	S2	S3	S4	S5	S6	Total (n)
	Appias libythea	2	6	9	0	4	0	21
	Appias nero	1	0	0	0	0	0	1
	Appias pandione	0	0	0	2	2	1	5
	Catopsilia pomona	0	2	2	8	10	4	26
	Cepora iudith	0	10	9	0	2	3	24
	Delias baracasa	0	0	0	1	2	0	3
	Delias descombesi	0	0	0	6	9	0	15
	Delias ninus	0	0	0	3	10	5	18
	Dercas verhuelli	0	0	0	4	7	0	11
	Eurema ada	6	6	9	15	0	0	36
	Eurema andersonii	2	2	5	13	7	0	29
	Eurema blanda	5	11	16	8	0	0	40
	Eurema brigitta	10	0	0	4	0	0	14
	Eurema hecabe	40	20	35	70	29	0	194
	Eurema lacteola	3	14	10	6	2	0	35
	Eurema sari	6	2	1	3	0	0	12
	Eurema simulatrix	2	4	6	22	0	0	34
	Gandaca harina	4	3	1	2	0	0	10
	Leptosia nina	22	23	8	13	9	0	75
	Parevonia valeria	0	1	0	0	0	0	1
	Saletara liberia	5	2	4	1	0	0	12
Hesperiidae	*Ancistroides armatus*	0	1	0	0	0	0	1
	Ancistroides gemmifer	1	0	0	0	0	0	1
	Ancistroides nigrita	1	1	2	1	0	0	5
	Caltoris bromus	1	0	0	0	0	0	1

Appendix 2 (Continued) The number of individuals of each species collected at six different sites in the Titiwangsa Mountains

Family	Species	Number of Individual Collected						
		S1	S2	S3	S4	S5	S6	Total (n)
	Hyarotis iadera	0	3	1	0	0	0	4
	Iambrix salsala	2	3	0	0	0	0	5
	Iambrix stellifer	0	1	0	0	0	0	1
	Idmon distanti	0	1	0	0	0	0	1
	Idmon obliquans	0	5	0	0	0	0	5
	Isma umbrosa	0	1	0	0	0	0	1
	Koruthaialos rubecula	2	2	2	0	0	0	6
	Koruthaialos sindu	2	6	0	0	2	0	10
	Notocrypta clavata	0	0	4	1	0	0	5
	Notocrypta paralysos	0	2	2	0	3	0	7
	Oriens paragola	0	1	1	2	0	0	4
	Potanthus ganda	3	1	0	0	0	0	4
	Potanthus hetaerus	1	1	0	0	0	0	2
	Potanthus lydia	0	1	0	0	0	0	1
	Potanthus omaha	0	2	0	3	0	7	12
	Pseudocoladenia dan	0	2	0	0	0	0	2
	Pseudokerana fulgur	0	0	0	0	0	1	1
	Psolos filigo	0	0	1	0	0	0	1
	Pyroneura latoia	0	1	0	0	0	0	1
	Quedara monteithi	0	1	1	0	1	0	3
	Salanoemia fuscicornis	1	1	0	0	0	0	2
	Taractrocera archias	0	0	1	0	0	0	1
	Zela zero	0	0	2	0	0	0	2
	Zela zeus	3	5	0	2	0	0	10
	Zizeeria karsandra	2	0	0	0	0	0	2

Appendix 2 (Continued) The number of individuals of each species collected at six different sites in the Titiwangsa Mountains

Family	Species	Number of Individual Collected						
		S1	**S2**	**S3**	**S4**	**S5**	**S6**	**Total (n)**
Papilionidae	*Zographetus rama*	0	0	0	1	0	0	1
	Graphium agamemnon	0	0	1	1	0	0	2
	Graphium doson	0	0	1	0	0	0	1
	Graphium sarpedon	1	0	3	3	4	2	13
	Pachiliopta aristolochiae	0	1	0	0	0	0	1
	Papilio demoleus	0	0	0	2	2	0	4
	Papilio demolion	1	1	0	1	0	0	3
	Papilio helenus	0	0	0	2	0	0	2
	Papilio memnon	2	0	0	1	0	0	3
	Papilio polytes	0	3	0	1	3	0	7
	Parides nox	0	0	0	1	0	0	1
	Pathysa antiphates	0	0	4	0	0	0	4
	Pathysa delessertii	2	0	0	0	0	0	2
	Pathysa macareus	0	1	0	0	0	0	1
	Troides aeacus	2	1	1	4	0	0	8
	Total individual	**402**	**596**	**420**	**676**	**569**	**313**	**2976**
	Total number of species	**97**	**118**	**82**	**97**	**47**	**34**	
	Cumulative number of species							**214**

LIST OF PRESENTATION AND PUBLICATION

Chia, K.H.M., Chen, C.D. and Sofian-Azirun. (2011). A preliminary checklist of butterfly (Insecta: Lepidoptera) collected from Coniferous Recreational Forest, Bentong, Pahang. Paper presented at *UMTAS 2011: Empowering Science, Technology and Innovation Towards a Better Tomorrow*. pp. 123. (ISBN 978-967-5366-60-4).

Karen-Chia, H.M., Chen, C.D. and Sofian-Azirun, M. (2012). The diversity of the butterflies (Insecta: Lepidoptera) in Genting Highlands, Pahang, Malaysia. Paper presented at *The Proceeding of The 4th Regional Conference on Natural Resources in the Tropics*. pp. 604.(ISBN 978-967-5418-273).

Karen-Chia, H.M., Chen, C.D. and Sofian-Azirun, M. (2012). The attractiveness of fruit baits against butterflies obtained from secondary forest in Gombak, Selangor. International Symposium on Insects, Entomological Society of Malaysia, 3-5 December 2012, Mines Wellness Hotel, Kuala Lumpur, Malaysia. (Poster Presentation, Abstract: Page 80).