



Faculty of Resource Science and Technology

**Biology, Spatial Distribution and Range Expansion of
Acraea terpsicore (Linnaeus, 1758) (Nymphalidae: Heliconiinae) in Borneo**

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Biology, Spatial Distribution and Range Expansion of
Acraea terpsicore (Linnaeus, 1758) (Nymphalidae: Heliconiinae) in Borneo

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DECLARATION

The thesis has not been accepted for any degree and is not concurrently submitted in candidature of any other degree.

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DEDICATION

Dedicated to my loving and caring husband Mr. Yasir Arafat Barech, my kids and my lovely parents Mr. and Mrs. Noor Muhammad.

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ABSTRACT

The Tawny Coster, *Acraea terpsicore* (Linnaeus, 1758) is one of the prominent species from the tribe Acraeini, which is widely distributed throughout the Indian and Sri Lankan region. In the past three decades, its distribution range has expanded southwards through Southeast Asia and Australia. This non-indigenous Nymphalid butterfly was first detected in 2013 in Sarawak, on the Borneo Island. This study aimed to the findings of biological and ecological aspects of *A. terpsicore* in support of its current establishment in Borneo. A comparative biology relative to habitat types, phenotype and gender was conducted on the populations of *A. terpsicore* in Sarawak. This study reveals that the biology of *A. terpsicore* is similar to those reported from other regions of its novel range (Southeast Asian and Australian). Biological control and pest management strategies are better performed if precise information about interactions of an invasive species in a newly invaded environment are available in real time. This non-indigenous species was found to feed voraciously on *Passiflora foetida* in Sarawak. The host plant association and feeding preference studies showed that this species prefers to feed on its primary host plant from the family Passifloraceae and currently, it is unlikely to pose as a pest of economic importance. Species Distribution Modeling (SDMs) forecasting the current and future climatically habitable range of *A. terpsicore* showed promising future range of this species, which is likely to expand further southwards to Sulawesi Island, the Philippines and northwards to Taiwan. The genetic variance of *A. terpsicore* among the population of Borneo and adjacent areas was executed at the level of partial mitochondrial genome (*COI*). Pairwise genetic distance and phylogenetic analysis among the populations of *A. terpsicore* showed no genetic variance. This is due to the species may be too recent and young with higher gene flow among the population and is still in the establishment phase of invasion. *A. terpsicore* should

be closely monitored in the future as this species is still in the initial phase of invasiveness and can be a threat at any point in future for becoming a pest of economic importance.

Keywords: Biological invasion, Borneo, *Acraea terpsicore*, biology, host plants, range expansion

**Biologi, Taburan Spatial dan Julat Pengembangan Acraea terpsicore (Nymphalidae:
Heliconiinae) di Borneo**

ABSTRAK

Acraea terpsicore (Linnaeus, 1758) atau dikenali sebagai Tawny Coster adalah salah satu spesies yang terkemuka dari tribus Acraeini yang mempunyai taburan secara meluas di seluruh rantau Sri Lanka dan India. Dalam tempoh tiga dekad yang lalu, taburan spesies ini telah berkembang ke arah selatan melalui Asia Tenggara dan Australia. Spesies rama-rama Nymphalid yang bukan asli di Sarawak, Borneo telah pertama sekali dikesan di Sarawak, pada tahun 2013. Kajian ini bertujuan untuk mengkaji aspek-aspek biologi dan ekologi A. terpsicore sehubungan dengan penetapan hidup spesies ini di Borneo. Kajian biologi perbandingan A. terpsicore relatif dengan jenis habitat, fenotip dan jantina Sarawak telah dijalankan ke atas populasi spesies ini di Sarawak. Hasil kajian menunjukkan bahawa biologi A. terpsicore adalah serupa dengan yang dilaporkan dari kawasan lain (Asia Tenggara dan Australia). Strategi kawalan biologi dan pengurusan perosak dapat dilakukan jika maklumat yang tepat tentang interaksi spesies invasif dalam persekitaran yang baharu boleh didapati dalam masa nyata. Di Sarawak, spesies yang bukan asli ini hidup dengan memakan Passiflora foetida. Kajian hubungan tumbuhan perumah dan pilihan pemakanan menunjukkan bahawa spesies ini lebih memilih tumbuhan perumah dari famili Passifloraceae dan sehingga kini, ia tidak bertindak sebagai spesies perosak. Model Taburan Spesies (SDMs) julat taburan habitat berdasarkan iklim meramalkan bahawa spesies ini akan mempunyai julat taburan yang baik yang berkemungkinan akan diperluaskan ke arah selatan Pulau Sulawesi, Filipina dan utara ke Taiwan. Varians genetik A. terpsicore di kalangan populasi Borneo dan kawasan berhampiran telah dilaksanakan pada tahap genom mitokondria separa (COI). Jarak genetik berpasangan dan analisis filogenetik populasi A. terpsicore tidak menunjukkan varians genetik. Ini adalah

kerana spesies ini mungkin sangat baru dan muda dengan aliran gen lebih tinggi di kalangan populasi dan masih berada dalam fasa awal pencerobohan. Pemantauan terhadap A. terpsicore perlu dilakukan dengan rapi di masa hadapan memandangkan spesies ini masih dalam fasa pencerobohan dan berkemungkinan akan menjadi perosak ekonomi yang penting.

Kata kunci: *Biologi pencerobohan, Borneo, Acraea terpsicore, biologi, tumbuhan perumah, julat pengembangan*

TABLE OF CONTENTS

	Page
DECLARATION	i
DEDICATION	ii
ACKNOWLEDGEMENT	iii
ABSTRACT	v
ABSTRAK	vii
TABLE OF CONTENTS	ix
LIST OF TABLES +	xv
LIST OF FIGURES	xvii
LIST OF ABBREVIATIONS	xxiii
CHAPTER 1: GENERAL INTRODUCTION	1
1.0 Introduction	1
1.2 Problem Statement	4
1.3 Research Objectives	5
1.4 Thesis Structure	6
CHAPTER 2: LITERATURE REVIEW	7
2.1 Taxonomic Position of <i>Acraea terpsicore</i> (Nymphalidae: Heliconiinae)	7
2.2 Biology of <i>A. terpsicore</i>	9
2.2.1 Morphology	9

2.2.2 Physiology	10
2.2.3 Life-cycle of <i>A. terpsicore</i>	11
2.3 Feeding Habit and Habitat	11
2.3.1 Feeding Habit	11
2.3.2 Habitat	13
2.4 Occurrence and Abundance of <i>A. terpsicore</i>	14
2.5 Climate-driven Range Expansion of <i>A. terpsicore</i>	16
CHAPTER 3: COMPARATIVE BIOLOGY OF <i>Acraea terpsicore</i>	19
(LINNAEUS, 1758) (NYMPHALIDAE: HELICONIINAE),	
RELATIVE TO HABITAT TYPES, PHENOTYPE AND	
GENDER	
3.1 Introduction	19
3.2 Materials and Methods	23
3.2.1 Data Collection	23
3.3 Data Analysis	27
3.4 Results	28
3.4.1 Courtship Activities and Mating Behavior	28
3.4.2 Host Plants and Oviposition Responses	28
3.4.3 External Morphology of the Immature Stages	29
3.4.4 The Adults	37

3.4.5 Natural Enemies and Defensive Behavior	43
3.4.6 Comparison of Developmental Cycle Relative to Habitat Types	43
3.4.7 Survival Rate Recorded Among All the Cohorts	46
3.4.8 Population Index	47
3.5 Discussion	49
CHAPTER 4: HOST-PLANT PREFERENCES OF <i>Acraea terpsicore</i>	53
(LINNAEUS, 1758) (NYMPHALIDAE: HELICONIINAE)	
AND NUTRITIONAL INDICES FOR	
PASSIFLORACEAE AND CUCURBITACEAE	
4.1 Introduction	53
4.2 Materials and Methods	56
4.2.1 Host Plants	56
4.2.2 Experimental Larval Instars	57
4.2.3 Experimental design for Feeding Preference Tests	57
4.2.4 Nutritional Indices Experiment	60
4.2.5 Oviposition Choice	63
4.3 Data Analysis	64
4.3.1 Feeding Preference Experiment	64
4.3.2 Nutritional Indices Experiment	64

4.4 Results	66
4.4.1 Feeding Preference Experiments	66
4.4.2 Nutritional Indices Experiments	71
4.4.3 Oviposition Choice of <i>A. terpsicore</i>	73
4.5 Discussion	74
CHAPTER 5: WING SIZE VARIATIONS IN <i>Acraea terpsicore</i>	77
(LINNAEUS, 1758) (NYMPHALIDAE: HELICONIINAE)	
ADULTS FOLLOWING EXPOSURE TO HAZE	
5.1 Introduction	77
5.2 Materials and Methods	80
5.3 Data Analysis	81
5.4 Results	82
5.4.1 Environmental conditions	82
5.4.2 Wing size and pattern variations in NorG and AbG males of <i>A. terpsicore</i>	83
5.4.3 Wing size and pattern variations in NorG and AbG females of <i>A. terpsicore</i>	86
5.5 Discussion	89

CHAPTER 6: SPATIAL DISTRIBUTION AND RANGE EXPANSION	92
OF <i>Acraea terpsicore</i> (LINNAEUS, 1758) (NYMPHALIDAE: HELICONIINAE) IN BORNEO	
6.1 Introduction	92
6.2 Materials and Methods	96
6.2.1 Collection of Distributional Data	96
6.2.2 Environmental Data and Climatic Predictors	97
6.2.3 Model Selection for Ecological Niche Modeling	99
6.3 Data Analysis	101
6.4 Results	102
6.4.1 Distribution records for Southeast Asia	102
6.4.2 Distribution records for Borneo	102
6.4.3 Rate of colonization	103
6.4.4 Range size of <i>A. terpsicore</i> in Borneo	105
6.4.5 Spatial modelling in South-East Asia and Borneo	106
6.4.6 Entrance into Borneo	108
6.5 Discussion	112
CHAPTER 7: GENETIC VARIATION AMONG <i>Acraea terpsicore</i>	116
(LINNAEUS, 1758) (NYMPHALIDAE: HELICONIINAE)	
FROM BORNEO AND ADJACENT AREAS	

7.1 Introduction	116
7.2 Materials and Methods	118
7.2.1 Sample Collection	118
7.2.2 DNA Extraction	120
7.2.3 Polymerase Chain Reaction (PCR Amplification) and Sequencing	120
7.3 Data Analysis	122
7.4 Results	122
7.4.1 Pairwise Genetic Distance	123
7.4.2 Phylogenetic Analysis	124
7.5 Discussion	127
CHAPTER 8: GENERAL SUMMARY	130
REFERENCES	132
APPENDICES	159

LIST OF TABLES

	Page
Table 3.1 Comparative summary of <i>A. terpsicore</i> developmental timeline (Body length and duration) from three study sites.	45
Table 3.2 Summary of One-way ANOVA performed for evaluation of two hypotheses.	46
Table 3.3 Population indices of <i>A. terpsicore</i> , relative to mean temperature, relative humidity and maximum precipitation at specific monthly time.	48
Table 4.1 Summary of host plant preference experimental data, compared for Passifloraceae.	65
Table 4.2 Summary of One-way ANOVA and post comparative Tukey's test for all three feeding preference tests.	70
Table 4.3 Nutritional indices results for all the test plants tested on third instar larvae of <i>A. terpsicore</i> .	72
Table 5.1 Environmental data during the study period in Sarawak.	82
Table 5.2 Male <i>A. terpsicore</i> wing length measurements (mm).	84
Table 5.3 Types of wing faults detected in Population A of male <i>A. terpsicore</i> .	85
Table 5.4 Female <i>A. terpsicore</i> wing length measurements (mm).	87

Table 5.5	Types of wing faults detected in Population A of female <i>A. terpsicore</i> .	88
Table 6.1	Environmental data of bioclimatic variables with respective codes.	98
Table 6.2	Distribution of <i>A. terpsicore</i> across Borneo (presence based data).	104
Table 6.3	Summary of models performance and parameters range.	107
Table 7.1	Samples of <i>Acraea terpsicore</i> analyzed for DNA (<i>COI</i>) sequence variation	119
Table 7.2	Master mixture components and volume of PCR amplification for one reaction.	121
Table 7.3	Amplification parameters for PCR	121
Table 7.4	Pairwise genetic distance of <i>Acraea terpsicore</i> using Kimura-2-Parameter.	125

LIST OF FIGURES

	Page
Figure 3.1 Map showing three sampling sites selected for comparative analysis study of immature stages based on urbanization levels.	25
Figure 3.2 Study sites and host plant: (a) & (b) the netted house (butterfly) used for the events of mating and oviposition in the study (outside and inside view, respectively); (c) Primary food plant (<i>Passiflora foetida</i>) of <i>A. terpsicore</i> showing the lavish growth in the study site (DI); (d) Wild Passion flower; (e) Urban site (JSB) and the plentiful growth of host plant.	26
Figure 3.3 Courtship behaviour and oviposition in <i>A. terpsicore</i> : (a) & (b) Male adults grasping each other before released to the netted house, eager for mating (after emergence from pupa); (c) Mating pair in netted house; (d) Female ovipositing on the upper side of leaf (<i>P. foetida</i>); (e) Patches of eggs laid at a single time on the same leaf of <i>P. foetida</i> ; (f) Oviposition event.	30
Figure 3.4 Eggs of <i>A. terpsicore</i> : (a) Lateral view of an egg with encircled area of glue (visible); (b) Top view of eggs under a Stereomicroscope; (c) Top view of eggs under SEM with a resolution of x30; (d) Single egg under SEM with a resolution of x130; (e) Micropyle of an egg under SEM with a resolution of x500; (f) cluster of eggs with discrepancy; mature (black head) and under development (pale	31

yellow); (g) Typical egg cohort (after oviposition); (h) cohort of mature eggs (8 hrs before hatching).

Figure 3.5	Immature stages of <i>A. terpsicore</i> : (a) Newly emerged 1 st instars and egg shells; (b) 1 st instar larvae after 12hrs; (c) gregarious group of 1 st instar larvae feeding on <i>Passiflora foetida</i> ; (d) earlier 1 st instar and newly moulted (encircled) 2nd instar larvae on the same spot.	33
Figure 3.6	Immature stages of <i>A. terpsicore</i> : (a) batch of 2 nd instars feeding gregariously, while the silken web can be visualized as well; (b) fully grown second instar stage.	34
Figure 3.7	Immature stages of <i>A. terpsicore</i> : (c) Newly moulted 3 rd instar; (d) fully grown third instar larva.	34
Figure 3.8	Immature stages of <i>A. terpsicore</i> : (a) full sized fourth instar stage	35
Figure 3.9	A fully grown fifth instar larva (last instar) of <i>A. terpsicore</i>	36
Figure 3.10	The pre-pupal stage and pupa of <i>A. terpsicore</i> .	38
Figure 3.11	The adults of <i>A. terpsicore</i> ; (a) Male adult emerged from pupa resting on the discarded pupal case	39
Figure 3.12	Female genitalia of <i>A. terpsicore</i> .	42
Figure 3.13	The male genitalia of <i>A. terpsicore</i> .	42
Figure 3.14	Natural enemies and defensive behavior of <i>A. terpsicore</i> .	43

Figure 3.15	The difference between male and female <i>A. terpsicore</i> , correlated with pupal size (mm), wingspan (mm) and pupal duration (days).	46
Figure 3.16	The overall survivorship rate of each developmental stage of <i>A. terpsicore</i> for 22 cohorts.	47
Figure 3.17	Population index of <i>A. terpsicore</i> , in relation to the average temperature, relative humidity and maximum precipitation throughout the duration.	48
Figure 4.1	Illustration of the three tests performed for the feeding preferences in <i>A. terpsicore</i> .	58
Figure 4.2	Feeding preference experimental design.	59
Figure 4.3	Illustration of nutritional indices feeding trial for <i>A. terpsicore</i> , the test plant (watermelon) leaf (petiole inserted in micro centrifuge tube cap and filled with water) in a plastic container with the third instar larva.	63
Figure 4.4	(a) After feeding trial the unconsumed dried leaf (test plant); (b) dried frass of <i>A. terpsicore</i> after feeding trial.	63
Figure 4.5	Equal opportunity feeding preference results for <i>P. foetida</i> .	67
Figure 4.6	Less opportunity feeding preference results for <i>P. foetida</i> .	69
Figure 4.7	No opportunity feeding preference results for <i>P. edulis</i> .	70

Figure 4.8	Nutritional indices of <i>A. terpsicore</i> compared between <i>P. foetida</i> and <i>P. edulis</i> .	72
Figure 4.9	Mann-Whitney's comparative analysis of <i>A. terpsicore</i> third-instar larval performance for <i>P. foetida</i> and <i>P. edulis</i> with SD (70±20).	72
Figure 4.10	Oviposition of <i>A. terpsicore</i> on <i>P. edulis</i> .	73
Figure 5.1	Attributes of the wing length measurement in <i>A. terpsicore</i> and the measurement types are given in the legend (all the measurements were taken parallel to each other i.e. left-right exact points).	81
Figure 5.2	Wing size deviations between left and right fore- and hindwing of abnormal male <i>A. terpsicore</i> .	85
Figure 5.3	Wing size deviations between left and right fore- and hindwing of abnormal female <i>A. terpsicore</i> .	88
Figure 5.4	Apparent size variations of <i>A. terpsicore</i> male (a) and female (b).	89
Figure 6.1	Model design for understanding the use of environmental data and models of current and future climate to estimate the suitable occurrence areas for <i>A. terpsicore</i> .	101
Figure 6.2	Distribution of <i>A. terpsicore</i> in South-East Asia (Thailand, Vietnam, Malay Peninsula, Indonesia and Australia) and particularly in Borneo, showing known locations (black circles) and years of detection.	105

Figure 6.3	Distribution and range size of <i>A. terpsicore</i> in Borneo, showing known locations (black circles) and extent of occurrence (EOO) (cross-hatched area) based on the records between 2012 -2016.	106
Figure 6.4	Potential range of distribution for <i>A. terpsicore</i> across the world, predicted under current climatic conditions.	109
Figure 6.5	Potential range of distribution for <i>A. terpsicore</i> in South-East Asia, predicted under current climatic conditions.	109
Figure 6.6	Potential range of distribution for <i>A. terpsicore</i> across the world, predicted for future 2050, climatic conditions.	110
Figure 6.7	Potential range of distribution for <i>A. terpsicore</i> in South-East Asia, predicted for future 2050, climatic conditions.	110
Figure 6.8	Potential range of distribution for <i>A. terpsicore</i> in Borneo, predicted under (a) current climatic and (b) future 2050 climatic conditions	111
Figure 7.1	Sample collection localities including those that were accessed from Genbank with their respective field ID's. The Sarawak samples are enlarged in the inset map	118
Figure 7.2	Gel electrophoresis using 2% Agarose gel and PCR products amplified at mtDNA <i>COI</i> in <i>A. terpsicore</i> samples; Lane 1: KUC01; Lane 2: KUC02; Lane 3: KUC03; Lane 4: KUC04; Lane 5: KUC05; Lane 6: negative control; Lane 7: KUC06; Lane 8: SRI01; Lane 9:	123

SER01; Lane 10: MOA01; Lane 11: KAL01; Lane 12: 100 bp DNA ladder.

- | | | |
|------------|---|-----|
| Figure 7.3 | Maximum Likelihood (ML) tree of <i>Acraea terpsicore</i> , constructed from partial <i>COI</i> gene and based on 1000 replications. | 126 |
| Figure 7.4 | Neighbour-Joining (NJ) tree of <i>Acraea terpsicore</i> , constructed from partial <i>COI</i> gene and based on 1000 replications. | 126 |
| Figure 7.5 | Minimum evolution tree for <i>Acraea terpsicore</i> , constructed from partial <i>COI</i> gene. | 127 |

LIST OF ABBREVIATIONS

EOO	Extent of occurrence
SDM	Species distribution models
AUC	Area under the curve
°C	Degree celsius
NorG	Normal group
AbG	Abnormal group
API	Air pollutant index
AQI	Air quality index
CTAB	Cetyl trimethyl ammonium bromide
DNA	Deoxyribonucleic acid
dNTP	Deoxyribonucleotides triphosphate
PCR	Polymerase chain reaction
MEGA	Molecular evolutionary genetics analysis
PAUP	Phylogenetic analysis using parsimony
μL	microliter
bp	base pair

CHAPTER 1

GENERAL INTRODUCTION

1.0 Introduction

Acraea terpsicore Linnaeus, 1758 (Lepidoptera: Nymphalidae), commonly known as the Tawny Coster is a non-indigenous Acraeini butterfly in Borneo. Originating from India and Sri Lanka, prior to its first occurrence in Borneo in 2012, this butterfly has expanded its geographical range from India and Sri Lanka to Southeast Asia and Australia. Within the last three decades it became widely distributed and established in Nepal (Smith, 1989), Thailand (West, 1996), Peninsular Malaysia (Arshad et al., 1996), Singapore (Khew, 2008) and more recently in Indonesia (Matsumoto et al., 2012), Timor and Australia (Braby et al., 2013). The most recent report of its occurrence was in Bhutan (Nidup, 2015), Western Kalimantan (Florida et al., 2014), East Kalimantan (Iqbal et al., 2015) and Sarawak (Borneo) (Abang et al., 2016).

During the latter half of the 20th century, globalization and anthropogenic disturbances had been resulted in significantly increased number of breaching of the natural dispersal barriers by non-indigenous species (Perrings et al., 2005). As the increasing number of non-indigenous (exotic, alien and invasive) species becomes established in new landscapes, eventually affects the ecosystems in their newly invaded ranges (Sakai et al., 2001). In rapidly changing environments, the effects of non-native species may vary with time and species that are not causing harm now might do so in the future and the same is true of the natives (Davis, 2011).

The rate of biological invasions, elucidation of their consequences are growing rapidly, and thus challenging the conservation of biodiversity and natural resources (TEEB, 2010; Simberloff et al., 2013). The role of alien species in conservation efforts brings together information, evidence and examples from many parts of the world to illustrate their impacts

directly on ecological changes and biotic homogenization (New, 2016). They affect the biogeochemical pools and fluxes of materials and energy, thereby altering the fundamental structure and function of ecosystems (Ehrenfeld, 2010) as well as cause decline of the native species abundance and habitat destruction (Wilson, 1992). It is important to address the issue as to what extent are invasive species a threat to ecosystems in their invaded ranges. Researchers have acknowledged that few invasive species are more successful than the others, which involves their bio-ecological as well as physiological characteristics in establishment (Peacock and Warner, 2008).

One of the characteristics of invasive species is their selection of similar ecological niche between native and invaded range (Petitpierre et al., 2012). In novel ranges, their considerable tolerance to abiotic and biotic environment is magnificent in terms of rapid population growth plus facing few competitors (Martin, 1999; Sanford et al., 2003; Jackobs et al., 2004). Hence, they can be remarkably wide-spread and ubiquitous in a short time (Orians, 1986; Sax et al., 2007). Among phytophagous insects, the abundance and similarity of biological and ecological resources or host plants is essential for successful colonization and establishment. The survival of phytophagous insect in a new location is much more certain if the potential host plant range is similar to that of invasive species native host plant range (Lattin and Oman, 1983; Simberloff, 1989; Peacock and Warner, 2008). Yet another apparently attractive trait in the invasion success is insect's small body size. For the record among animal invaders, social insects have been rated the most successful, reaching to highest population densities and establishing over broad geographic ranges (Gaston and Lawton, 1988; Holway et al., 2002).

Among the facets of global change, rapid climate change probably has the most potential to influence research direction in invasion science (Richardson, 2011). In today's world

biological invasions are considered more vital when persuaded with the influence of climate change (Hanski, 1998). The common response of species to survive climatic change is directly proportional to their ability of adaptation, either by adapting those changes *in situ* or by colonizing suitable regions that lies outside of their geographic ranges (Thomas et al., 2004; Fitzpatrick et al., 2008). According to Thomas et al., (2004) approximately 15-37% of animal and plant species are believed to be at risk of extinction after 2050. Accounting for the main causes of species extinction are their widespread range expansion to shift and track suitable environment and adaptive changes (Buckley et al., 2012). Species that keep up with the rate of climate change have greater dispersal ability to expand their range margins and establishing new populations (labelled with exotic, alien and invasive species) (Hughes et al., 2003; Simmons and Thomas, 2004).

In the past two decades, empirical studies conducted on the colonized populations of the butterflies revealed their increased dispersal ability (Thomas et al., 2001; Simmons and Thomas, 2004; Hill et al., 2011). During the last century, 35 butterfly species in Europe have expanded their range northwards 35-240 km (Parmesan et al., 1999; Mitikka, 2010). Butterfly species with the ability of expanding range boundaries are reported to have populations with larger adults and broader thorax shape (for example; *Pararge aegeria*). Evidences of these traits have proved that such individuals possess impressive flight and dispersal ability and may invade faster (Hughes et al., 2003 and 2007; Hill et al., 1999). One of the well-known cases documented for climate-driven range expansion with a role of invasive species is the outbreaks and abundance (potential damage) of *Acraea acerata* (Sweet Potato Butterfly) in Uganda. During the past 10 years this species has been recorded as a major pest of sweet potato (Okonya et al., 2015).

1.2 Problem Statement

A significant increase of human trade and tourism has preferably triggered the rates of exotic species introduction into new geographical ranges (directly or indirectly). Invasive species has been assigned as the most significant component of global change (Vitousek et al., 1997) and their impacts on native species, communities and ecosystems has been extensively accredited around the world (Elton, 1958; Sakai et al., 2001; Simberloff, 2011). The invasion of exotic species into novel environment might result into the extinction of local flora and fauna (Vitousek et al., 1997). Once established in novel range they can become a major threat to the local biodiversity.

Therefore, range expansion (due to climate change) and invasion of exotic species had been recognized as two important issues of interest in biodiversity conservation. The key for successful management is to predict the rate at which species spread in order to minimize the impacts of these species on ecosystem (Elliot and Cornell, 2013). During invasion, phenotypic changes occurs under non-equilibrium demographic conditions and may reflect the influences of evolutionary history, chance events and selection. Whereas, established populations of non-native species often experience genetic alterations in phenotypic traits and changes in selection regime that results in the viability of populations (Keller and Taylor, 2008). Biological invaders can be handled with accurate management to reduce the impacts on the ecosystems that they threaten by studying the aspects of physiology, demography, life history, species interactions, dispersal, colonization, range dynamics and environmental variation (Urban et al., 2016). In addition to that for conservation efforts, it is essential to protect the native insect biodiversity by controlling the exotic invaders (Wagner and Driesche, 2010).

Butterflies have worldwide distribution and their tracking is possible due to their past known geographic ranges. Newly invaded butterfly species in any region can be identified if the monitoring methods are precise. The range expansion of *A. terpsicore* and its establishments in the Indo-Australian region is possibly a result of climate change. Because of its success in extending its distribution throughout wide geographic regions southwards over the equator, it is possible that *A. terpsicore* could become established in Southeast Asia including Borneo (Braby et al., 2013). Since its first documentation in Peninsular Malaysia, *A. terpsicore* was only reported in Borneo after almost two decades.

1.3 Research Objectives

Being an exotic species and its impact on the novel ecosystem the objectives were as follows;

- i. To examine the biology, host-plants association and feeding preference of *A. terpsicore* on Passifloraceae and Cucurbitaceae
- ii. To find out the spatial distribution and range expansion of *A. terpsicore* in Borneo pertaining to its rate of colonization in the recently invaded range
- iii. To evaluate the genetic variation among *A. terpsicore* species of Borneo and adjacent areas through *COI* subunit of mitochondrial genome

The intention of this study was to highlight the incidence of *A. terpsicore*'s long-distance migration and invasion into Borneo (with possible mode of entrance). Primarily the first objective aimed at investigating aspects of the life history traits of *A. terpsicore* including the morphology, size variation and population indices. The first objective focused on the innate host findings and colonizing strategies, which would hence, give a picture of establishment levels. This could further indicate its status as an invasive species while pertaining to the most susceptible adaptation of larval food plants.

The second objective was to determine the spatial distribution of *A. terpsicore* was determined by calculating EOO (Extent of Occurrence) across the Island of Borneo. Geographic maps were executed for range expansion under current and future climatic conditions with the help of ArcGIS (10.4) providing forecasts of its current status across Borneo and Southeast Asia and predicting future range margins.

For the third objective a comparative analysis of genetic variation of *A. terpsicore* at level of *COI* subunit of mitochondrial genome was quantified among the samples of different localities from Borneo and adjacent areas for the prospective of species range expansion. Lastly in order to predict this species further range expansion, Species Distribution Modelling was used to identify the likelihood of *A. terpsicore* of expanding its range from Borneo Island towards Philippines and the neighboring islands.

1.4 Thesis Structure

This thesis is arranged in eight parts, which includes five chapters (research articles) written for publication in a scientific journal. A general introduction and a comprehensive review of literature proceeds Chapter 3 which represents first research article. Each article includes an introduction, materials and methods, data analysis, results and discussion. The overall summary and findings of thesis are deliberated in Chapter 8.

CHAPTER 2

LITERATURE REVIEW

2.1 Taxonomic Position of *Acraea terpsicore* (Nymphalidae: Heliconiinae)

Acraea terpsicore is currently classified in the subfamily Heliconiinae. The subfamily Heliconiinae (Michener, 1942) consists of four major tribes namely, Acraeini, Heliconiini, Vagrantini and Argynnini (Penz and Peggie, 2003). Through all the recent approaches in the systematics of Nymphalidae, the affinities within the tribe Acraeini are still controversial and unrevealed (Silva-Brandao et al., 2008). The Acraeini tribe corresponds to the Muller's group IA, Acraeini *sensu* Ehrlich (1958) and Acraeini *sensu* Pierre (1987) (Harvey, 1991). Pierre (1985, 1986, and 1987) treated the Acraeini group for a single genus (Harvey, 1991). The Acraeini tribe is believed to embrace seven genera, amongst which the greatest diversity is found in Africa. Neotropics has the distribution of three genera namely, *Abananote*, *Altinote* and *Actinote* (s. str.) whereas the remaining four genera of *Acraea*, *Bematistes*, *Miyana* and *Pardopsis* are Palearctic in distribution. Although the existing genus of *Acraea* is a paraphyletic group related to the Acraeini genera of South America. *Acraea* species are believed to belong to the *Old World Actinote* (sister group) of Neotropical genera (Silva-Brandao et al., 2008).

The tribe Acraeini retains the disco cellular cross veins and is probably a little more primitive than the subfamily Nymphalinae (Fox et al., 1965). Members are characterized by the number of morphological characters possessed by the larva, pupa, wing veins and scales of adults and the male, female genitalia and abdomen (Penz and Peggie, 2003; Freitas and Brown, 2004). Acraeini butterflies are unpalatable and unpleasant to their predators and generally show distasteful properties to birds and lizards (Brower et al., 1963 and Boyden, 1976). It is suggested

that the unpalatability is due to the presence of cyanoglycosides secreted by thoracic glands (Nahrstedt and Davis, 1983; Kassarov, 2001). *Acraeini* species have slow flight and are mimetic models for several other families such as *Pieridae*, *Papilionidae*, *Lycaenidae* and some moths (Brown, 1992; Larsen, 2005).

There is a growing body of published literature discussing the taxonomic classification and phylogeny of *A. terpsicore*. A debate on the taxonomy was initiated by Eltringham, (1912), in the Monograph of African species of Genus *Acraea*, (Fabricius) with supplements to those in Oriental region. *A. terpsicore* was outlined in very detail as to harboring African regions particularly Madagascar (and adjacent islands), Congo, Rhodesia, German East Africa, British East Africa and Uganda. While *Acraea violae*, which in more recent studies is distinguished as a senior synonym (Pierre and Bernaud, 1997), was discussed as an oriental species existing mainly in Ceylon and the Indian regions. Henning and Williams, (2010) reviewed the taxonomic position of *A. terpsicore*, redefined the nomenclature confusion as in recent studies, *A. serena* replaces *A. eponina* and *A. terpsicore* replaces *A. violae*. However, this controversial species in Nigeria is described as *A. violae* (Fab. 1793), suggesting that it is the same species as *A. terpsicore* (Ogedegbe et al., 2014). On the same note, Anbalgan (2015) ranks *A. terpsicore* and *A. violae* as separate individual species but using the same common name of Tawny Coster. The most recent taxonomic classification of *A. terpsicore* to that of Braby et al., (2014) is as follows;

Order: Lepidoptera

Superfamily: Papilionoidea

Family: Nymphalidae

Subfamily: Heliconiinae

Tribe: *Acraeini*

Genus: *Acraea*

Species: *A. terpsicore* (Linnaeus, 1758)

Synonyms: *Acraea violae* (Fabricius, 1775)

Common name: Tawny Coster

2.2 Biology of *A. terpsicore*

2.2.1 Morphology

Acraea terpsicore possesses a distinctive morphology compared to that of related species from the genus. It has a small thorax but long and narrow abdomen. Male adults are brighter in color than the female, which are dull in coloration. This butterfly is evident in the field due to its bright red coloration and lazy flight with distinctive wing shape. Male wing span is about 40-50mm and exhibits reddish orange to golden yellow color. The female wing span is about 50-65mm and possesses a dusky ochreous color (Eltringham, 1912). The forewings are broad, long and rounded at the apex. Both pairs of wing are bordered black, which on the hind wings become much broader and possess a series of white spots in it (Kunte and Gadgil, 2001; Hoskins, 2014). With regards to the disguise and concealment, *Acraeini* pupae are easiest to identify. The pupae are attached only by the tail and are usually white colored with a row black spots down sides (MiGdoll, 1997).

2.2.2 Physiology

Evidence on the biochemical analysis of *A. terpsicore* has been documented by Shodhganga (2007) whose investigation revealed that the total carbohydrate, protein, lipids and trehalose contents of the haemolymph of pre-pupal larvae, early pupae and 24-hour old adults. Studies on the differential haemocyte count revealed six major types of haemocytes in the haemolymph of *A. terpsicore* and were classified on the basis of morphological and cytological features.

These six types of haemocytes were recognized as prohaemocytes (PRs), plasmatocytes (PLs), granulocytes (GRS), sphernocytes (SPs), oenocytes (OEs) and adipohaemocytes (Ads).

Studies about the global warming effect on the impurity of DNA content of *A. terpsicore* were performed by Anbuselvi (2013). The butterfly species were sampled from different areas, prominently from high temperature polluted areas to medium temperature non-polluted areas. His investigation proposed that Global Warming can cause the extinction of small creatures notably the insects, due to impurities detected in the DNA contents and at levels of gene expression in *COI* subunit of *A. terpsicore*.

The distasteful properties shown by members of Heliconiinae to birds and lizards have very little evidences of biochemical basis (Brower, 1963; Boyden, 1976). The only observational proof of the toxicity of these butterflies is the presence of cyanoglycosides in adults (Nahrstedt and Davis, 1981a). Physiological proof of the two types of cyanoglycosides identified from *H. erato* and *H. dosoris* were linamarin and lotaustralin (suggested to be shared among Heliconiinae members). The larval food plant for Heliconiinae is Passifloraceae, which is considered to be cyanogenic, but the associated cyanoglycosides with this larval host plant are quite different both biogenetically and structurally and hence are unrelated (Conn, 1980). Some *Passiflora* species contain other cyanoglycosides including linamarin (Fischer et al., 1982). The larvae are not exclusively dependent on cyanoglycosides as a dietary supply and thus, it is confirmed that these species are able to synthesize linamarin and lotaustralin (Nahrstedt and Davis, 1981b). Studies on the biosynthesis of cyanoglycosides in the genera of Heliconiinae including the two species of *Cethosia* and two species of *Acraea* (including *A. terpsicore*) were found to be cyanogenic. The larvae of all the six genera were observed to feed upon Passifloraceae and led to sustain the evidence for morphological relationship that all the *Heliconiines*, *Cethosia* and *Acraea* possess the same cyanoglycosides. But the same food plant

used by other Lepidopteran's *Parthenos sylvia* and *Vindula arsinoe* were not cyanogenic, hence confirming the presence of two cyanoglycosides (linamarin and lotaustralin) only arise from *de novo* synthesis (Nahrstedt and Davis, 1983).

2.2.3 Life Cycle of *A. terpsicore*

Scientific literature available on the life cycle studies of the genus *Acraea* documents certain species possessing five to seven larval instars most likely present in the same cohort (Van Son, 1963). Khew (2008) documented the developmental time of *A. terpsicore* passing through six larval instars before pupation in Singapore. However, five larval instars with similar size as reported by Khew, (2008) to that of the Australian species of *A. terpsicore* has been documented by Braby et al., (2014).

2.3 Feeding Habit and Habitat

2.3.1 Feeding Habit

The species of *Acraea* have been reported to feed primarily on Passifloraceae. Those of the *Acraea* species belonging to the basal clade feeds on the plants of family Violaceae, while the remaining *Acraea* of “*Old World Actinote*” are characterized by feeding on family *Urtiaceae* (Silva-Brandao et al., 2008). Phylogenetic studies based on the host plants used by all ancestral *Acraeini* appears to be Passifloraceae as its larval host plants and thus, is considered to be the ancestral plant for all *Heliconiinae* clade (Ackery, 1988; Wahlberg et al., 2003; Willmott, 2003).

The larval host plants of *A. terpsicore* are characterized by numerous families, with most dominant evidences from the family Passifloraceae (*Passiflora*, *Adenia*) (Matsumoto et al., 2012; Das et al., 2010; Kaur et al., 2009) and Cucurbitaceae (Wikarmayanke and Wikramayanke, 2006). *Passiflora* genus consists about 500 species across the globe with great

genetic diversity. *Passiflora foetida* (Stinking/ Wild passionfruit) is widespread throughout the tropics and predominantly in Malaysia and Brunei (Waterhouse, 1994). *Passiflora* species are heliophilous climbers (mostly) and nearly 20 species inhabit the Southeast Asian region, in particular Malaysia, Northeast and East Australia with Pacific Islands (Leyden, 1972). In tropical countries *P. foetida* is considered as of much economic value where the leaves and petals are used in preparation of tea, juices and ice-cream (Lade et al., 2015). However, the young fruit of *P. foetida* is categorized as cyanogenic and the stem and leaves have been reported to be poisonous to the livestock.

As the egg hatches, the larvae's first meal is the egg shell itself (Shodhganga, 2007; Bhagwat and Pejaver, 2011) but in the reported novel ranges, it does not consume the discarded egg shell (Braby et al., 2014). During the first few days, the larva takes a fine spot on the lower side of the leaf and cutoff a complete circle around itself. The larva acquires this feeding manner to avoid the poisonous sap of host plant (*P. foetida*) from flowing into the area inside the circle. Then the upper cuticle is left intact and only the lower surface area is consumed. As the larvae grows, it eats both the lower and upper cuticle leaving small circular holes in the host plant leaves (Shodhganga, 2007). The larvae are reported to chew the leaves completely leaving it porous mash like, as well as consumes the stem, tendrils and flower buds (Lade et al., 2015).

In India, a new larval host plant from the family Passifloraceae, *Turnera ulmifolia*, has been reported for *A. terpsicore* despite the abundantly presence of *P. foetida* (Khot and Gaikawad, 2011; Bhagwat and Pejaver, 2011). *T. ulmifolia* is also known for cynogenesis. If damaged it has the ability to release (HCN) hydrogen cyanide gas.

Subsequently in Ghana, *A. terpsicore* is considered as the dominant pest of *Corchoru olitorius* (Tossa jute) (Duodu and Lawson, 1983). Tossa jute is considered as one of the best

raw materials for the country's fiber bag factory, but it is not protected from its common pest, *A. terpsicore*. Introduction of any new species either accidentally or purposefully have threats of becoming invasive species and more likely pests. In devising a pest management program the knowledge of natural enemies is considered very useful. Hence, the parasites and natural enemies of *A. terpsicore* in Ghana were specifically identified as three predators (species) of the larvae namely, *Platynopus rostratus* Dru, *Macrorhaphis acuta* (Dall) and an unidentified mite. The tachinid *Carcelia normula* (curran) and ichneumonid *Charops diversipes* were also reported to be the common parasites of *A. terpsicore* larvae.

The adults of *A. terpsicore* were reported to be regular visitors of *Tridax procumbens* (a medicinal herb) where they collect nectar and indirectly involves in the pollination of the plant (Raju and Varalakshmi, 2013). The detailed study of the nectar revealed the presence of five essential and ten non-essential amino acids. Predominantly this plant is well adapted for occupation of disturbed habitats. Alturi, (2011) mentioned *A. terpsicore*'s adult nectar resources is *Eupatorium odoratum* and its percent contribution in the butterfly composition of Anacardium plantation was 4.14%. Deepika et al., (2014) compiled the study about the larval and nectar host-plants of the butterflies at Vishakhapatnam. Investigating a total number of 74 host-plant utilized by 43 species of butterflies, the documentation indicated that *A. terpsicore* feed on the *Mangifera indica*, *Hibiscus cannabinus*, *Musa paradisiaca*, *Passiflora foetida* and *Hybanthus enneaspermus*.

2.3.2 Habitat

Acraea terpsicore is a small leathery winged butterfly found in grasslands as well as scrub habitats (Kunte and Gadgil, 2001; Larsen, 2005). Ramana (2010) chose four study sites in Eastern Ghats of India and concluded his work on butterfly's oviposition, host plants, wing

position, proboscis length and different stages of developmental cycle. The presence of *A. terpsicore* was highlighted in all the sites, in addition to its linear population indices throughout the year. An interesting fact mentioned was the tracking of adults way back to more than 200 km to the same host plant on which they have developed to fulfil the sole purpose of its life cycle (oviposition) (Lade et al., 2015).

2.4 Occurrence and Abundance of *A. terpsicore*

Most of the online records on the occurrence of *A. terpsicore* have been reported in distribution studies of Indian Wildlife Sanctuaries. Narayanankutty et al., (2014) documented the butterfly diversity in the Shendurney Wildlife Sanctuary and reported the presence of a total 265 species. Nymphalidae was the most dominant and diversified family in the sanctuary with 81 species encountered. *A. terpsicore* was mentioned as one of the most common species in the Wildlife Sanctuary. Mishra et al., (2010) studied the butterfly diversity inhabiting Wildlife Sanctuary of Orissa, India and found a collection of 92 species of butterflies. Nymphalidae was the most diversified family. The study also found that the habitat of *A. terpsicore* was characterized by scrub forest and open grasslands in the sanctuary.

Shashikumar and Venkatesha (2008) discussed on the abundance of butterflies with respect to seasonal variation in Cubbon Park Bangalore. In a duration of two years of study they recorded 31 species of butterflies in winter, 24 in summer and 40 in the monsoon season. From the tribe Acraeini, *A. terpsicore* was recorded as one of the representative species and much more abundant throughout the seasons. Aneesh et al., (2013) investigated the relative abundance of butterflies between plantations and natural habitats of Kerala Agriculture University Campus Kerala, India. They found that the nymphalid butterflies in natural habitats

are more abundant compared to man-made habitats. The relative abundance of this species was common in both study areas.

Urban greeneries contain a high abundance and variety of butterflies (Arun, 2010). Remarkable work on the richness and biodiversity of butterflies in urban sprawls of Mumbai pointed out the low population levels of all the butterfly fauna during the late summer season and abundance in post monsoon months. *A. terpsicore* was thus more common in the post monsoon period. Seasonal variation and relative abundance of butterflies inhabiting a scrub jungle of Tamilnadu have been studied in detail by Alagumurugan et al., (2011). They briefly documented the abundance of butterflies was due to the accessibility of sufficient food, favorable climate and calm atmosphere. The relative abundance of *A. terpsicore* was 1.53% of the total of eight families, with seasonal occurrence from early monsoon to late monsoon.

A checklist of butterflies from 30 different localities of Northern Western Ghats was compiled by Padhye et al., (2013). The checklist included 191 species of butterflies, classified into 117 genera and six families. Amongst 30 localities, *A. terpsicore* was reported from seven localities and constitute the most common species from the tribe Acraeini. Shashikumar and Venkatesha (2010) analyzed the butterfly communities in relation to the tree density in Bangalore, India. Their conclusion was that butterfly species sampled from closed canopy had more restricted geographical distribution than in an open area. The study indicated that the wide geographical distribution of *A. terpsicore* was attributed to its attraction to tree shades. It also concluded that the seasonal variation of butterfly fauna is dependent on availability of host plants and suitable temperatures.

Larsen (2002) stated that *A. terpsicore* was not a permanent resident of Dehli and is instead a regular migrant. Larsen (2004) documented 234 species of butterflies from

Bangladesh. *A. terpsicore*'s existence in Bangladesh has been observed since 1968 (Ameen and Choudery, 1968). Islam et al., (2011) investigated the butterfly diversity and occurrence of newly recorded species from four different localities in Dhaka, Bangladesh. *A. terpsicore* was commonly found in one of the four localities. In Bhutan *A. terpsicore* was encountered by Kehimkar (2008) and Nidup (2015) first recorded this species from the southern hills of the country. Whereas, Saji et al., (2014) indicated that *A. terpsicore* rarely inhabits the northern and north-eastern areas of southern India.

Vane-Wright and Jong (2003) compiled the annotated checklist of butterfly fauna from the Sulawesi Island. They mentioned the presence of two species of *Acraea* (Fabricius, 1802) and reported that this genus is absent from the Malay Peninsula, Borneo and Philippines and weakly represented in the Indo-Pacific region with four to five species found in India, Burma and China. The host plants mentioned for this genus includes families Passifloraceae, Malvaceae, Violaceae and Urtiaceae. . The first appearance of *A. terpsicore* in the open wasteland of North Eastern Singapore was reported in 2006 (Khew, 2008). Highlighting that the rapid spread of this butterfly across Singapore was due to the plentiful availability of host plant *P. foetida*.

2.5 Climate-driven Range Expansion of *A. terpsicore*

Matsumoto et al., (2012) recorded *A. terpsicore* as one of the newly established species among the ten new butterfly species in Lombok. The colonisation of *A. terpsicore* in Sekaroh (East Lombok Regency) occurred in 2011 and this species feeds and oviposit often in assemblages around *P. foetida*.

Braby et al., (2013) documented the spatial distribution and range expansion of *A. terpsicore* determining its range size under current climatic conditions which is likely to increase in 2050, following the climate change. *A. terpsicore* became established in Indo-china during 1980's and has continued its journey to spread into other parts of South-east Asia. The study also reported the range size of *A. terpsicore* across the equator has expanded approximately by 6,000 km at a rate of 170-230 km per year. This butterfly has become established in the northern parts of Australia and is continuously expanding its ranges rapidly (Braby et al., 2014). Most prominently Braby et al., (2014) pointed out that *A. terpsicore* breeds primarily on *Hybanthus enneaspermus* (Violaceae; a native herb and natural food plant) as well as on *P. foetida* L. (Passifloraceae; which is an introduced vine) in Australia. They also concluded that Australian population of *A. terpsicore* is restricted to Passifloraceae and lacks the ability to consume Cucurbitaceae.

Malaysia is a biodiversity hotspot in which Peninsular Malaysia supports about 1,182 species of butterflies while the eastern part of Malaysia have records of 939 species. The butterfly fauna of Borneo is very similar to that of Peninsular Malaysia, Sumatra and Java (Abang, 2006). These butterflies include cosmopolitan species often those that recently arrived in Peninsular Malaysia or are documented with invasive potential (Wilson et al., 2015). Prior to 2012, no records of butterfly invasion into Borneo has been reported, except for one such occasion of a well-known Southeastern butterfly *Papilio demoleus* (Lime/citrus Swallowtail) (Cordero, 2001). This butterfly was previously lacking in Sumatra, Java, Borneo, Philippines and the Moluccas (Cobert and Pendlebury, 1978). Today this species is widely distributed in the tropical and subtropical regions of Asian continent, Australia, Taiwan, Hainan, New Guinea and Lesser Sunda Islands (Ishii, 1987; Homziak and Homziak, 2006).

Malaysian Borneo has a highest diversity of plant and animal as well as high level of endemism (Cavendish, 2008). However, such biodiversity is under serious threats due to human activities as well as biological invasions. Taking early steps to study biological invasions have positive impacts in the biodiversity conservation. This study was initiated in response to the invasion of a newly reported butterfly “*Acraea terpsicore*” in Borneo to appraise the global problem of biological invasions and its significance.

CHAPTER 3

COMPARATIVE BIOLOGY OF *Acraea terpsicore* (LINNAEUS, 1758) (NYMPHALIDAE: HELICONIINAE), RELATIVE TO HABITAT TYPES, PHENOTYPE AND GENDER

3.1 Introduction

The cosmopolitan Nymphalidae represents one of the largest butterfly families, with about 7,200 species (Freitas and Brown, Jr., 2004). Among Nymphalidae; Heliconiinae was reported exclusive to the Neotropics (Fox et al., 1965). In this subfamily, the tribe Acraeini is characterized by distinctive morphologies of both the immature and adult stages (Willmott et al., 2009). Acraeini have been reported in all tropical and subtropical regions of the world, especially in the Ethiopian region (Von Son, 1963). The genus *Acraea* is widely distributed across the Paleotropics, with Africa recording greater prevalence compared to Asia (Larsen, 2005). Eltringham (1912) divided the genus into nineteen groups while, Von Son (1963), divided the Southern African species into 23 groups based on their genetilia (Henning, 1992). The genus *Acraea* is almost confined to the Ethiopian region, with the presence of about 250 species and only four to five species are recorded from the Oriental and Indo-Australian regions (Van Son, 1963). *Acraea terpsicore* and *A. issoria* (Yellow coster) are the only species found in India (Kunte and Gadgil, 2001; Larsen, 2005; Hoskins, 2014). *Acraea issoria* is quite well established in the Indo-China region up to Taiwan (Emmel and Heppner, 1990). Though from Sulawesi Island the records of *A. moluccana dohertyi* and *A. moluccana parce* were reported but no clear observation of *A. terpsicore* has been documented (Vane-Wright and Jong, 2003). In Australia only one species, *Acraea andromacha* was described (Braby, 2014).

Henning and Williams (2010) reviewed the taxonomic history and the early stages of *A. terpsicore* (Linnaeus, 1758), which was formerly investigated by Pierre and Bernaud (1997). This species at that time was significantly designated as a senior synonym of the *Acraea serena* (Fabricius, 1775) (*Papilio*), although upon detailed investigation it was learned to be the common Indian species illustrated by Linnaeus whereas Fabricius (1793) erroneously restated it as *Papilio violae*. Later (*Fabricius*) *Papilio serena* was also found to be the common *Acraea eponina* which had been described as *Papilio eponina* by Cramer (1780) and was in common use (until 1999). Therefore, *Acraea serena* replaced *Acraea eponina* and *Acraea terpsicore* replaced *Acraea violae*. In the comprehensive monograph of African genus *Acraea*, both *A. terpsicore* and *A. violae* have been described separately (Eltringham, 1912). Ogedegbe et al., (2014) mentioned this controversial species from Nigeria, as *A. violae*, suggesting that it is the same species as *A. terpsicore*. The literature available on the systematic's of *A. terpsicore* (Linnaeus, 1758) solves the mystery that *A. violae* (Fabricius, 1775) is the senior synonym (Braby et al., 2014) and is the same species as *A. terpsicore* as mentioned by various authors in their scientific work (Ackery, 1988; Pierre and Bernaud, 1997).

Acraea terpsicore avoids dense vegetation and shades and is more commonly encountered along the vicinity of the larval host-plant where the larvae infest *Passiflora* species, usually in open grasslands, wooden wreckage along roadsides and dumped areas. It is a small leathery winged butterfly found in grasslands as well as scrub habitats (Kunte and Gadgil, 2001; Larsen, 2005). It is considered amongst the boldest fliers in India because of its very slow but long-sustained wing beats. Even after attacks from its predators it flies unaffected in the same manner (Kunte and Gadgil, 2001). *Acraea terpsicore* is a colony forming species, noticed in marginal habitats of Sri Lanka with more than half a dozen flying together and often on hot sunny days, in the form of swarms flying in savannah/ woodland mosaics (Hoskins, 2014).

In nature, for individuals with a complex life cycle experiencing the sudden ontogenetic transformations (metamorphosis) is ubiquitous (Werner, 1984; Rowe and Ludwig, 1991). Most poikilotherms, particularly insects are described by their life cycles which are less than a year in length (Roff, 1980). The larval and adult stages of holometabolus insects varies morphologically and ecologically. For example, larvae have a limited capacity to disperse but have a great capacity to gain weight while feeding on plant tissue (Stamp and Casey, 1993). On the other hand, adults are greatly mobile and do not grow anymore. While feeding on nectar and sugar rich resources such as honey dew and decaying fruits (Gilbert, 1972 and Gotthard, 2008). Being holometabolus insects, Lepidopterans do not acquire any further growth once they become adult. In the lifetime of female individuals, oviposition may be traced by different chronological patterns, but occurs as a single event (Garcia-Barros, 2000). During the transformation and growth period the larval stage undergoes a varying number of instars (Gotthard, 2008). Individuals of a single species may also vary with the growth rates and instars. Under different photoperiods and between different phenotypic classes, the growth rates of similar individuals varies considerably (e.g. males vs. females) (Nylin, 1992 and Abrams et al., 1996). The life cycle studies of *Acraea* species from the Neotropics have been described with varying number of instars (same species) depending on the time of the year (photoperiod), seasonality and mainly temperature (Henning and Williams, 2010). *A. terpsicore* was also reported to have six larval instars in Singapore (Khew, 2008). Given the background and the literature related to *A. terpsicore*, there was a need to further investigate this invasive species in the context of Borneo landscape where the species has gained a foothold somewhat not yet fully understood. Therefore, the objectives of this study were specifically:

- i. To investigate comparative biology of *A. terpsicore*'s immature stages (egg-larval instars) relative to habitat types.

- ii. To assess the developmental outcomes in *A. terpsicore* with respect to the phenotype and gender.
- iii. To monitor this species in one of its established locations for population indices throughout the year.

3.2 Materials and Methods

The present study was carried out between April 2015 to 2016, at the netted butterfly house and laboratory of UNIMAS campus (N 1° 27' 53.8927", E 110° 25' 36.644"). Observations on the behaviour of the immature stages were conducted in the laboratory while for mating and oviposition the adults were kept in the netted house (Figure 3.2 a and b). Three study sites were selected based on the urbanization levels for the comparative study of immature stages and behaviour (Figure 3.1).

- a) Desa Ilmu (1°27'10.498"N, 110°27'26.83"E); Rural site (RS).
- b) BDC (1°30'34.164"N, 110°21'34.149"E); Suburban site (SUS).
- c) Jalan Stampin Barat (1°30'54.037"N, 110°20'46.286"E); Urban site (US).

Eggs of *A. terpsicore* were collected from the primary host plant *P. foetida* at three sampling sites and brought back to the laboratory, where they were maintained in plastic containers (17cm x 12cm x 6cm) with *P. foetida* as a host plant. The life cycle study was carried out under controlled conditions ($T^{\circ} = 28^{\circ}\text{C}$ and photoperiod of 12:12 hrs of light and dark). For the adult feeding and oviposition, sufficient supply of nectar flowered plants (*Lantana camara*, *Ixora coccinea* and common *Hibiscus rosa sinensis*) and larval host plant (*P. foetida*) were provided.

3.2.1 Data Collection

The observations of the developmental time (eggs to adult) were performed in the laboratory, while the adults were released in the netted house for mating and oviposition. The adults released in the netted house were keenly observed for mating behaviour and oviposition, if failed (found dead) for both, the described events were then removed from the experiment (when found dead). From the firstly collected egg clusters, the F1 generation was utilized in the comparative study of immature stages. Among all the clusters obtained, six eggs were randomly separated from each cluster and observed for the developmental timeline (egg-adult). The

percent (%) survival of all the clusters at each stage was also recorded. The rest of the individuals of F1 generation, in their third instars were utilized in chapter 4 of this study. Body length for each instar was measured using a millimeter scale and duration for each stage was recorded in days. For each larval instar, moulting was ensured by examining the exuviae.

Desa Ilmu (RS) was selected as the permanent site for monitoring *A. terpsicore*'s population indices. This site was selected based on its nearest distance to the university campus and the abundance of its primary host plant *P. foetida* (Figure 3.2 c and d). The site (DI) was visited twice weekly from April 2015 to 2016, and the presence and abundance of immature stages and adults were recorded separately with respect to mean temperature, relative humidity, and maximum rainfall throughout the observed duration.



Figure 3.1: Map showing three sampling sites selected for comparative analysis study of immature stages based on urbanization levels (Source: Google Maps).



Figure 3.2: Study sites and host plant: (a) and (b) the netted house (butterfly) used for the events of mating and oviposition in the study (outside and inside view, respectively); (c) Primary food plant (*Passiflora foetida*) of *A. terpsicore* showing the lavish growth in the study site (DI); (d) Urban site (JSB) and the plentiful growth of host plant.

3.3 Data Analysis

The developmental data of *A. terpsicore* was analyzed and compared from three sites to evaluate the variance for body length (mm) and duration (days) from egg-larval instar stages of the life cycle. To compare any relevant relationship between the gender's body size (pupa-adult stage) and duration, two hypotheses were proposed and variance for two sets of variables were evaluated separately by One-way ANOVA.

The two hypotheses proposed were;

1. H_0 : There is no correlation between genders pupal size and adult wingspan of *A. terpsicore*.

H_a : There is correlation between genders pupal size and adult wingspan of *A. terpsicore*.

2. H_0 : There is no correlation between genders pupal size and pupal duration.

H_a : There is a correlation between genders pupal size and pupal duration.

Since the interpretation of One-Way ANOVA suggests that if;

$F\text{-value} < F\text{ critical value (from F distribution table)}$ or if $p\text{-value} \leq 0.05$, H_0 will be rejected.

Alternatively the correlation between the two variables will be defined as per criteria with the acceptance of H_a hypotheses or vice versa.

3.4 Results

3.4.1 Courtship Activities and Mating Behavior

Copulation was often aerial where male usually grasped the female adult with the tarsal claws of thoracic legs and the pairing occurred end-to-end abdominally and normally lasted up to 2-3 hours (Figure 3.3c). The mating pair generally hangs upside down holding themselves with the help of supporting leaf or branch of a plant. The females after mating were observed feeding on nectar and resting for approximately 24 hrs. Later the event of oviposition was observed, often at late morning hours until noon (1000-1200). On several occasions the newly emerged males were observed eagerly grasping other male *A. terpsicore* for mating, before they were released to the netted house (Figure 3.3a and 3.3b). This might be due to the fact that males have only one chance of copulation in their life time and were intensely impatient to mate.

3.4.2 Host Plants and Oviposition Responses

Passiflora foetida is a heliophilous herb climbing with tendrils, very common in thickets. It is widely distributed in South-east Asia, Malaysia, North-east Australia and Pacific Islands. In all study sites *P. foetida* was frequently abundant and healthier (Figure 3.2c and 3.2d). After 24hrs of copulation or at least 18 hours later, the female was observed to be fluttering from plant to plant seeking for a suitable leaf for oviposition. The surface areas of leaf for oviposition were selected (observed) with very little or no trichomes (plant hairs) at all (Figure 3.4f). Females alighted on the underside of host plant for oviposition around noon and this event lasted up to 2-3 hours (Figure 3.3f). In the field, eggs were found laid on host plant that either already had larvae or egg batches (but not on the same leaf). However, on few occasions the eggs were observed in 2-3 clutches on the same leaf and twice the eggs were found on the upper side of the leaf as well (Figure 3.3d and 3.3e). This might have been due to some sort of interruption

that had occurred during the oviposition event or the surface area of the leaf might have ended further (as butterfly generally choose the marginal area of *P. foetida*).

3.4.3 External Morphology of the Immature Stages

(a) The Eggs

Figure 3.4 shows the eggs of *A. terpsicore*, which were generally laid in clusters and the number of eggs varied from 30-80 per cohort. The typical egg measured about 0.5-0.7 mm in diameter and 0.8-0.9 mm height. Eggs were barrel / olive shaped with pale yellowish in color. They were mostly laid in clusters on the underside of host plant leaves. The eggs were adhered to the underside of leaves in an upright position and the glue surrounded the base of each egg is molded in a circular shield (Figure 3.4a). The barrel shaped egg has 20 longitudinal ridges finely sculptured, runs from the base to apex and each ridge is separated with transverse grooves (Figure 3.4c and 3.4d). Micropyle at the apex of the egg usually forms a rosette with tiny ridges surrounded by a ring of polygonal cells. At the time of oviposition, eggs are pale yellowish in color (Figure 3.4g) and takes about 48-60 hrs to change into black color (Figure 3.4h). The egg stage in the life cycle of *A. terpsicore* lasted 2-3 days.

The eggs became translucent about 8-12 hrs prior to hatching. Figure 3.4b and 3.4h shows the change in color of the eggs, which is due to the development of immatures and formation of head that gives the egg its black appearance. Generally, in some cohorts few of the eggs that did not turned black failed to hatch and can be seen in Figure 3.4f. The eggs usually have a transparent protective liquid that covers the eggs throughout the embryonic period preventing it from dryness. Any fungal/ bacterial attack to the egg or drying out might result in the failure of suitable development /hatching.

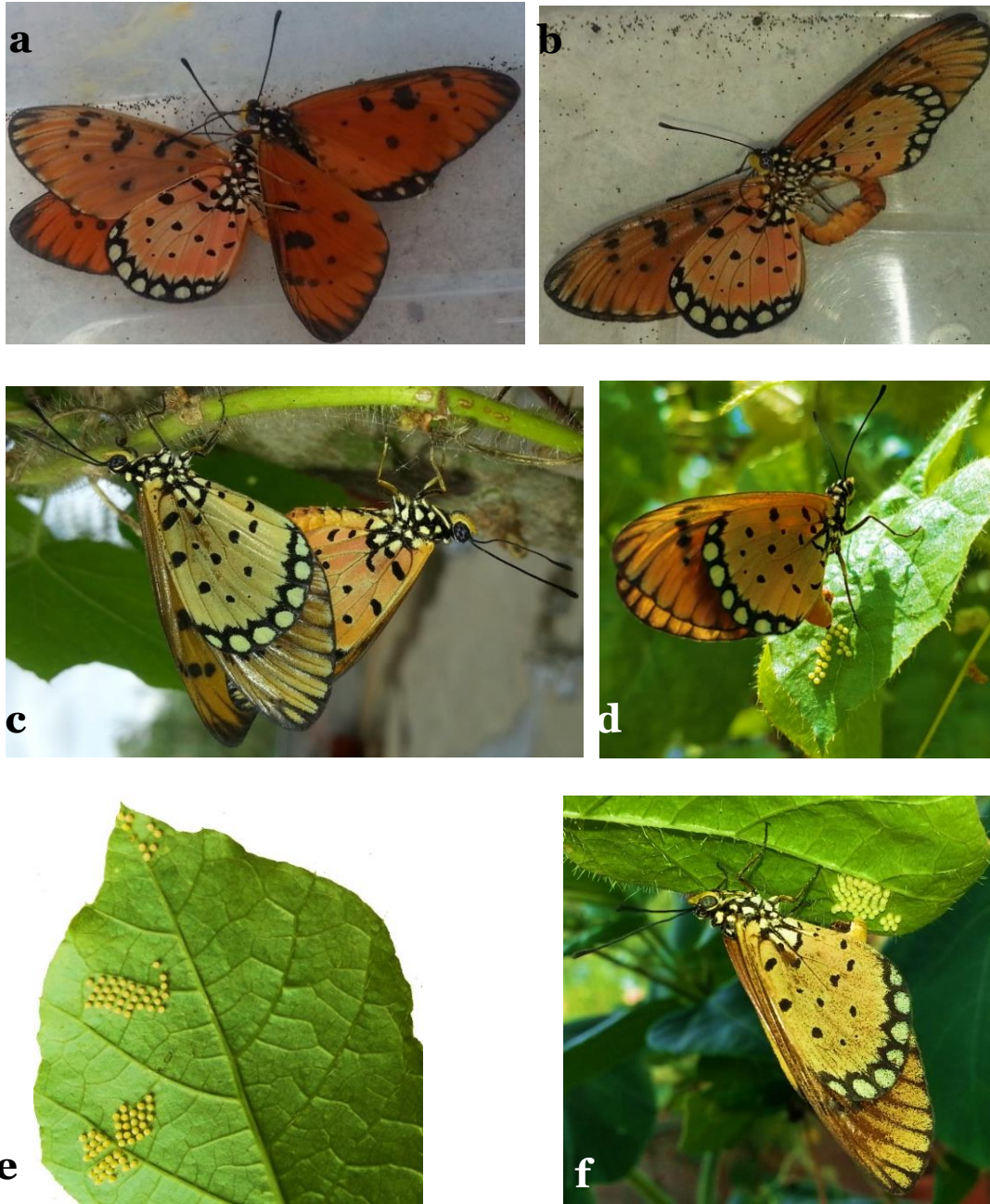


Figure 3.3: Courtship behaviour and oviposition in *A. terpsicore*: (a) and (b) Male adults grasping each other before released to the netted house, eager for mating (after emergence from pupa); (c) Mating pair in netted house; (d) Female ovipositing on the upper side of leaf (*P. foetida*); (e) Patches of eggs laid at a single time on the same leaf of *P. foetida*; (f) Oviposition event.

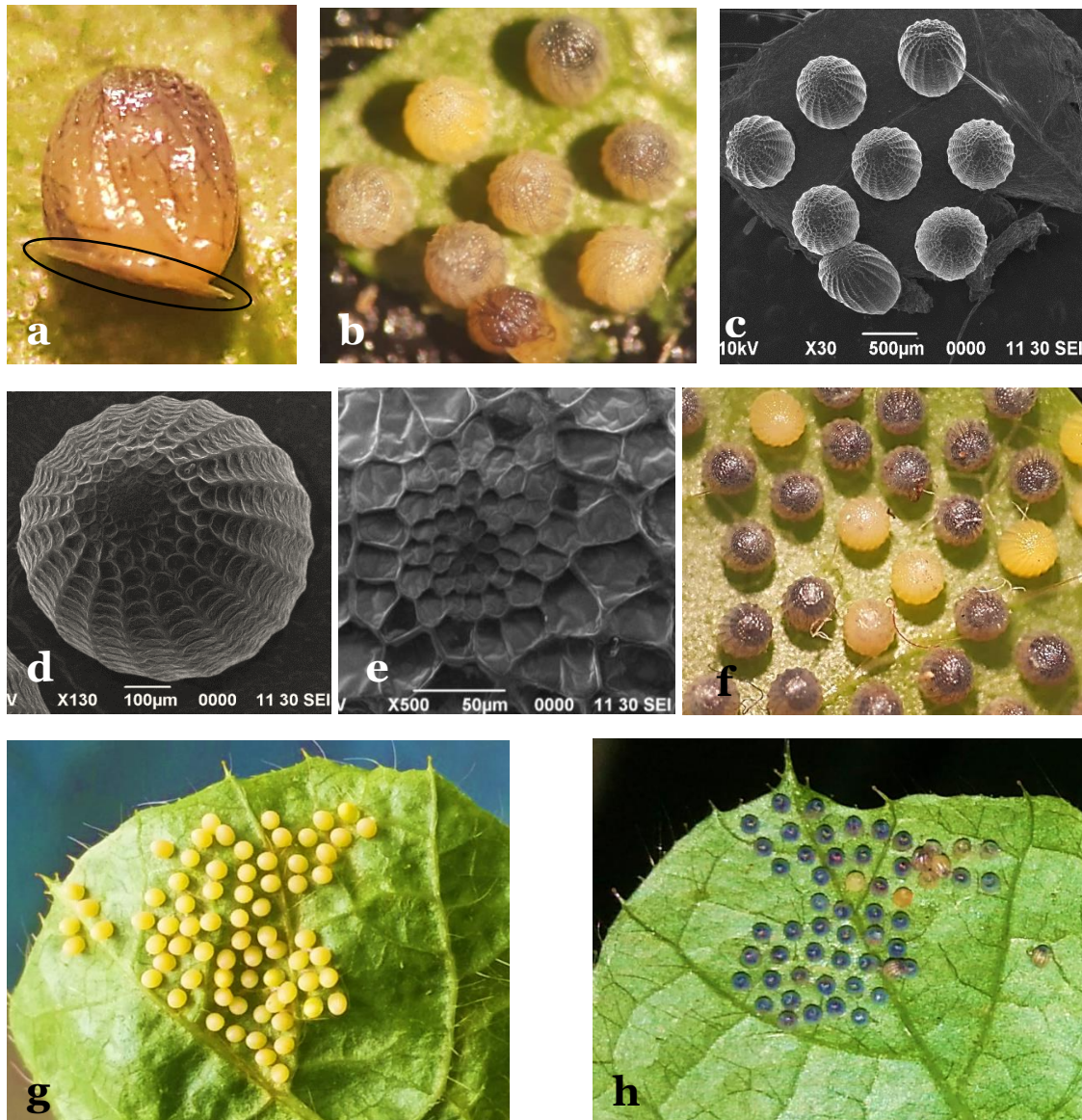


Figure 3.4: Eggs of *A. terpsicore*: (a) Lateral view of an egg with encircled area of glue (visible); (b) Top view of eggs under a Stereomicroscope; (c) Top view of eggs under SEM with a resolution of x30; (d) Single egg under SEM with a resolution of x130; (e) Micropyle of an egg under SEM with a resolution of x500; (f) cluster of eggs with discrepancy; mature (black head) and under development (pale yellow); (g) Typical egg cohort (after oviposition); (h) cohort of mature eggs (8 hrs before hatching).

(b) The First-instar Larvae

As the eggs hatched, the newly developed larva emerged through the micropyle and devoured the egg shell from inside (Figure 3.5a). The first-instar larvae had a smooth round black head and pale yellowish cylindrical body covered with greyish black setae (Figure 3.5b). Each seta arises from the sclerotized pinacula that protruded from the skin. Immediately after emergence the first meal of larvae was its discarded egg shell. Once the food passes through the translucent skin, its color changed into creamy yellow. The prothoracic and anal shields turned dark brown within 6-12 hrs, while the rest of the abdominal body remained creamy yellow. The first-instar fed on the *P. foetida* leaves by eating the cuticle and making a hole near the surface where they emerged. Newly emerged larvae measured about 1-2 mm in length and grows up to 3.5 mm. Approximately 24 hrs later the 1st instar turned into orange color and the stage lasts 3-4 days (Figure 3.5c).

(c) The Second-instar Larvae

The second-instar larvae (Figure 3.5d) as it molts exhibit a burnt orange body colour and a brown to black smooth head. The thoracic and anal segments were blackish in color. The thin and long setae of 1st instar larva was transformed into dorso-laterally black spines arranged in a row of six (numbers) on each body segment. The second-instar larvae possess the same cylindrical body and measures about 9-11 mm in length and the stage lasted 3-4 days (Figure 3.6a). The individuals of second-instar remained together in crowd and fed gregariously. The second-instar larvae secreted a silken web around the leaf area as they fed along to prevent them from falling down (Figure 3.6b).



Figure 3.5: Immature stages of *A. terpsicore*: (a) Newly emerged 1st instars and egg shells; (b) 1st instar larvae after 12hrs; (c) gregarious group of 1st instar larvae feeding on *Passiflora foetida*; (d) earlier 1st instar and newly moulted (encircled) 2nd instar larvae on the same spot.

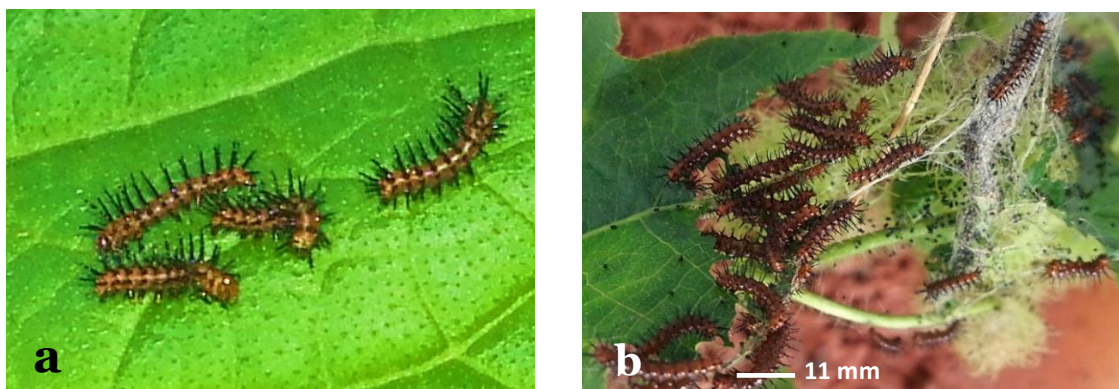


Figure 3.6: Immature stages of *A. terpsicore*: (a) fully grown second instar stage; (b) batch of 2nd instars feeding gregariously, while the silken web can be visualized as well.

(d) The Third-instar Larvae

The third-instar larva (Figure 3.7a and 3.7b) possessed the same features as the second instars and grew in size of up to 11-16 mm but slightly variable in coloration. The head, thorax and last abdominal segments were paler than the rest of the body color which was dark chestnut. The spines grew in size with multiple lateral sub branches. On the ventral side of larvae the color remained pale yellowish. This stage lasted up to 4-4.5 days, and the larvae were observed feeding irrepressibly throughout the time period eradicating the traces of leaves. Upon disturbance the larva usually exuded dark yellowish liquid from the posterior part of the body. The modification in coloration of the newly moulted third-instar and mature stage can be seen in Figure 3.7a and 3.7b.

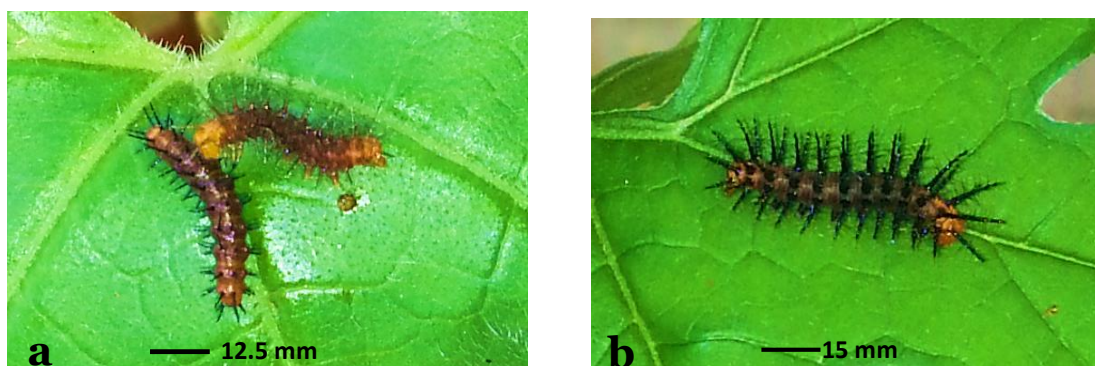


Figure 3.7: Immature stages of *A. terpsicore*: (c) Newly moulted 3rd instar; (d) fully grown third instar larva.

(e) The Fourth-instar Larvae

Figure 3.8a shows the fourth-instar larvae, which displayed no major differences apart from the lightening of body color from chestnut brown to purplish brown. The head of the larva remained globular and the three thoracic and two last abdominal segments retained tawny orange color. On the ventral side pale yellow color transformed into greenish yellow. The fully grown fourth-instar extended up to 21 mm in length and lasted in this stage about 3-4 days. After moulting from the third into fourth-instar stage, the larvae inflates its spines and were completely developed. Figure 3.8b shows each of the spines have 8-12 sub lateral branches of black color. The larvae of *A. terpsicore* were known to feed in groups on the host plant until second-instar but becomes completely solitary in its fourth-instar. The larvae fed and grew gradually in size day by day.



Figure 3.8: Immature stages of *A. terpsicore*: (a) full sized fourth instar stage; (b) close up of the branched spines present in rows over each body segment on fourth instar larva.

(f) The Fifth-instar Larvae

The fifth-instar larvae of *A. terpsicore*, as shown in Figure 3.9 was very similar to that of fourth-instar in appearance with a body length of about 28-31 mm for a fully matured individuals. The body color retained purplish brown on the dorsal surface, whereas the ventral surface had the

greenish yellow color. The head was bright orange, whereas the thoracic and the last two abdominal segments displayed a deep orange color. There were several short black setae on the head and two long, branched spines on the mesothorax. While on the prothorax and metathorax the spine/scoli were long and branched into four. This stage lasted for 4-5 days and the body grew massively while feeding on leaves, stems and flower buds of the host plant.



Figure 3.9: A fully grown fifth instar larva (last instar) of *A. terpsicore*.

(g) The Pre-pupal Stage:

Figure 3.10a shows the pre-pupal stage of *A. terpsicore*, which started after the fifth instar larva spent about 4-5 days in that stage. The larva then ceased feeding and wandered around in search of fine place such as twigs of the host plant, on the underside of leaves and sometimes away from the host plant in the grass/bushes. Pre-pupal stage lasted for 1-1.5 days and on several occasions, the larvae were observed moulting while the pupation starts. During the pre-pupal stage the larvae shrank about 2-3 mm in length and remained stationary if it found a suitable substratum. In laboratory the quiescent larvae usually chose side walls of plastic containers in which they were kept. White patches appeared on the lateral sides of thoracic segments during the last hours of pre-pupal stage.

(h) The Pupa

The pupation as shown in Figure 3.10 b and 3.10d of *A. terpsicore*, took place during the quiescent stage as the last instar suspended vertically with the help of a short cremastral silk pad attached to the substrate. It took about 2-4 hrs for the pupae to reach full sclerotization and coloration. The pupae were white in color with one lateral (on both sides) and two dorsally arranged black bands. Each attained seven tawny orange spots at an equal distance. The ventral side of the pupa also had two black bands whereas, the costal and dorsum margins of the wing case possessed black stripes as well. The pupal stage lasted for 5-6 days. Approximately about 24 hrs prior to eclosion, the pupa turned darker in the wing case and thorax region. Male individuals usually pupated a day earlier than the female individuals, while no major discrimination in the measurement of both sexes was observed. The length of the pupae measured about 20-23 mm. Eclosion of the pupa took place during the 0600-1000 hrs and 1800-1100 hrs of the day. The emergence of adult/ imago occurred from the preapical area of the pupa and the adult rested on the pupal case for about 3-4 hours to allow the wings to dry completely as shown in Figure 3.11a.

3.4.4 The Adults

(i) Head: Figure 3.11b shows the head of adult *A. terpsicore*, which was hypognathus type and was entirely black with yellow scales arranged centrally on the fronto-clipial part. The globular compound eyes were reddish brown along with ocular margins of bright yellow and hairy labial palps were present. The antennae were clubbed, black, and possessed the flagellum that had the width of half of the scape, making it the distinguishing feature present amongst Acraeini.

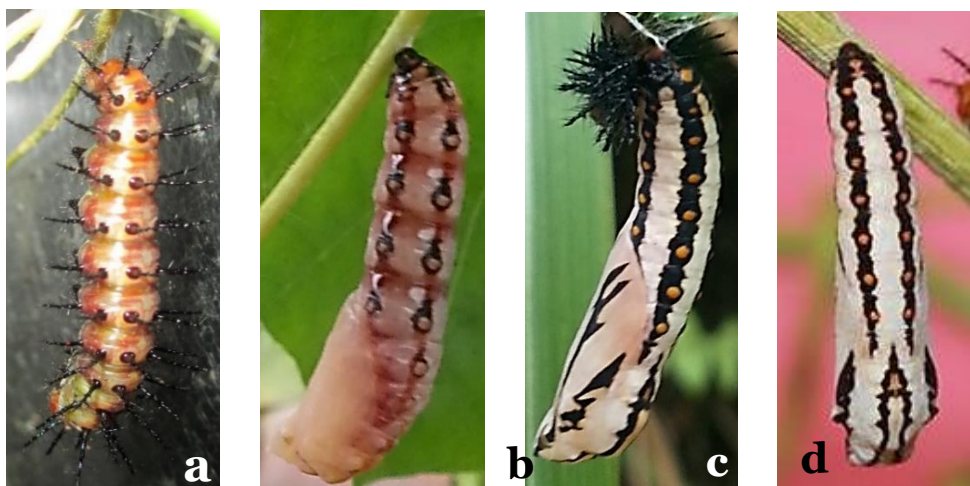


Figure 3.10: (a) Pre-pupal stage with visible white patches over the body; (b) Pupation stage and formation of wing case; (c) completely sclerotized pupa with last instar exuvia attached at the base (lateral view); (d) dorsal view of the fully matured pupa.

In Figure 3.11c and 3.11d, the female had tapered black thorax but possessed a long and thick, dorsally amber brown abdomen. The female wing span measured about 50-65mm and possessed an ochreous orange color, while the broad forewings were round at the apex in both sexes. In contrast to the female, male as shown in Figure 3.11e and 3.11f had a small thorax but the abdomen was long and narrow. Males were brighter in color as compare to females, which were dull in coloration. The wing span of males was recorded to be about 40-50 mm and appeared reddish orange to dark orange color. Regardless of coloration and size both the male and female adults had the same wing pattern and could be distinctly identified in its usual habitat. In the field *A. terpsicore* was more apparent owing to its bright tawny red coloration and lazy flight with distinctive wings shape. Both pairs of the wing were bordered black at the margins and turned out to be thicker for about 3 mm at the apex of the forewings. On the hind wings the black margins were much extensive and possessed a distinctive series of seven white spots in it. Adults were observed flying low and alighting on the ground frequently in the

morning and afternoon hours of sunny days. While feeding on the nectar the adults can be caught easily by hand.

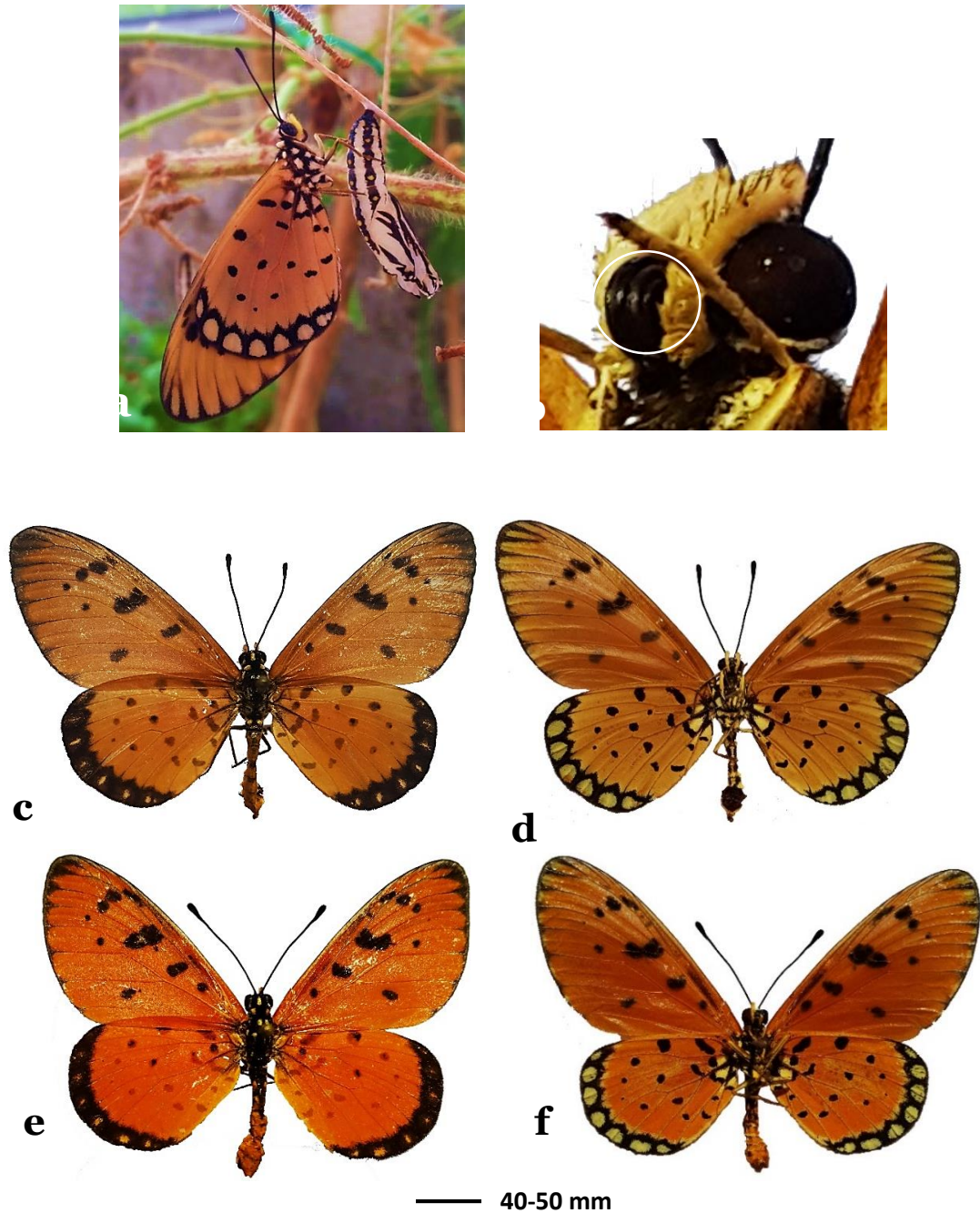


Figure 3.11: The adults of *A. terpsicore*: (a) Male adult emerged from pupa resting on the discarded pupal case; (b) head of the imago with visible compound eyes, labial palps (hairy, yellow) and coiled proboscis (encircled); (c) Female upper side; (d) female under side; (e) Male upper side; (f) Male under side.

(ii) Genitalia

The genitalia in Lepidopteran insects functions in a “key and lock” mechanism to prevent cross-specific mating. The courtship behavior in butterflies is executed with visual cues but typically lifted up by the emission of olfactory scents of male for nearby females (or vice versa). The genus *Acraea* is remarkably known for its distinctive feature of courtship plug in females that appears after pairing and is called “Sphragis” or “Mating plug/ seal”.

Female Genitalia

In *A. terpsicore* it is comprised by the modification of eight to ten abdominal segments (tergum). Commonly during courtship, the female receives a spermatophore from the male, which is usually stored in the Bursa copulatrix, but *Acraea* species also receives a seal on the external abdominal segments. This “seal/ plug” as shown in Figure 3.12a and 3.12b is formed as the secretion of waxy material by the male and is shaped like an appendage/ hollow tile and shiny dark brown in coloration. The Sphragis under SEM as shown in Figure 3.12c, 3.12d and 3.12f, had the same scales and hairs as those of present on the male genitalia. The presence of similar scale and variation in the structure of Sphragis is believed to be used in taxonomic identification of Acraeini. Besides, the primary opening of “corpus bursae” (receptacle of the female) three other holes were present on the Sphragis and were assumed to serve as two openings for clasper (indicated as “C” in the Figure) present on the sides and one centrally located hole (indicated as “U” in the Figure) for Uncus. The sole purpose of the Sphragis is hypothetically to serve three to four general functions and were summarized as:

- i. To prevent seminal fluid leakage from the bursa copulatrix
- ii. To anchor the spermatophore in its place
- iii. To serve as signals for other males that the female has already been mated, or

- iv. To inhibit the emission of strong sexual scents from female genitalia making them free from further attention.

Female butterflies comprises two genital openings, the Sphragis, covering the female's copulatory opening without obstructing the event of oviposition. Whereas, the last abdominal segment modifies into “Ovipositor”, surrounded by intensely broad anal papillae covering the ovipore externally as well (Figure 3.12e).

Male Genitalia

The male genitalia of *A. terpsicore* as shown in Figure 3.13(a-c). It is comprised of a modification of the eighth to tenth abdominal segments. Apparently it consists of the uncus, located centrally at the tegumen (dorsal plate/ sclerotization of the last abdominal segment in male) and curved ventrally into a hook shape. The top area of the uncus is two-tipped and can be seen in Figure 3.13c. It is laterally covered with hairs similar to the size of those present on the valva (ventral plate/ sclerotization of last abdominal segment in male). Figure 3.13a and 3.13b, displays the valva as a curved/ conical shape, covered entirely with bristles and scales from the inner side. The penis in male butterflies is sclerotized into aedeagus and is cylindrical in shape. The aedeagus in *A. terpsicore* has a globose bulb with anteriorly pierced ejaculatory duct, which helps in inseminating the female and generally extends through an eversible sheath or “vesica”.

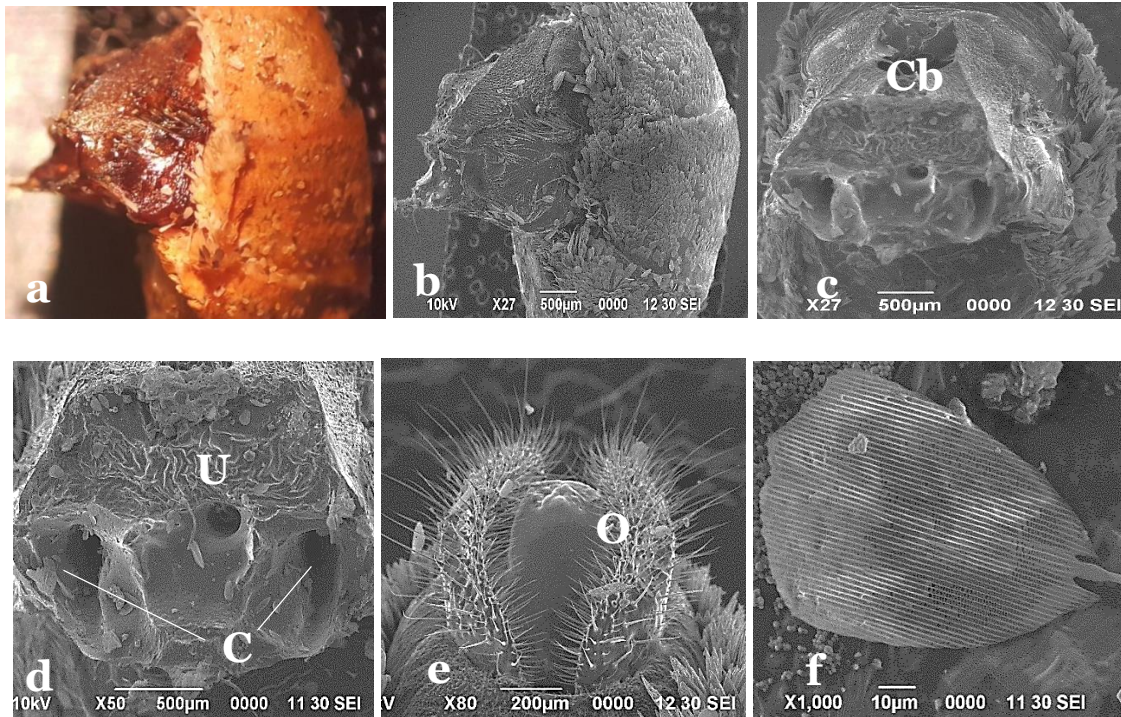


Figure 3.12: Female genitalia of *A. terpsicore*: (a) Lateral view of Sphragis under stereomicroscope; (b) Lateral view of Sphragis under SEM with a resolution of x27; (c) top view of Sphragis under SEM (x27) indicates the opening of Corpus bursae; (d) U = indicates the uncus opening, C = indicates the claspers opening; (e) Ovipositor/ ovipore; (f) Scale on the Sphragis with a resolution x1000 (similarly present on male genital).

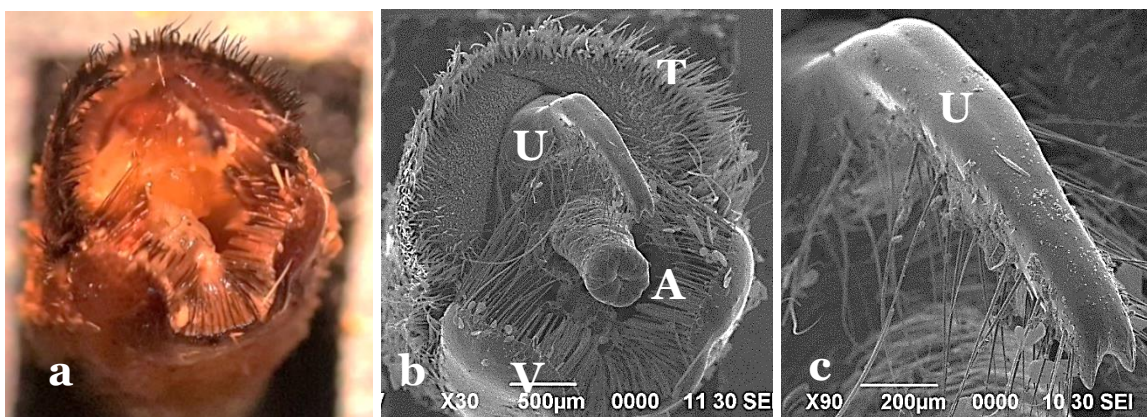


Figure 3.13: The male genitalia of *A. terpsicore*: (a) Male genitalia under a stereomicroscope; (b) Male genitalia under SEM with a resolution of x30, U= indicates the uncus, A= indicates the aedeagus, V= indicates the valva and T= indicates the tegumen; (c) Close up ciew of the Uncus (hook) under SEM with a resolution of x90.

3.4.5 Natural Enemies and Defensive Behavior

Acraeini larvae are considered unpalatable and unpleasant to their predators. This significant reason is due to the presence of cyanoglycosides secreted by thoracic glands. Yet to survive in the cruel world, the secreting of defensive fluid (cyanoglycosides) might not help in preventing them from the attack of natural enemies. Thus, on several occasions in the field both the eggs and larvae were observed to have been attacked by lizards. Mostly instars falling to the ground were immediately attacked by ants (Figure 3.14a) and the first instars were preyed by ants more oftenly in the field. Besides attack of natural enemies the individual larvae (both 4th and 5th instars) of the same cohort were found feeding on the pupa that attempts to pupate earlier (Figure 3.14b) despite the presence of the larval food.



Figure 3.14: Natural enemies and defensive behavior of *A. terpsicore*: (a) fourth instar larva attacked by group of ants; (b) group of larvae attacking the pupa of the same cohort.

3.4.6 Comparison of Developmental Cycle Relative to Habitat Types

The period of development from egg to adult was approximately 30-32 days that passed through five larval instars. The body length of first to fifth larval instars analyzed for the three habitat types was averaged to 3.231 ± 0.025 , 9.578 ± 0.0636 , 14.802 ± 0.0721 , 20.533 ± 0.0721 and 29.92 ± 0.0915 , respectively. Table 3.1 shows the rural site (RS), individuals duration in larval instars

was averaged to 3 ± 0 , 3.083 ± 0.189 , 4.086 ± 0.191 , 4.176 ± 0.323 and 4.288 ± 0.396 . The development time averaged for the suburban site (SUS) immatures in the five larval instars were 3 ± 0 , 3.083 ± 0.192 , 3.972 ± 0.469 , 3.821 ± 0.249 and 4.346 ± 0.427 , respectively. Cohorts from the urban site (US) studied for the developmental cycle took an average of 3 ± 0 , 3.167 ± 0.241 , 3.854 ± 0.403 , 3.979 ± 0.179 and 4.182 ± 0.363 days, among first to fifth larval instars. No significant difference was observed in the body length and duration taken by each stage (days) of *A. terpsicore* in life cycle studies relative to habitat types. One-way ANOVA was performed, to evaluate any significant correlation between gender (male and female) pupal size to that of adult wingspan as well as a relationship between gender pupal size and pupal duration. Table 3.2, summarizes the results of two hypotheses tested for any positive or negative correlation between male and female *A. terpsicore*. The means of the two sets of variables tested (pupal size and wing span), were not all equal. The first and second hypotheses tested at a significance level of 95% and a p -value = 0.05 showed very insignificant results and are described in Table 3.2. For the first hypothesis tested, results suggested the rejection of null hypotheses and a positive association was recognized which specifies, that the bigger gender's pupal size, the larger sized adults would emerge and vice versa. Post Anderson-Darling Normality test suggested the pupal size of male and female individuals were also not much variant in this correlation. The second test resulted in no significant correlation between the gender's pupal size and pupal duration. Post Anderson-Darling Normality test highlighted the pupal duration of male and female individuals were not significantly variant enough. The difference between the mean pupal size, wingspan and pupal duration of both male and female *A. terpsicore* is represented in Figure 3.15.

Table 3.1: Comparative summary of *A. terpsicore* developmental timeline (Body length and duration) from three study sites.

Sampling sites	Stages	Length (mm)					Duration (days)					
		Min	Max	Mean	± SD	SE	Min	Max	Mean	±SD	SE	Range (days)
Rural site (RS)	Eggs						2.5	3	2.75	0.25	0.042	1-3
	L1	3	3.5	3.278	0.252	0.042	3	3	3	0	0	3-7
	L2	9	11	9.708	0.59	0.098	3	3.5	3.083	0.189	0.031	7-11
	L3	14	16	15.157	0.65	0.11	4	4.5	4.086	0.191	0.032	11-14
	L4	19	22	20.456	0.838	0.144	4	5	4.176	0.323	0.055	14-18
	L5	28	31	29.97	0.918	0.16	4	5	4.288	0.396	0.069	18-23
	Pre-Pupa	23	28	26.288	1.571	0.274	0.5	1.5	0.879	0.251	0.044	23-24
	Pupa	19	23	21.561	1.044	0.182	4.5	6.5	5.379	0.573	0.1	24-30
	Adult(B.L)	14	16.5	15.288	0.673	0.117						
	Wingspan	38	49	43.182	2.893	0.504						
Suburban site (SUS)	Eggs						2.5	3	2.833	0.243	0.057	1-3
	L1	3	3.5	3.25	0.257	0.061	3	3	3	0	0	3-7
	L2	9	11	9.528	0.581	0.137	3	3.5	3.083	0.192	0.045	7-11
	L3	14	16	14.972	0.776	0.183	3	4.5	3.972	0.469	0.11	11-14
	L4	19	22	20.643	0.864	0.231	3.5	4	3.821	0.249	0.066	14-18
	L5	28	31	29.615	0.961	0.266	4	5	4.346	0.427	0.119	18-23
	Pre-Pupa	24	28	25.885	1.46	0.405	0.5	15	1	0.289	0.08	23-24
	Pupa	20	23	21.5	1.08	0.3	5	6	5.615	0.506	0.14	24-30
	Adult(B.L)	14	16.5	15.154	0.851	0.236						
	Wingspan	38	49	43.308	3.881	1.076						
Urban site (UB)	Eggs						2.5	3	2.667	0.241	0.049	1-3
	L1	3	3.5	3.167	0.241	0.049	3	3	3	0	0	3-7
	L2	8	11	9.5	0.737	0.15	3	3.5	3.167	0.241	0.049	7-11
	L3	13	16	14.279	0.737	0.15	3	4.5	3.854	0.403	0.082	11-14
	L4	19	22	20.5	0.978	0.2	3.5	4.5	3.979	0.179	0.037	14-18
	L5	28	31	30.182	0.867	0.185	4	5	4.182	0.363	0.077	18-23
	Pre-Pupa	24	28	26.25	1.193	0.254	0.5	1.5	1.023	0.188	0.04	23-24
	Pupa	20	23	21.432	0.623	0.133	4	6.5	5.432	0.563	0.12	24-30
	Adult(B.L)	14	16.5	15.432	0.695	0.148						
	Wingspan	38.5	54	45.614	4.879	1.04						

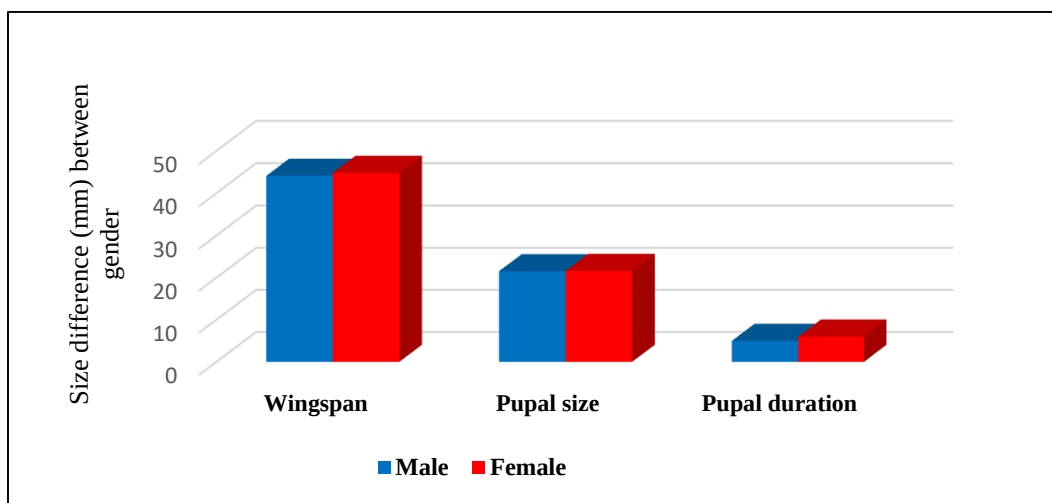


Figure 3.15: The difference between male and female *A. terpsicore*, correlated with pupal size (mm), wingspan (mm) and pupal duration (days).

Table 3.2: Summary of One-Way ANOVA performed for evaluation of two hypotheses.

Sex	Pupal size and Adult wingspan			Pupal size and Pupal duration		
	F	F critical	<i>p</i> -value	F	F critical	<i>p</i> -value
Female	971.0378	4.001191	9.331	8035.088	4.001191	1.2897
Male	1099.9924	4.001191	2.710	8.47.666	4.001191	1.2310

3.4.7 Survival Rate Recorded Among All the Cohorts

The overall data of this study was compiled for the percent (%) survivorship of each stage conquering the developmental phases to become an adult, among all the cohorts of *A. terpsicore*. The total survival rate recorded was 71.8% for successful adults. Figure 3.16 shows the gender ratio among the survived adults was almost equal. For stages egg-third instar the ratio of survival was approximately 90%. However, the percentage dropped in the fourth and fifth larval instars. Consequently, about 5% of the individuals failed to pupate and the emergence ratio to the adult stage was 71.8 %, hence, less than 2% of pupae being compromised to emerge successfully. (see Appendix I as well for the data records)

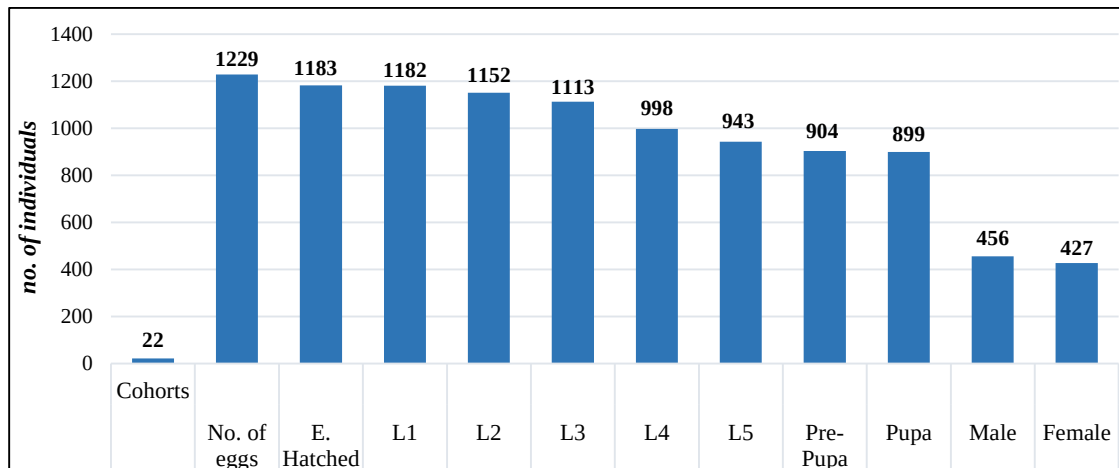


Figure 3.16: The overall survivorship rate of each developmental stage of *A. terpsicore* for 22 cohorts.

3.4.8 Population Index

Being an immigrant lepidopteran fauna of Borneo, *A. terpsicore* could serve a threat to become a potential invasive species. Therefore, for future reference its population indices were recorded for a duration of one year in this study. The rural site (DI) was visited for its population indices and data record is summarized in Table 3.3. The detection of *A. terpsicore* in the study site was evaluated for the observation of both the immature stages and adults if sighted. Whereas, if undetected does not necessarily related to the absence of a species, but might not be detected while present. Overall the presence of immature stages compared to the adults was recorded more frequently, as the immatures tend to remain gregarious and were not too mobile. While the adults mobility (flight) makes them harder to be detected at a specific location. Therefore, the observational data were arranged with respect to sighting, once, twice and thrice times in four weeks of a month. Figure 3.17 represents the population indices with relation to the monthly mean temperature, relative humidity and maximum precipitation.

Table 3.3: Population indices of *A. terpsicore*, relative to mean temperature, relative humidity and maximum precipitation at specific monthly time.

2015-2016	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar
Larval stage	***	***	*	**	***	***	***	***	**	*	**	**
Adults	***	***	**	**	***	***	***	***	**	—	*	**
Mean temperature (°C)	32	33	32	33	33	32	32	32	31	31	30	31
(Av.) R. humidity %	62.8	59.3	62.7	59.3	59.3	62.7	62.7	62.7	66.4	70.5	70.3	70.5
Precipitation	21.1	38.1	54.1	66	35	39.9	36.1	111	94	58.9	77	77

(– not found, * Rare, ** Common, *** Very common) (Weather data retrieved from www.weatherunderground.com)

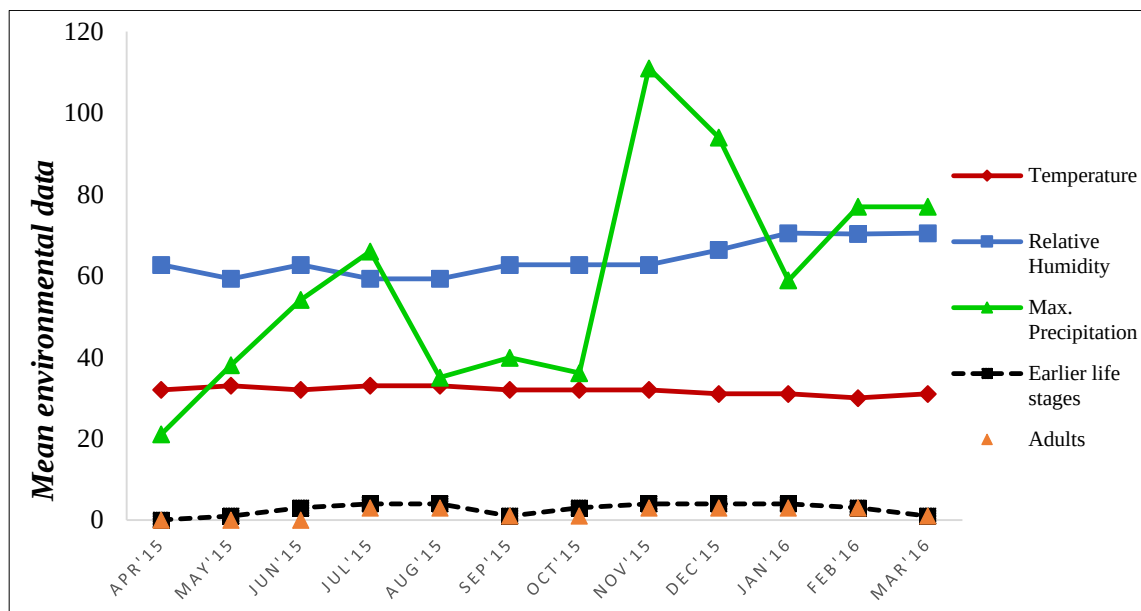


Figure 3.17: Population index of *A. terpsicore*, in relation to the average temperature, relative humidity and maximum precipitation throughout the duration.

3.5 Discussion

Acraea terpsicore was recorded to be one of the prominent species from the tribe Acraeini and is distributed widely through the Indian and Sri Lankan region. With the passage of time, the effect of climate change and habitat modification forced this species to invade geographical regions that lie far more beyond than its native range (Arshad et al., 1996; Khew, 2008; Braby et al., 2013 and 2014). It took about three decades for *A. terpsicore* to invade the Southeast region leading to Australia through the path of Greater Sunda Islands (Matsumoto et al., 2012; Braby et al., 2013; Florida et al., 2015 and Iqbal et al., 2016). Since its first sighting in Sarawak, Borneo (2013) detected as a non-indigenous as well as an addition into the list of invasive species (Abang et al., 2016).

The host plant in Borneo was initially *P. foetida*, which is also referred to as the common ancestral plant for the tribe Acraeini (Pierre and Bernaud, 1997 and Iqbal et al., (2016). The oviposition response encountered at all the sites varied between 30-85 eggs in number and were laid on the underside marginal areas of soft leaves (Braby et al., 2014). Comparative analysis of life cycle studies revealed that the time duration taken by *A. terpsicore* from the three study sites was approximately 30 ± 3.01 days. However in 2014, for general observation the developmental duration was obtained about 30-40 days at the same rural site (DI). Since, in butterflies the overall development time and instar duration are influenced mainly by temperature (Braby, 2003). However, the record annual mean temperature in 2014 was recorded at 28°C, which was similar to that of 2015. This could possibly be due to arriving into new geographic land, decision making was essential to adopt better choices for survival by prioritizing establishment and later to spread.

The total development time taken by each individual in a single cohort to complete one life cycle was directly related to the duration spent by each stage and the number of instars. Among the members of the Acraeini, most of the species were reported to exhibit five to seven larval instars in the same cohort (Henning and Williams, 2010). As for *A. terpsicore* in Singapore, (Khew, 2008) a total of six larval instars were reported. In this current study five larval instars with similar body length as documented by Khew (2008) were recorded. Eggs took almost three days to hatch, and the first meal of the newly hatched instars was the discarded chorion. Whereas, in Australia few of the cohorts kept in captivity were reported to last seven days in their egg stage and the newly hatched larvae did not consume the egg shell (Braby, 2014). The earlier larval instars (1st to 3rd) were gregarious and became more isolated in the last instars. This is a common behaviour among other Acraeini species (Kunte, 2001; Henning and Williams, 2010 and Velez et al., 2011). The average body length for the first to fifth larval instars was attained as 3.231 ± 0.025 , 9.578 ± 0.0636 , 14.802 ± 0.0721 , 20.533 ± 0.0721 and 29.92 ± 0.0915 , respectively. No significant difference was recognized among the immatures of *A. terpsicore* in body length and duration compared for the three habitat types. Development of the larvae was accomplished without any interruption of diapause (Braby et al., 2014). Hypothetical tests conducted through One-way ANOVA, resulted in a positive correlation between the pupal size and wingspan and also among pupal size and pupal duration, regardless of the sex differentiation in *A. terpsicore* (Wiklund and Karlsson, 1984). The males usually pupated a day earlier than the females and this finding is supported by Kunte and Gadgil (2001).

In female adults of *A. terpsicore*, wingspan was about 50-60 mm between the apical margins of the front wings with ochreous orange color. Compared to the females, the male displayed a much more brighter orange color and has a wingspan of about 40-50mm. Regardless of coloration and size, both male and female adults were observed to have the

similar wing patterns and can be distinctly identified in its usual habitat. This is in accordance to Eltringham (1912); Pierre and Bernaud (1997) and Braby et al., (2014). In the field *A. terpsicore* was more apparent owing to its bright tawny red coloration and lazy flight pattern with distinctive wing shape (Kunte and Gadgil, 2001; Hoskins, 2014). Adults were observed to be flying low at about 2-3 meters from the ground frequently in the morning and afternoon hours of sunny days (Braby et al., 2014).

An interesting character of Acraeini members is the female mating plug, the Sphragis received after copulation and is composed of waxy material secreted by the males (Eltringham, 1912; Henning, 1992; Paluch et al., 2003 and 2008). Under the SEM, Sphragis displayed scales similar to those of the present on the male genitalia. However the male has a two-tipped hook-shaped, uncus and the aedeagus was tubular with globose apical area and anteriorly pierced for the passage of spermatophore (Paluch, 2003). The sole purpose of the Sphragis is still under hypothetical conclusions, which is to serve as protection of female preventing them from (again) mating, anchoring the spermatophore or to inhibit the emission of copulatory scents from the female genitalia (Dickinson and Rutowski, 1989; Eltringham, 1912).

The common natural enemies observed in the field were lizards and ants that attacked the eggs and larvae without hesitancy, regardless of the yellowish fluid that is reported to be secreted in result of a defensive behavior (Nahrstedt and Davis, 1983; Kassarov, 2001). Besides the common behavior and characters of *A. terpsicore* for the survivorship rate of each stage conquering the developmental phases successfully to become an adult was 71.8% overall. However the ratio for both male and female adults during this study quite significantly remained in equal proportion (52% males and 48% females, overall) which has been also reported from some other members of Heliconiinae (Clarke and Sheppard, 1975). Population indices for the

abundance was evaluated as to be common during the yearly duration but drops during the months with higher precipitation.

In conclusion, no major discrimination was obtained in the comparative biology studies relative to that of three habitat types based on urbanization levels. In this novel range it also has the similar developmental duration (days) as described in other recent studies. This species was found to feed voraciously on its primary host plant *P. foetida* in this newly invaded range. In general, *A. terpsicore* has already been reported from several locations of Borneo and its future distribution to the nearby regions will likely to happen. This might create a chance of overlapping the geographical range and increases the chance of competition for the native biodiversity. Which could possibly lead to become either an invasive pest or to the extinction of the other species.

CHAPTER 4

HOST PLANT PREFERENCES OF *Acraea terpsicore* (NYMPHALIDAE: HELICONIINAE) AND NUTRITIONAL INDICES FOR PASSIFLORACEAE AND CUCURBITACEAE

4.1 Introduction

Throughout the world phytophagous species are capable of utilizing food plants that exist widely in their geographical and ecological range (Dethier, 1954). Since butterflies are tropical group, so their utilization of food stock is primarily tropical rather than temperate plant families (Ehrlich and Raven, 1964). Today, the diversity of phytophagous insect is estimated to be about a quarter of the total number of species on earth (Strong et al., 1984). The significant reason behind the success of herbivorous insects is believed to be their distribution, abundance and specific response to habitat and climate change which determine the insect host plant association and oviposition choice (Söderlind, 2012). The evolutionary studies of insect-host plant association revealed great knowledge about host ranking correspondence among female preference, offspring preference and offspring performance (Nylin et al., 2015). Experimental evidence suggests that females are of central focus in the relationship of phytophagous insects and their host plants. The females are under great pressure for preferring suitable host plant selection that will permit favorable growth and development of their offspring (Thompson and Pellmyr, 1991; Gripenberg et al., 2010 and Nylin et al., 2015) and to avoid any natural enemies as well as to maximize fecundity rate (Nylin and Janz, 1996; Soler et al., 2012).

In certain cases, the oviposition mistake leads to host range shifting with fitness advantage and the association occur in chemically similar food plants (Forister et al., 2012). But alterations and adaptations of chemically different food plants occurs in cases where there

is limited supply of “preferred” plant and serves as the last option for the survival of larvae (Ehrlich and Raven, 1964).

Although the selection of specific ranged food plants had a significant value for phytophagous insects, there is also a level of preference present for different parts of the food plant in individual genotypes with phenological and physiological conditions (Ehrlich and Murphy, 1988; Wiklund and Karlsson, 1984). The association of host plants and phytophagous insects is often modified by/or restricted to the mechanical and structural characteristics of the food plants (Ehrlich and Raven, 1964). However, chemical factors are the main determinants of food selection (Gordon, 1961). An insect’s food plant selection is a part of its metabolic adjustment thus making the selection of other food plants inefficient for utilization (Thorsteinson, 1960; Ehrlich and Raven, 1964). The immatures of phytophagous insects are attracted to a variety of nutrients assembled in their nutritionally heterogeneous environment. The nutrients in their food plants are highly responsible for growth and reproduction (Tobashnik and Slansky, 1987). In herbivorous insects, the fecundity and oviposition are mainly influenced by the quality and quantity of secondary metabolites they consume. In certain groups of butterflies secondary metabolites are responsible for influencing the insect’s performance. Secondary metabolites are the chief factor in food plant selection and utilization are usually defined by two types of properties, either they are toxic such as glycosides or have antifeedent/repellent properties. (Ehrlich and Raven, 1964; Awmack and Leather, 2002; Aryal et al., 2007).

The second largest lepidopteran family, Nymphalidae embraces about 6000 species with a divergent host plant range (Brower, 2000). The Acraeini tribe (in the past treated as subfamily Acraeinae) is a tropical group and most often reported to be associated with Passifloraceae, Flacourtiaceae, Violaceae and Turneraceae (Ehrlich and Raven, 1964).

In its native land of India, *A. terpsicore* has primarily larval feeding evidences on Passifloraceae (Kunte, 2000; Das et al., 2010; Kaur et al., 2009) and Violaceae (Ramana, 2010; Alturi et al., 2011). Subsequently in Sri Lanka the immatures of *A. terpsicore* were reported to feed on Cucurbitaceae (Wikarmayanke and Wikramayanke, 2006). In India, a new larval host plant *Turnera ulmifolia* has been reported but still found to be unsuccessful in their adjustment of extant conditions (Khot and Gaikawad, 2011; Bhagwat and Pejaver, 2011). In Ghana, *A. terpsicore* is nominated as a principal pest of *Corchorus olitorius* (Tossa jute: Malvaceae) (Duodu and Lawson, 1983). In the Southeast Asian region the larval host plant of *A. terpsicore* in newly invaded locations are also chiefly Passifloraceae and Violaceae (*Passiflora*, *Adenia*, *Hybanthus enneaspermus*) (Matsumoto et al., 2012; Braby et al., 2014). Various studies have been conducted to evaluate the host plant preference of *A. terpsicore* in its native regions but there is only one potential evidence in relation to its invaded range provided by Braby et al., (2014). Modern studies of phytophagous insects and their host plant relationship are quantified for description of variables such as consumption, growth and efficiency of food conversion into insect's biomass (Greenberg et al., 2001). In this current study extensive experiments were conducted to investigate the interactions of host-plant preference and the nutritional indices under semi-natural conditions as well as in the laboratory. Specifically, the two objectives addressed in this part of the study were:

- a) to determine the host plant preferences of *A. terpsicore* among Passifloraceae and Cucurbitaceae
- b) to determine the stage specific nutritional indices, larval consumption rate, growth rate, approximate digestibility and efficiency of food utilization of Passifloraceae and Cucurbitaceae

- c) to describe the oviposition choice acquired by *A. terpsicore* among the Passifloraceae and Cucurbitaceae

4.2 Materials and Methods

4.2.1 Host plants: The five plant species investigated for feeding preference and nutritional indices studies selected were *Passiflora edulis* (Passion fruit) (Passifloraceae), *Cucumis sativus* (cucumber), *Citrullus lanatus* (watermelon), *Cucurbita moschata* (Pumpkin) and *Momordica charantia* (bitter gourd) (Cucurbitaceae). Selection of Cucurbitaceae plants was based on their economic importance as cultivated crops as well as based on the documentation of *A. terpsicore* as a minor pest (melons) in Sri Lanka. Since this species was primarily observed and obtained in the field from *P. foetida*, hence it was used as a control during the feeding experiments.

Passiflora species were planted by the method of grafting in 30 cm pots using a local soil mixture (soil + fertilizer). Whereas for Cucurbitaceae plants, seeds were obtained from a local market and cultivated in 30 cm pots. Plants were grown in the netted (butterfly) house at 27-29 °C, 65-75% RH with a photoperiod of 12:12 (L: D) hours, following Greenberg et al., (2001). A total of four pots for each plant were cultivated to avoid any lessening of the consummated species. Subsequently, alternative pots were prepared for selected plants when required during the trials. All the Cucurbitaceae plants reached to suitable height and growth in 2-3 weeks. Feeding preference trials were performed when the host plants reached to an average height of 1-1.5 m with well-developed leaves.

4.2.2 Experimental larval instars: All the feeding preference trials and nutritional indices studies were conducted on the third instar larvae of *A. terpsicore*. The third instar larvae were selected due to their intermediate stage of developmental cycle and were able to feed or adopt host plants with certainty. Since the neonate larvae were obtained from cohorts of different

locations (as described in part 1), the test caterpillars were used randomly for all the tests and trials. All the larvae were obtained and reared initially on *P. foetida* and were utilized for all the trials.

4.2.3 Experimental design for Feeding Preference Tests

Feeding preference of *A. terpsicore* was evaluated in relation to the position and quantity of host plant community. Generally, three tests were proposed, which include:

- i. Equal opportunity: The larvae of *A. terpsicore* were given a chance to choose amongst preferred host plant and test plant.
- ii. Less opportunity: The number of alternative plants is increased compared to that of preferred host plant.
- iii. No opportunity: The preferred host plant is absent.

In order to better understand and to make it convenient all the test plants were categorized symbolically as shown in Figure 4.1. Four replicates were performed for each test plant and test objective with respect to changes in the position of plants after 24 hrs. A total of four pots of plants were utilized in a single trial. The test area (circular) selected was in semi natural condition (netted house) with a diameter of 3 meters and the distance between each plant was 1.5 meters apart. 16 individual larvae of *A. terpsicore* were introduced in the middle section of the test area to allow the selection of food plant they prefer the most. All the larvae before the trials were starved for six hours to clear up their digestive tract.

The experimental design for the feeding preference tests can be better understood with the help of Figure 4.2.

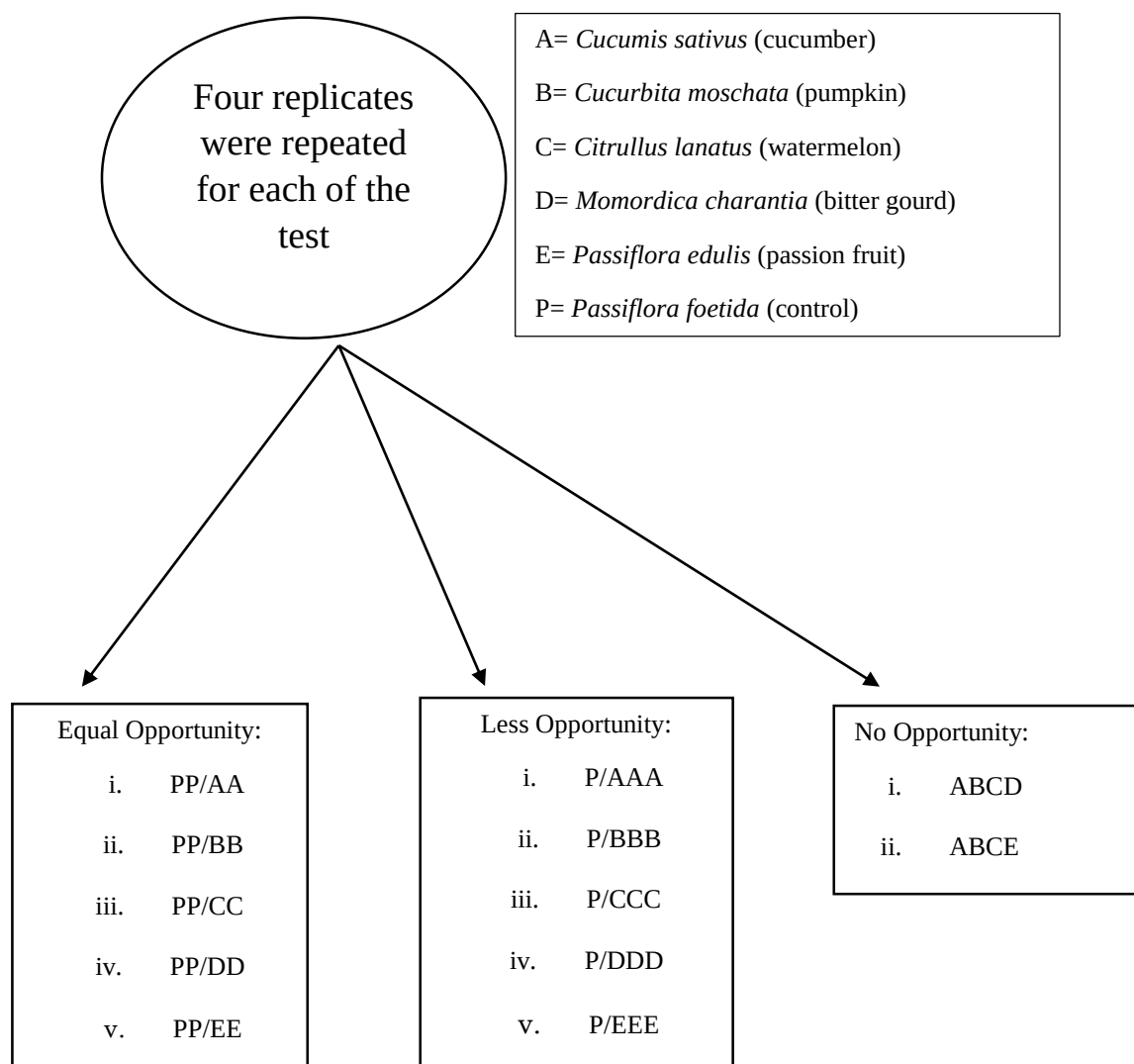


Figure 4.1: Illustration of the three tests performed for the feeding preferences in *A. terpsicore*.

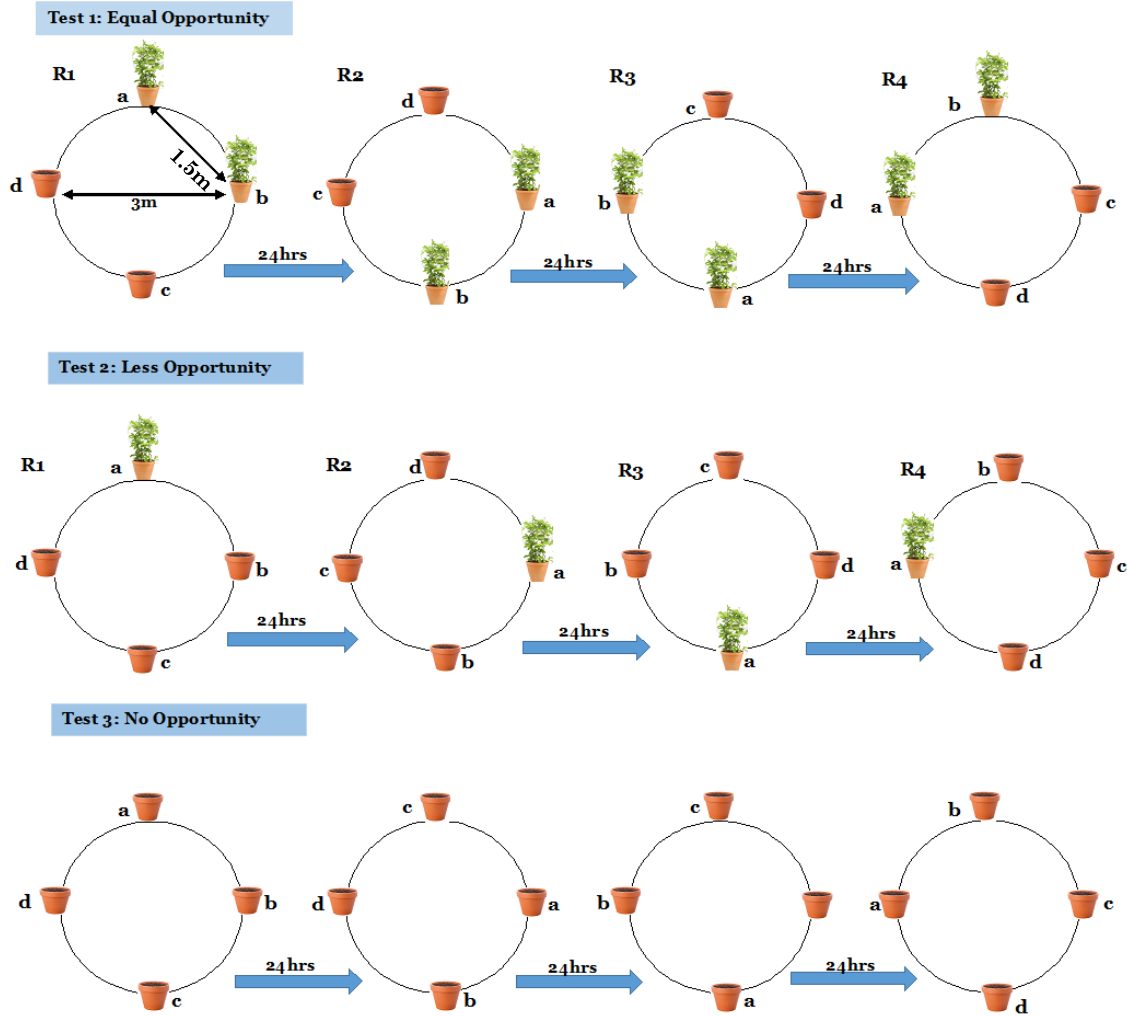


Figure 4.2: Feeding preference experimental design: Test 1; Equal opportunity, diameter of test area and the distance between individual pots mentioned 3m and 1.5 m respectively. The green plant in the Figure indicates *P. foetida*, whereas the empty pots are indicated as test plants. The positioning of the food plants was changed after 24 hours.

Test 2: Less opportunity, shows only one pot of *P. foetida* was utilized during all the trials;

Test 3: No opportunity, no preferred host plant was used in trials.

Five parameters for each replicate and trial were measured which include:

- a) Plants on which larvae were found
- b) Number of larvae present on each plant
- c) Number of leaves attacked on each plant
- d) Number of feeding points attacked per leaf
- e) Position of leaves attacked on each plant (top/ middle/bottom)
- f) Part of the leaves attacked (edge/ midrib/ apical margin/ petiole)

During the trial either the attacked leaves were removed from the plants or they were replaced with new plant pots (if they had less number of leaves left in) after 24 hours. Since all the tests were carried out in a more or less confined area but still during the trials the missing number of larvae were replaced with new additions after 24 hours to adjust the balance. But prior to the addition (to make up 16 test larvae), all the individuals were collected back from their food plants and mixed up in a plastic container before being released in the middle section of the test area. However, *A. terpsicore* was tested for five food plants and subsequently the tests were performed on Cucurbitaceae plants first and then trials were repeated with *P. edulis* by removing one plant (bitter gourd).

4.2.4 Nutritional Indices Experiment:

i). Collection and Standardization of Test Plant Leaves: Leaves from *P. foetida*, *P. edulis*, *C. sativus*, *C. lanatus*, *C. moschata* and *M. charantia* were collected based on equal size and quality. Ten leaves of each species were measured (on an electronic precision balance with an accuracy: 0.000 grams) prior to the trial. The petioles of leaves were inserted (through a hole in cap) in a (2 ml) micro centrifuge tube filled with water to prevent any moisture loss during the experiment and to maintain the natural state possible following Carson et al., (2008).

ii). Standardization of Larvae: Newly molted third instars larvae (neonate of F2 generation, as mentioned in chapter 3) were used for the nutritional indices experiment. To avoid any bias from measurements the third instar larvae was selected, which usually lasts 3-4 days without moulting. Usually, the caterpillars of the same cohort were selected and were starved for six hours to clear their digestive tract. Ten larvae were weighed, frozen, dried and re-weighed to estimate dry weight of the test caterpillars (wet to dry conversion factor). Initial weight of the larvae was measured, followed by freezing them for 24 hours at -70 °C to ensure least painful death. They were weighed again and dried at 62 °C for 72 hours followed re-weighed. From this the average percent of water content in caterpillars was estimated as initial dry weight.

(a) Feeding trial

The nutritional indices experiment was performed in the laboratory under a controlled environment with 28 °C, 60-70% of relative humidity (RH) and a photoperiod of 12:12 (L:D) hours. The ten leaves per species set were placed in a plastic container of about (17cm x 12cm x 6cm) (Figure 4.3). A total of ten replicates were carried out for each of the plant species and the experiment was run for 72 hours. The larvae were checked twice a day (morning and evening) to ensure that caterpillars had enough of food stock and leaves were fully hydrated. If necessary, additional leaves were provided to the larvae and weighed prior to use.

(b) Determining Growth, Consumption, Digestion and Efficiency of Conversion

After 72 hours of feeding trials for all the test plants the larvae were removed from their feeding containers and were kept for three hours in smaller plastic containers (3x3cm). This was done to ensure the clearing of digestive tract and to eliminate any undigested food which could be included in calculations. After 3 hours the larvae were weighed, frozen, dried and re-weighed to obtain the final dry weight.

All the uneaten food mass upon end of trial was also weighed, dried at 62 °C and re-weighed to obtain the dry weight of food ingested as shown in Figure 4.4a. The digestive efficiency of caterpillars was obtained by the collection of frass from all the test caterpillars (separately) at the end of trial (Figure 4.4b). To estimate the dry weight of frass the above mentioned weighing options were performed (following Carson et al., 2008).

For all the test plants and *P. foetida* the nutritional indices of *A. terpsicore* larvae was compared to determine the growth, consumption, digestion and conversion efficiency. The parameters of nutritional indices were calculated adopting Waldbauer (1968) and modified by Karowe (1989). These included:

RCR= Relative consumption rate,

$$\text{RCR} = \frac{\text{Weight of ingested food}}{(\text{Average larval weight during the trial}) \times (\text{number of days})}$$

RGR= Relative growth rate

$$\text{RGR} = \frac{\text{Larval weight gain}}{(\text{Average larval weight during the trial}) \times (\text{number of days})}$$

%AD= Approximate digestibility

$$\% \text{ AD} = \frac{\text{Weight of food ingested} - \text{weight of frass}}{\text{Weight of food ingested}}$$

ECD= Efficiency of conversion of digested food into tissue

$$\text{ECD} = \frac{\text{Larval weight gained}}{\text{Weight of food ingested} - \text{weight of frass}}$$



Figure 4.3: Illustration of nutritional indices feeding trial for *A. terpsicore*, the test plant (watermelon) leaf (petiole inserted in micro centrifuge tube cap and filled with water) in a plastic container with the third instar larva.



Figure 4.4: (a) After feeding trial the unconsumed dried leaf (test plant); (b) dried frass of *A. terpsicore* after feeding trial.

4.2.5 Oviposition Choice

The adults of *A. terpsicore* were given a choice for the event of oviposition among the test plants of Passifloraceae and Cucurbitaceae. The adults were released for this purpose in the netted house for 3 – 4 days after mating.

4.3 Data Analysis

4.3.1 Feeding Preference Experiment

Due to some non-numeric parameters and less/ not at all larval feeding performance, the data was analyzed for only three selective parameters. There were only two observations recorded for *C. sativus*, but with no feeding attack on the leaves. Therefore, the two observations were omitted from analysis. Summary of host plant preference experimental data compared for Passifloraceae is given in Table 4.1. A One-way ANOVA was performed to evaluate any significant variance for position of leaves on the plant (attacked), number of larvae attacking and number of leaves attacked. Since data sets have unequal number of observations, hence, post-comparison Tukey's test was applied.

4.3.2 Nutritional Indices Experiment

Before applying any statistical analysis, the data was reviewed for inaccuracy.

For all the Cucurbitaceae plants negative consumption was calculated for larval performance. Due to negative consumption and zero digestion, the data was statistically compared only between *P. foetida* and *P. edulis*. All the four nutritional indices were compared between the Passifloraceae plants by Mann-Whitney U tests.

Table 4.1: Summary of host plant preference experimental data, compared for Passifloraceae.

Experiment type	Test Plant	No. of Larvae found			No. of leaves attacked		
		Top	Middle	Bottom	Top	Middle	Bottom
Equal opportunity	<i>Passiflora</i>	6	8	1	11	17	4
	<i>foetida</i>	3	3	5	11	15	6
		5	7	3	7	9	3
		3	7	2	5	14	2
		8	8	2	14	20	3
		7	5	2	11	9	2
		3	6		4	10	
		2	6		9	9	
		9	7		11	16	
		4	7		7	13	
		4	6		9	9	
		4	9		4	18	
		3	7		5	8	
			5			12	
			9			11	
			6			6	
			6			8	
			2			2	
Less opportunity	<i>Passiflora</i>	1	5	2	1	8	3
	<i>foetida</i>	4	8	4	4	16	5
		2	5	4	3	9	4
		4	5	2	6	14	3
		4	4	5	5	5	5
		4	6	2	8	10	2
			3	6		3	6
			7	5		11	6
			7	2		7	3
			5	2		5	2
			4			6	
			3			4	
			6			6	
No opportunity	<i>Passiflora</i>	3	4	2	3	6	2
	<i>edulis</i>	3	4	1	3	5	1
			4			4	

4.4 Results

4.4.1 Feeding Preference Experiments

Equal Opportunity Test: Among all the test plants the data was only analyzed for *P. foetida* since there was no observation recorded for all the other plants. There was significant variance recorded among all the three positions of plant and the number of larvae that were found. One-way ANOVA computed the p value = 0.0005 (significance level of 95%), which was significantly low. Tukey's post comparison showed significant variant position was the bottom position of the plant at which relatively lesser number of larvae were found as shown in Figure 4.5a. The most attacked position of the plant was the middle section with a larger number of larvae found. There was not much difference found between the top and middle position in relation to the number of larvae. Similarly, Figure 4.5b shows the results obtained for the number of leaves attacked at three positions that varied significantly. The p value = 0.0003 which was significantly low and Tukey's test showed the bottom position leaves that were attacked in relatively low quantity. The results for equal opportunity tests revealed that the larvae showed preference for *P. foetida* at the middle position where a larger number of larvae attacked and alternatively damaged the highest number of leaves.

Less Opportunity Test: Similar data analysis pattern was followed due to no observational data for test plants. Therefore, the data of *P. foetida* was compared for the number of larvae and the number of leaves attacked relative to the top, middle and bottom section of the plant. The ANOVA results for the number of larvae found (at significance level of 95%) showed very low p -value = 0.0082.

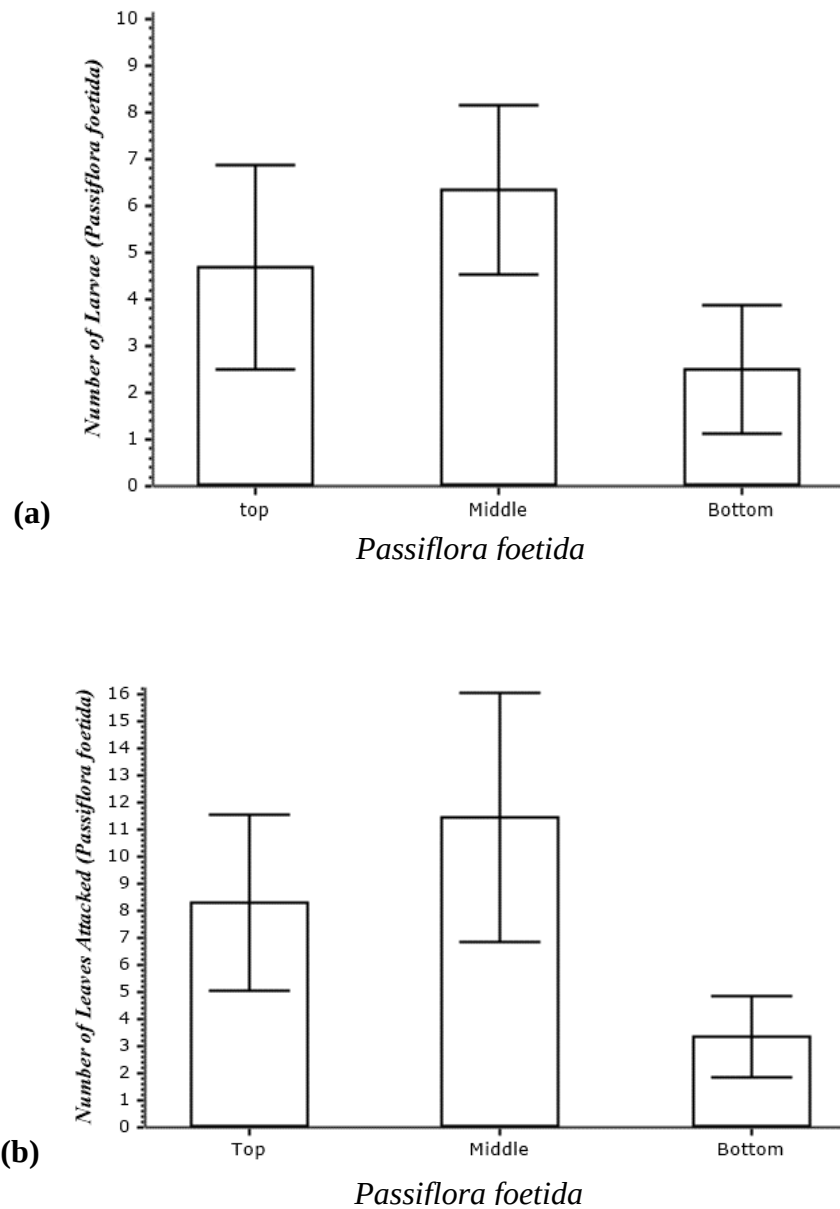


Figure 4.5: Equal opportunity feeding preference results for *P. foetida* (a) graphical bars of the number of larvae found in relation the top, middle and bottom position (8 ± 3); (b) graphical bars of number of leaves attacked at three positions of tests plant. Middle section was ranked as highly attracted position for the *A. terpsicore* larvae (16 ± 5).

Tukey's post comparison analysis indicated the middle position was most attractive for the *A. terpsicore* larvae as shown in Figure 4.6a. There was not much variance found between the top and bottom position of the plant when compared to the number of larvae attacked. The results for the number of leaves attacked at three positions of *P. foetida* were almost similar to that of number of larvae found. The p -value = 0.0067 was significantly low and post comparative Tukey's test revealed the middle position was significantly attractive for the larvae of *A. terpsicore* as shown in Figure 4.6b.

No Opportunity Test: For this trial the observational data was obtained only for *P. edulis*. The results of ANOVA for the number of larvae at three sections of the test plant computed a p value = 0.0039. Tukey's post comparative analysis showed the bottom position was significantly less attacked by *A. terpsicore* larvae. Moreover, there was not much difference between attraction among the top and middle section of test plant (Figure 4.7a). In terms of the number of leaves attacked in relation to their position on the plant, the ANOVA test was significant at a p value of = 0.0199. Tukey's test indicated the bottom position was significant for the number of leaves attacked, but not much discrimination was found for the top and middle section. However, in the less opportunity tests, the middle section on *P. edulis* also showed the higher rank for attraction and attack by the *A. terpsicore* larvae as shown in Figure 4.7b. The summary of ANOVA results and post comparative Tukey's test are given in Table 4.2.

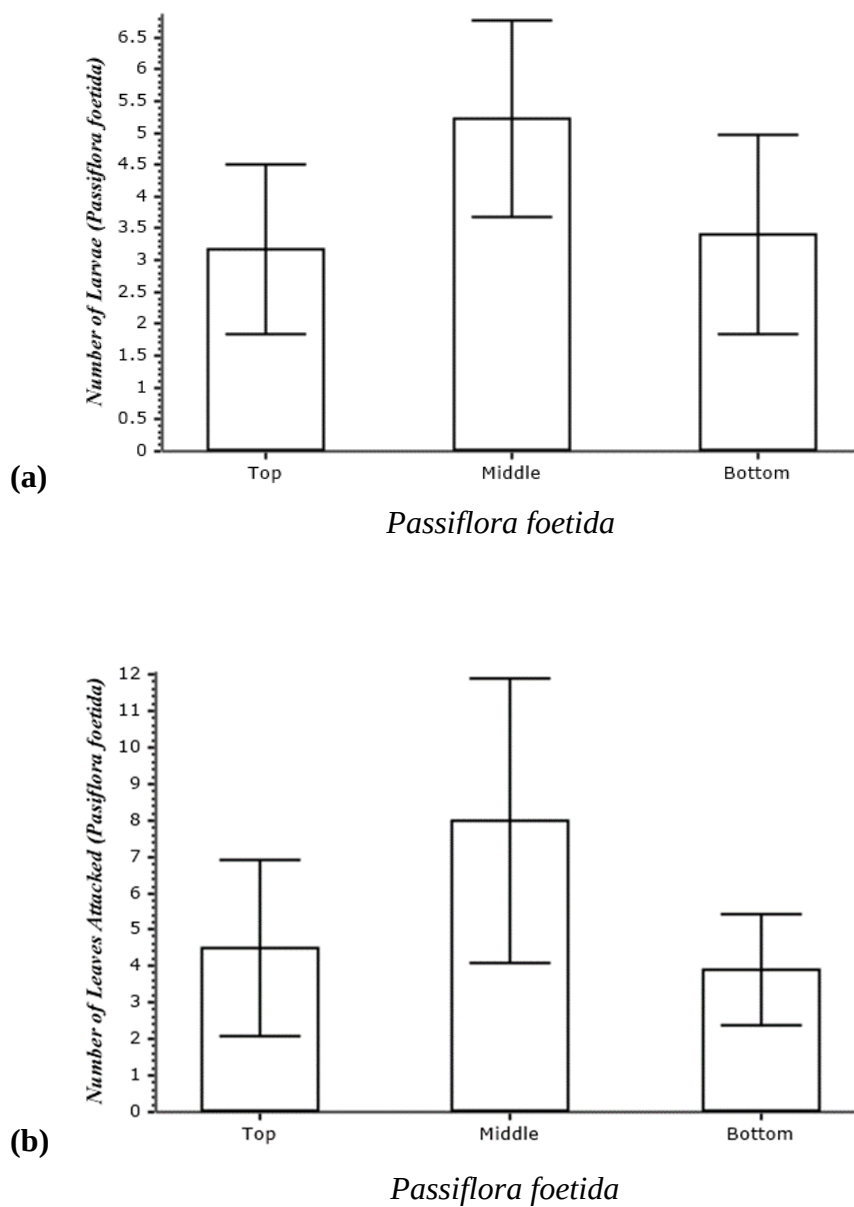


Figure 4.6: Less opportunity feeding preference results for *P. foetida* (a) graphical bars of number of larvae found in more/less equally relative to the top and bottom position (6 ± 2.5); (b) graphical bars of number of leaves attacked at top, middle and bottom position of tests plant. Middle section was ranked as highly attracted position for the larvae of *A. terpsicore* (12 ± 6).

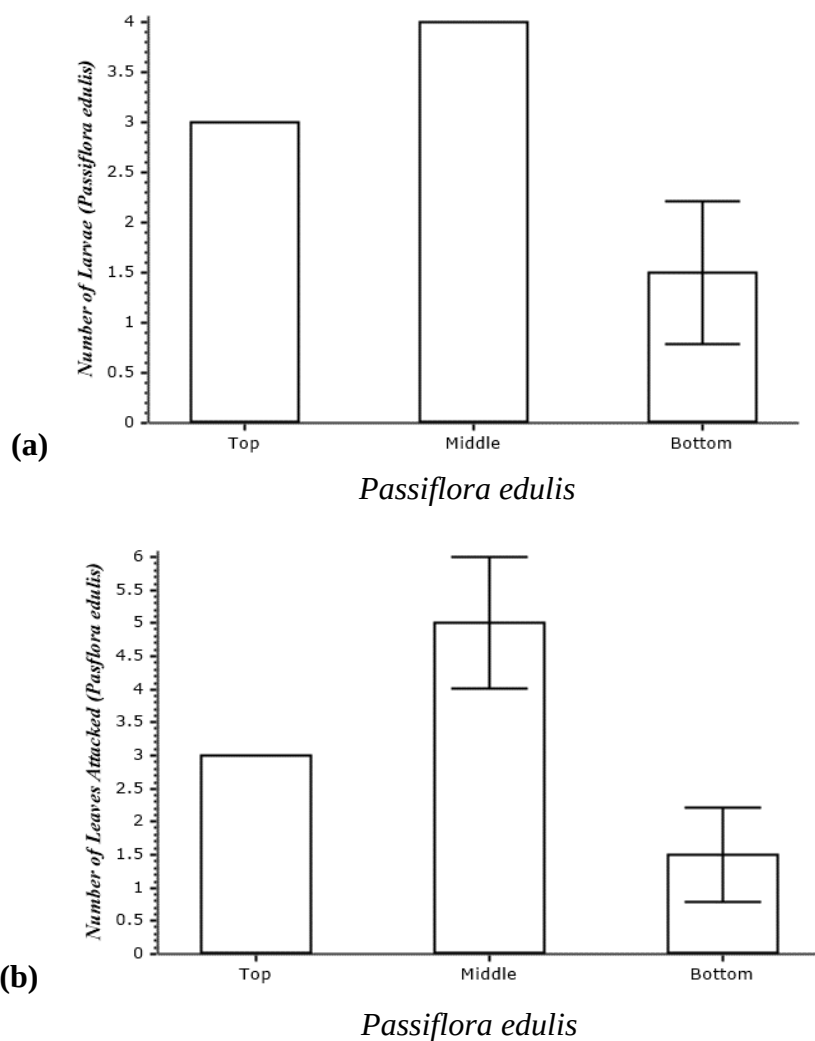


Figure 4.7: No opportunity feeding preference results for *P. edulis* (a) graphical bars of number of larvae found in more/less equal means for top and bottom position (4 ± 1.5); (b) graphical bars of number of leaves attacked at top, middle and bottom position of tests plant. Middle section was ranked as highly attracted position for the larvae of *A. terpsicore* (with 6 ± 2.5).

Table 4.2: Summary of One-way ANOVA and post comparative Tukey's test for all three feeding preference tests.

Experimental type	Plants Evaluated	Number of larvae found (<i>p</i> -value)	Number of leaves attacked (<i>p</i> -value)	Tukey's test (Significant position)
Equal	<i>Passiflora foetida</i>	0.0005	0.0003	bottom
Opportunity	<i>Passiflora foetida</i>	0.0082	0.0067	middle
Less opportunity	<i>Passiflora foetida</i>	0.0039	0.0199	bottom
No opportunity	<i>Passiflora edulis</i>			

4.4.2 Nutritional Indices Experiments

The data for Cucurbitaceae was excluded due to high negative value and all the larvae died during the trial rather than attempting to consume the non-preferred plants. Hence, the data for nutritional indices analyzed through Mann-Whitney's U test revealed not much discrimination among all the four parameters. Table 4.3 shows the summary of nutritional indices for all the test plants. The RCR of *A. terpsicore* for *P. foetida* was 0.205 gm and for *P. edulis* was calculated as 0.053 grams (comparatively negligible). The RGR was also more or less calculated at 0.285 gm for *P. foetida* and 0.12gm to that of *P. edulis*. However, the RGR for all the plants with negative consumption rate was also similar. Therefore, the difference in value of RGR was not much significant compared among all of the plants tested except for *P. foetida*. In the case of (%) AD as shown in Figure 4.8, *P. foetida* showed a higher ratio relative to that of *P. edulis* with 92.1% and 72.54%, respectively. The efficiency of converting digested food into insect biomass (ECD) was negative for *P. edulis* and therefore, was considered zero. Whereas the third-instar larva of *A. terpsicore* had an ability to convert about 1.51 grams of digested food into its biomass. Mann-Whitney test (comparing two data medians) resulted with a p -value =1, indicated that medians of both *Passiflora* species, among four nutritional parameters are not at all different. Result of Mann-Whitney's U test compared for *A. terpsicore* nutritional performance as shown in Figure 4.9.

Table 4.3: Nutritional indices results for all the test plants tested on third instar larvae of *A. terpsicore*.

Nutritional parameters	<i>Passiflora foetida</i>	<i>Passiflora edulis</i>	<i>Cucumis sativus</i> (Cucumber)	<i>Cucurbita moschata</i> (Pumpkin)	<i>Citrullus lanatus</i> (watermelon)	<i>Momordica charantia</i> (bitter gourd)
RCR	0.205	0.053	-0.157	-0.107	-0.144	-0.086
RGR	0.285	0.12	0.33	0.33	0.32	0.31
%AD	92.1	72.54	100	100	100	98
ECD	1.51	-1.13	-2.11	-3.12	-2.302	-3.67

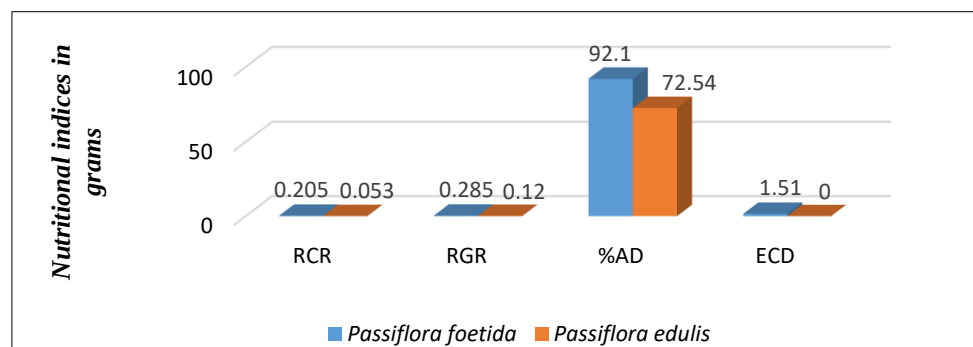


Figure 4.8: Nutritional indices of *A. terpsicore* compared between *P. foetida* and *P. edulis*.

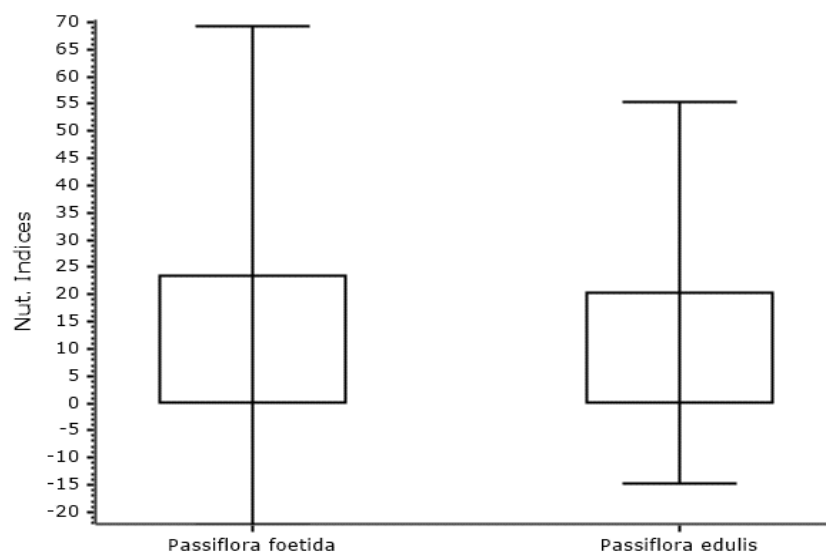


Figure 4.9: Mann-Whitney's comparative analysis of *A. terpsicore* third-instar larval performance for *P. foetida* and *P. edulis* with SD (70 ± 20).

4.4.3 Oviposition Choice of *A. terpsicore*

Prior to conducting the feeding preference experiments the adults of *A. terpsicore* were given a choice of oviposition. All the test plants were abundant in number at the netted house including *P. foetida*. The oviposition event was recorded three times for *P. edulis* and the eggs of a single cohort was found to be 20-40 in number. Among the three batches of eggs only two successfully hatched on time while one of the cohort of 19 eggs were found desiccated instead of hatched. The oviposition response on *P. edulis* was also a little different from that of *P. foetida*. The size of the eggs encountered on *P. edulis* was relatively smaller and were laid at a distance rather than in a compact batch which can be seen in Figure 4.10a. Also the newly hatched larvae did not consume the discarded chorion. Figure 4.10b shows the newly hatched larvae after hatching moved to the edge of leaves and started consuming the lower cuticle of a leaf leaving the upper part intact.

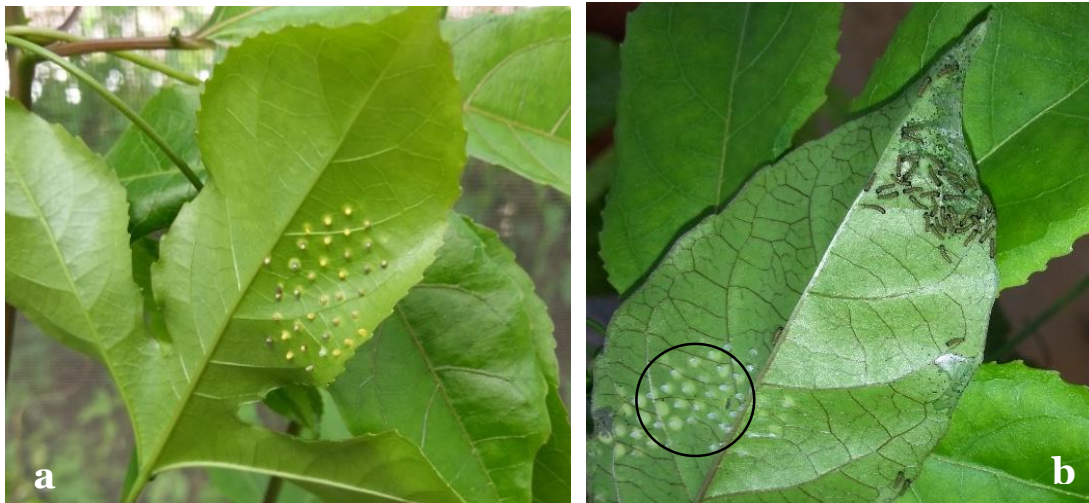


Figure 4.10: Oviposition of *A. terpsicore* on *P. edulis*; (a) cohort of eggs, eggs are distinctly apart; (b) newly hatched larvae from the cohort consuming the edges on underside cuticle of leaf, encircled area shows the unconsumed egg shells.

4.5 Discussion

Major larval food plants of *A. terpsicore* had been reported from Passifloraceae (*Passiflora*, *Adenia* species) and Cucurbitaceae in its native Indian and Sri Lankan regions (Bingham, 1905; Eltringham, 1912; Wynter-Blyth, 1957; Kunte, 2000 and Robinson et al., 2001; Wikramayanke and Wikramayanke, 2006). The larvae of *A. terpsicore* in India has been reported to exploit *Hybanthus enneaspermus* (Violaceae) (Alturi, 1999; Kaur et al., 2009 and Das et al., 2010). In India it has also recently been found to adapt to a new host plant, *Turnera ulmifolia* (Passifloraceae) for oviposition and larval food (Khot and Gaikwad, 2011; Bhagwat and Pejaver, 2011). The Acraeini tribe is mainly Neotropical in distribution and the species of *Acraea* from the African region has been noted for feeding on Moraceae, Passifloraceae, Tiliaceae, Sterculiaceae, Urticaceae, Verbenaceae and Violaceae (Owen, 1971). Whereas in Ghana, *A. terpsicore* is prominent as the principal pest of *Corchorus olitorius* (Malvaceae) (Tossa jute) (Duodu and Lawson, 1983).

Along the course of migration from native lands of India and Sri Lanka, *A. terpsicore* has followed the legacy of seeking its ancestral food plant of the family Passifloraceae while invading new geographic areas. The immatures are found to feed voraciously on *P. foetida* in Vietnam (Pierre and Bernaud, 1997), Malaysia and Singapore (Arshad et al., 1996 and Khew, 2008) and Lombok, Indonesia (Matsumoto et al., 2012).

The recent detection of *A. terpsicore* in Sarawak, Borneo (Abang et al., 2016) also showed that *P. foetida* is its primary host plant in this region. At present, *A. terpsicore* has been established in the Southeastern and Western part of Borneo and continues to disperse further northwards successfully. In a newly invaded geographic area, species interaction in ecosystem and feeding specialization provide information to identify the target species (Fisher, 1998). The

oviposition preference observations highlighted *A. terpsicore*'s potential effort of acquiring *P. edulis* instead of Cucurbitaceae plants. Feeding preference experiments carried out for *A. terpsicore* suggest its strong potential attraction towards Passifloraceae (especially *P. foetida*). So far, there is still no tendency of *A. terpsicore* to pose a threat of becoming vegetable crop pest (Cucurbitaceae). For *P. foetida* and *P. edulis* plants, the immatures of *A. terpsicore* particularly found to be more attracted and causing damage to leaves at the middle position of the plant compared to the leaves of top and bottom position. The nutritional indices study revealed that *A. terpsicore* has a RGR of 0.285 gm and 0.12 gm for *P. foetida* and *P. edulis*, respectively. The genus *Passiflora* has about 500 species distributed across the world but is widespread throughout the tropics, predominantly in Malaysia and Brunei (Waterhouse, 1994). *P. foetida* has a potential value of pharmacological drug and the fruit is considered edible (wild passionfruit). In rural parts of East Malaysia, Surinam and Java, the young leaves are also utilized as vegetable (Lade et al., 2015). Passifloraceae is considered to be cyanogenic, but the associated cyanoglycosides are moderately different both biogenetically and structurally than those present in *Heliconiinae* species and hence are unrelated (Conn, 1980). Furthermore, the immatures are not dependent on cyanoglycosides for dietary supply and thus this species (*A. terpsicore*) is able to synthesize linamarin and lotaustralin independently (Nahrstedt and Davis, 1981).

In a newly invaded region the invasive species have to face a challenge of introduction, establishment and dispersal to achieve its full invasive range (Elith, 2013). The information on interactions in newly invaded environment can be helpful in terms of using specialists for biological control of invasive species (Futuyma and Agrawal, 2009). In conclusion, currently *A. terpsicore* has the least affinity of becoming a horticulture vegetable pest (cucumber, pumpkin, watermelons and bitter gourd). Extensive feeding preference test results suggested its

likelihood towards Passifloraceae is persistent. But overall, *A. terpsicore* being a species labelled as “exotic” species, may have a significant role for modifying the native ecosystem and may pose a biodiversity threat to the other species in contact within its natural habitat. In general, to invade, establish and spread in the novel range, it is possible that *A. terpsicore* at present if not causing harm now may have a tendency to do so in future.

CHAPTER 5

WING SIZE VARIATIONS IN *Acraea terpsicore* (LINNAEUS, 1758) (NYMPHALIDAE: HELICONIINAE) ADULTS FOLLOWING EXPOSURE TO HAZE

5.1 Introduction

The second largest order in Insecta is Lepidoptera, comprising of butterflies and moths and cover more than 174,250 species (so far identified) (Mallet, 2007). The characteristic property of Lepidopteran insects is that their wings are shielded with minute scales and bristles. Each scale serves as an image unit or pixel and when combined together creates assorted mosaic patterns on wings. These patterns have the same randomness and distinctiveness as the human finger-prints ridge patterns. On the fore- and hindwings these patterns develop independently and are arranged over both dorsal and ventral surfaces. The diversification in butterfly wing arises due to the presence or absence, shape, size and color of the elemental patterns. Therefore, all the individuals of a species possess uniqueness of elements in wing patterns, which can be traced from species to species, through genera or even families (Nijhout, 2001). Among butterflies, Nymphalidae is ranked the most diversified color patterned family from which the nymphalid ground plan; a common overall color pattern has been deliberated (Nijhout, 1978, 1991). Wing patterns serve multiple functions such as in mimicry (defense), mate recognition and courtship which contribute vastly to genetic isolation and speciation (Jiggins et al., 2001, 2004). Henning (1992) proposed a group of “mimicry rings” in Acraeinae (Acraeini, present day) of the Afrotropical region, in which the convergence of multiple species wing patterns emits visual signals (defense) for predators (Staude, 1997). Apart from chemical signals, Heliconiinae butterflies are recognized for their brightly colored wings emanating a visual

signal of distastefulness towards predators (Langham, 2004). Majority of the *Acraea* species are accounted for their striking orange-reddish colored wings with black marks, emanating signals of avoidance towards a common predator or predator spectrum (Staude, 1997).

To study the evolutionary biological patterns and development, diversification in color patterns of butterfly wings serves at their best (Nijhout, 1991). Generally, lepidopteran body size is measured by their wing length, which is correlated to the wing area and width (Miller, 1977, 1991; Altizer and Davis, 2010). Alternative body measurements include antennal length and thorax width (Arango, 1996). In lepidopterans, body size dimension are better indicated through wing length measurements because the lipid and water content does not vary in contrast to the body mass (Miller, 1977). In studies of Monarch butterflies wing length is commonly incorporated with usual aspects (Hook et al., 2012). Sexually dimorphic *A. terpsicore* have ochreous orange females whereas males possess brighter orange colored wings. Their wing span ranges about 50-65 mm and 40-50 mm in female and male individuals, respectively. Males are comparatively smaller than the females, and the fore-wings are broad and round at the apex in both sexes. Regardless of coloration and size both male and female adults have the same wing pattern and can be distinctly identified in its common habitat (Eltringham, 1912).

In September and October of 2015 massive bush fires in Sumatra and Kalimantan (Indonesian, Borneo) were triggered due to burning of farming land for agriculture activities (in contribution of El Nino and Positive Indian Ocean Dipole condition), which lead to hazardous levels of smoke pollution across Equatorial Asia. This transboundary haze episode was the worst since 1997. Across Indonesia, to prepare the land for plantations and small farms, induced fires are most commonly used to clear forest and agricultural residue (Saliluddin, 2015 and Koplitz et al., 2016). This haze brought deterioration to the air quality which reached unhealthy levels. Haze typically consists of tiny smog-like solid or liquid suspended particles.

The haze episodes in Malaysia has been attributed to “smoke haze” due to the forest and peat fires of Indonesia. The Air quality index (AQI) of Malaysia is usually measured using the air pollution index (API) through the measurement of fine particulate matter (below 10µm), carbon monoxide (CO), sulfur dioxide (SO₂), nitrogen oxide (NO₂) and ozone (O₃). The effects of these pollutants cause severe disturbance in the insects via tri - trophic interactions (Warrington, 1987). Empirical studies of larval growth and immune responses in terms of relative growth rate and pupal weight were reported to be significantly lower in (heavy metal) polluted environment (Van-Ooik et al., 2007). Environmental stress conditions such as air pollution are responsible for atmospheric hypoxia (lower oxygen concentration) which adversely cause smaller body size in insects (Klok and Harrison, 2009; Harrison et al., 2010).

Therefore, this current study was set to investigate the variations in wing pattern and size of *A. terpsicore* populations during a haze episode in the Malaysian part of Borneo. The objective of the study was:

- a) To describe an association of environmental stress condition to that of insect morphology.

5.2 Materials and Methods

To improvise the current study data on temperature, precipitation and air quality were obtained from ASMC (ASEAN Specialised Meteorological Centre) and Weatherunderground.com.

The samples of *A. terpsicore* used in this study originated from field-collected eggs that were reared in netted house with permanent exposure to the haze. Four populations of *A. terpsicore* were used in this study relative to temporal haze episode progress. The adult population that derived from haze-exposed immature stages which eclosed on 8th September 2015 was referred to as population A; those of adults that resulted from pupae which eclosed on 27th September, 16th October and 3rd November of the same year were referred to as populations B, C and D, respectively (Tables 5.2 and 5.3).

Males and females from the different populations (unequal number of individuals) were measured for their sizes using 3R USB Handheld Digital Microscope and Anyty SEE software (1.0 version, Japan). All wing measurements were performed under the same scale magnification and calibration (real time magnification) at a millimeter scale, using dried specimens and adopting others (Hook et al., 2012). Each measurement was replicated 3 times for each of the six measurement types as displayed in Figure 5.1.

This include the inter-apical distance between forewings was defined as forewing, wing span. For the left and right forewing the distances from the wing base to the apex were considered as left and right wing lengths. Hindwing, wing span was defined as the distance between the middle outer marginal points of the hindwings. For the left and right hindwing, the wing lengths were described as the distance from the base to the middle point of outer margin.

For convenience, forewing wing span, left forewing wing length, right forewing wing length, hindwing wing span, left hindwing and right hindwing were referred to as FWWS, LFW, RFW, HWWS, LHW and RHW, respectively.

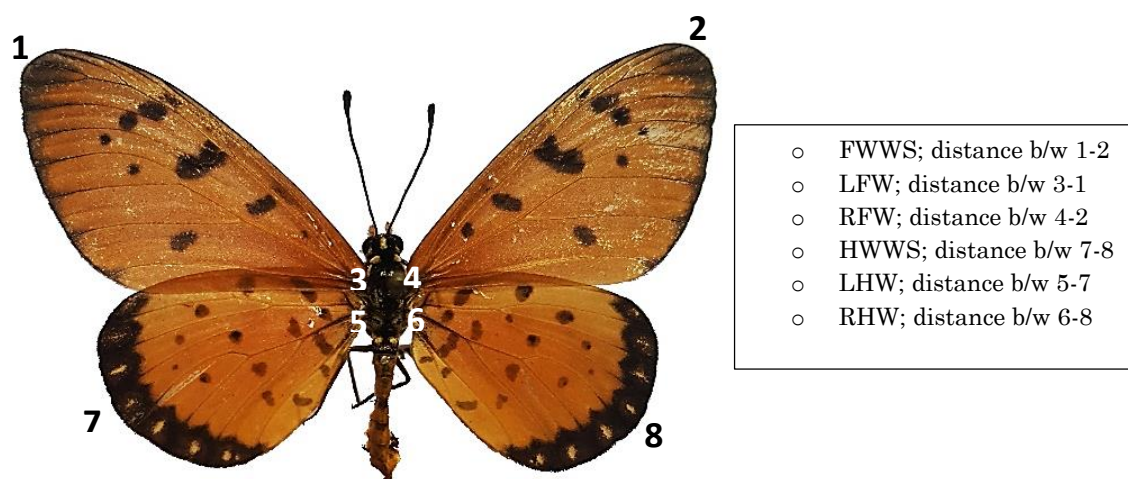


Figure 5.1: Attributes of the wing length measurement in *A. terpsicore* and the measurement types are given in the legend (all the measurements were taken parallel to each other, that is left-right exact points).

5.3 Data Analysis

In environmental condition study, an Air Pollutant Index (API) reading of 0 to 50 indicates good air quality; 51 to 100; moderate; 101 to 200, unhealthy; 201 to 300 very unhealthy and 300 and above is categorized as hazardous.

For both male and female, separately the mean values of different wing lengths were analysed incorporating all replicating measurements. In each sex the different mean values were compared using paired t-test with p value ≤ 0.05 as statistical significance. Where necessary Anderson-Darling Normality test was applied to separate mean values. Because the data for abnormal individuals of both sexes were very little, that data was not analysed in comparative analysis. Due to potential error reactive to magnification focus and eye handling, any difference

in wing length between left and right fore- and hindwing which was less than 1 mm was not considered significant. An individual of which the difference in length between the left and right fore- and hind-wings was over 1 mm and/or with an unusual wing pattern was considered as abnormal.

A group of individuals with no wing defects was considered as normal group (NorG), whereas those of individuals with wing faults was referred to as the abnormal group (AbG). In situations where the data set could not meet minimum sample size for paired t test, graphical illustrations were used to highlight differences. In some cases wing size variations were illustrated using graphical bars.

5.4 Results

5.4.1 Environmental conditions

In early September (2nd and 3rd) 2015, a strong haze event occurred in Sarawak. A brief entomological survey conducted from the 7th to 8th of the same month in a residential area revealed the presence of *A. terpsicore* individuals with unusual wing size. All the relevant environmental data during the study period is given in Table 5.1. During the study period (September – October 2015) the weather was warm, mostly dry and the atmosphere was highly polluted as shown by the increased APIs.

Table 5.1: Environmental data during the study period in Sarawak.

Period	Average Temperature	Average precipitation	API
September 2015	28 °C	2.8 (mm)	~68 - ~205
October 2015	28 °C	4.0 (mm)	~60 -~269
November 2015	28°C	16.1 (mm)	~40 -~81

(Weather data retrieved from www.weatherunderground.com and API from ASMC)

5.4.2 Wing size and pattern variations in NorG and AbG males of *A. terpsicore*

NorG Males

All the measured parameters data for wing lengths in male *A. terpsicore* is presented in Table 5.2. The mean wing length of the left forewing of *A. terpsicore* normal males (28.785 ± 1.881 mm) did not differ significantly to that of the right forewing (28.702 ± 1.970 mm) ($p = 0.4072$). For the hindwing, the left and right wings were similar in lengths (28.785 ± 1.881 vs. 28.702 ± 1.970 mm; $p = 0.073$). In these males, the mean FWWS showed different sizes across the study period, averaging 44.01 ± 1.074 mm. The largest size and smallest size adults were recorded within the population B and C (49.06 and 39.9 mm, respectively; Table 5.2).

AbG Males

In all the four populations of *A. terpsicore*, wing abnormality was only detected among the males that emerged in early September 2015 (Population A). In this group of males, 25% (5/20) showed either differences in length between paired wings that was more than 1 mm or unusual wing patterns relative to normal ones. Such wing defects were not found in the other populations, especially among adults that emerged during November 2015, a period that recorded lowest API. Wing defects exhibited different patterns (Table 5.3). The graphical comparison of these distinctions in AbG of male *A. terpsicore* is shown in Figure 5.4.

Table 5.2: Male *A. terpsicore* wing length measurements (mm).

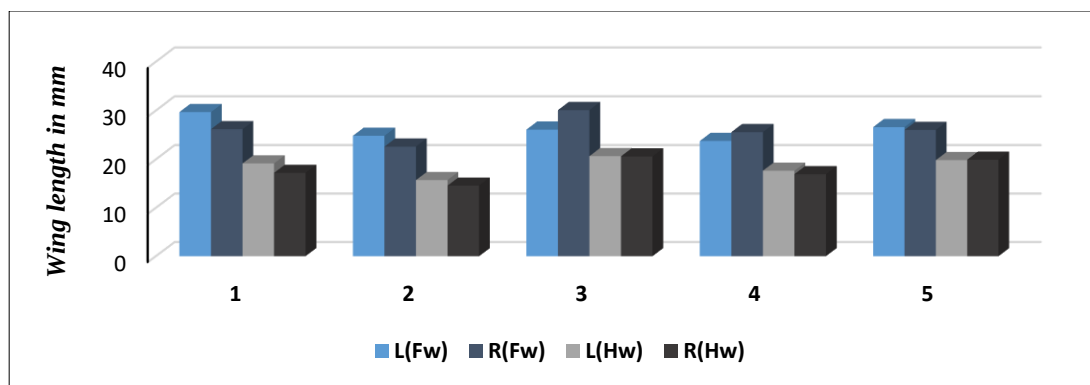
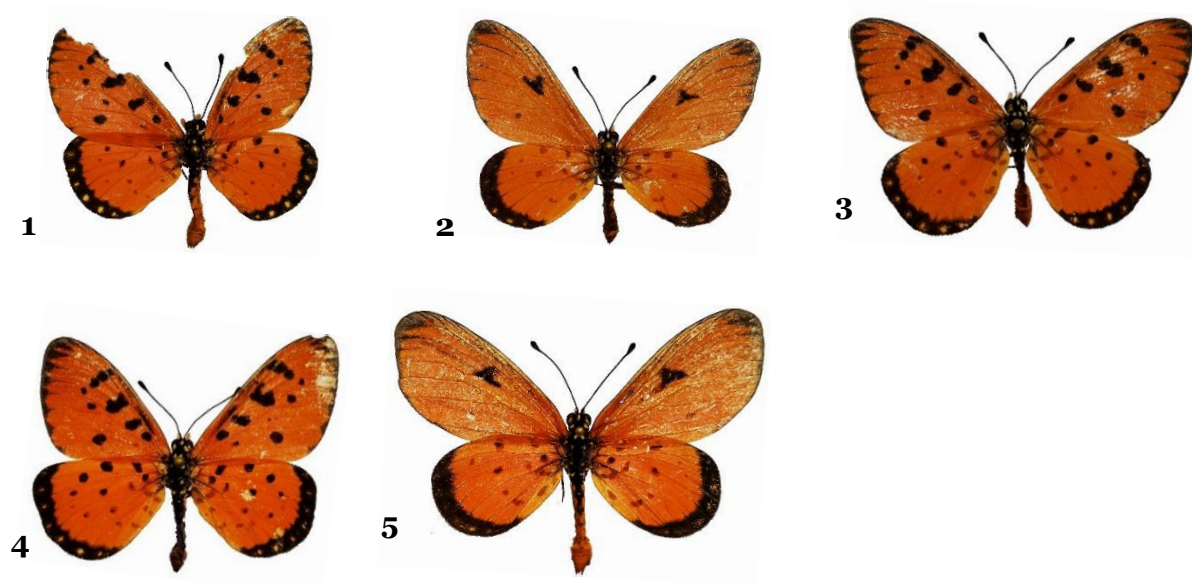
No.	Date	Remarks	FWWS	LFW	RFW	HWWS	LHW	RHW
1	8th Sep	Ab	39.94	29.7	26.19	34.49	19.16	17.22
2	(Population A)	Ab	41.78	24.84	22.62	31.34	15.73	14.58
3		Ab	42.84	26.1	30.1	37.94	20.67	20.61
4		Ab	43.46	23.78	25.59	31.19	17.65	16.94
5		Ab	46.18	26.62	26.04	33.48	19.85	19.94
6		Nor	41.11	28.04	27.25	33.45	18.44	17.05
7		Nor	40.98	26.78	26.63	32.41	17.53	16.25
8		Nor	41.01	26.44	26.15	33.36	16.72	16.7
9		Nor	44.47	31.49	30.07	40.04	20.35	19.46
10		Nor	42.61	26.56	26.79	33.34	18.38	17.45
11		Nor	45.52	30.85	29.76	38.78	21.48	21.24
12	27th Sep	Nor	42.95	26.58	26.62	32.28	18.27	18.27
13	(Population B)	Nor	40.34	26.61	25.56	34.89	19.09	18.49
14		Nor	44.68	29.6	29.7	38.23	21.65	21.81
15		Nor	45.33	30.4	30.71	38.49	21.08	21.31
16		Nor	48.63	30.3	30.7	38.64	21.84	21.68
17		Nor	49.06	31.31	31.7	39.55	21.37	21.26
18	16th Oct	Nor	39.9	26.1	25.82	33.72	18.78	18.66
19	(Population C)	Nor	43.57	30.18	29.84	39.72	20.13	19.89
20		Nor	44.47	29.9	29.91	38.96	21.13	21.18
21		Nor	43.76	29.81	29.9	38.72	21.31	21.58
22		Nor	41.04	26.78	26.46	32.55	18.37	18.26
23		Nor	47.63	29.3	29.7	37.62	20.9	21.11
24		Nor	46.71	30.26	30.38	38.64	21.84	21.68
25	3rd Nov	Nor	47.08	28.51	28.93	37.17	20.45	20.55
26	(Population D)	Nor	41.29	26.8	26.92	31.63	18.46	18.58
27		Nor	46.04	29.69	29.9	37.91	21.46	21.76
28		Nor	44.81	30.56	30.55	36.35	20.42	20.47
29		Nor	41.96	25.44	25.53	31.67	18.25	18.35
30		Nor	45.71	30.09	30.65	38.18	21.18	21.22
31		Nor	44.04	30.03	30.11	36.99	20.68	20.87

(Ab= abnormal, Nor= normal; FWWS= forewing wingspan, LFW= left forewing, RFW= right forewing, HWWS= hindwing wingspan, LHW= left hindwing, RHW= right hindwing)

Table 5.3: Types of wing faults detected in population A of male *A. terpsicore*.

Fault type	Description	Illustration
Size	• Small body size with LSW > RSW	Figure 5.2 (1)
	• RFW > LFW	Figure 5.2 (3)
	• Inconsistent sizes of both paired wings	Figure 5.2 (4)
Pattern	• Less black markings with round wing apex	Figure 5.2 (5)
Size and pattern	• Less black markings with round wing apex and LSW > RSW	Figure 5.2 (2)

(LSW = Left side wings; RSW = Right side wings)



(L(FW)= left forewing, R(Fw)= right forewing, L(Hw)= left hindwing and R(Hw)= right hindwing)

Figure. 5.2: Wing size deviations between left and right fore- and hindwing of abnormal male *A. terpsicore*.

5.4.3 Wing size and pattern variations in NorG and AbG females of *A. terpsicore*

NorG Females

In the Nor group, the mean wing length of the right forewing did not differ significantly to that of the left forewing (28.979 ± 2.804 mm and 28.702 ± 1.970 mm, respectively) ($p = 0.292$). In contrast, the mean left hindwing (20.126 ± 2.034 mm) was significantly longer than its right counterpart (19.832 ± 2.069 mm) ($p = 0.0227$). During the study period, the mean FWWS among these females showed varied sizes averaging 47.63 ± 2.973 mm with the largest (56.71 mm) and smallest (34.04 mm) sized females being encountered within the populations A and B (Table 5.4).

AbG Females

In this group, 20% of individuals that emerged in early September 2015 had size-related wing defect of different types. However, the females that reached the adult stage two months later (November 2015) had normal sized wings (Table 5.5; Figure 5.3 and 5.4).

Table 5.4: Female *A. terpsicore* wing length measurements (mm).

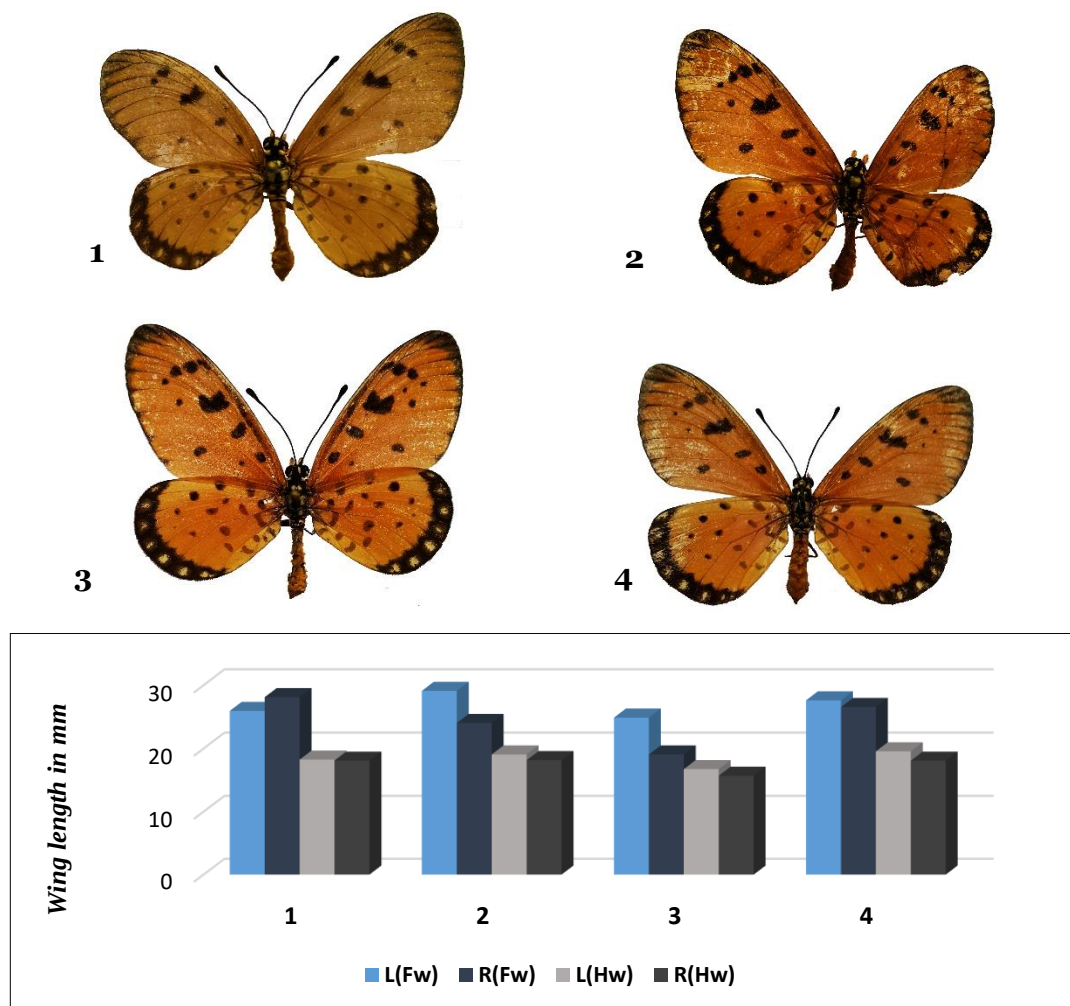
No.	Date	Remarks	FWWS	LFW	RFW	HWWS	LHW	RHW
1	8th Sep	Ab	44.56	25.94	28.12	35.3	18.25	18.08
2	(Population A)	Ab	40.46	29.11	24.04	35.9	19.06	18.16
3		Ab	41.09	24.88	19.07	29.65	16.75	15.7
4		Ab	38.3	27.61	26.55	37.88	19.56	18.08
5		Nor	36.4	26.06	25.69	33.83	16.9	16.1
6		Nor	43.73	26.99	26.39	34.46	20.57	20.67
7		Nor	48.5	30.18	30.57	38	20.4	20.91
8		Nor	44.53	27.29	27.01	36.46	20.04	19.72
9		Nor	56.71	31.1	31.24	40.65	20.97	21.02
10	27th Sep	Nor	52.3	31.92	31.04	39.46	21.82	20.78
11	(Population B)	Nor	50.93	31.22	31.52	39.33	21.12	20.43
12		Nor	42.32	26.42	26.68	36.5	19.2	17.88
13		Nor	34.04	24.15	23.85	30.5	16.07	15.52
14		Nor	44.78	25.48	25.56	33.98	16.99	17.28
15	16th Oct	Nor	53.97	31.52	31.48	40.3	21	21.02
16	(Population C)	Nor	54.9	35.02	34.46	42.3	22.52	21.66
17		Nor	48.37	29.11	29.47	40.24	21.46	21.28
18		Nor	51.31	30.6	30.37	40.43	21.62	21.18
19	3rd Nov	Nor	46.93	28.45	28.76	38.2	21.58	20.75
20	(Population D)	Nor	39.99	25.64	25.28	34.27	16.22	16.3
21		Nor	50.94	30.14	30.15	40.29	21.21	21.44
22		Nor	51.02	30.74	30.58	41.12	21.34	21.49
23		Nor	51.52	30.3	30.5	40.6	21.37	21.37

(Ab= abnormal, Nor= normal; FWWS= forewing wingspan, LFW= left forewing, RFW= right forewing, HWWS= hindwing wingspan, LHW= left hindwing, RHW= right hindwing)

Table 5.5: Types of wing faults detected in population A of female *A. terpsicore*.

Fault type	Description	Illustration
Size	• Abnormal wing shape with LFW < RFW	Figure 5.3 (1)
	• Abnormal wing shape and LSW > RSW	Figure 5.3 (2)
	• RSW < LSW	Figure 5.3 (3)
	• Smaller sized adult with LSW > RSW	Figure 5.3 (4)

(LSW = Left side wings; RSW = Right side wings)



(L(FW)= left forewing, R(Fw)= right forewing, L(Hw)= left hindwing and R(Hw)= right hindwing)

Figure 5.3: Wing size deviations between left and right fore- and hindwing of abnormal female *A. terpsicore*.

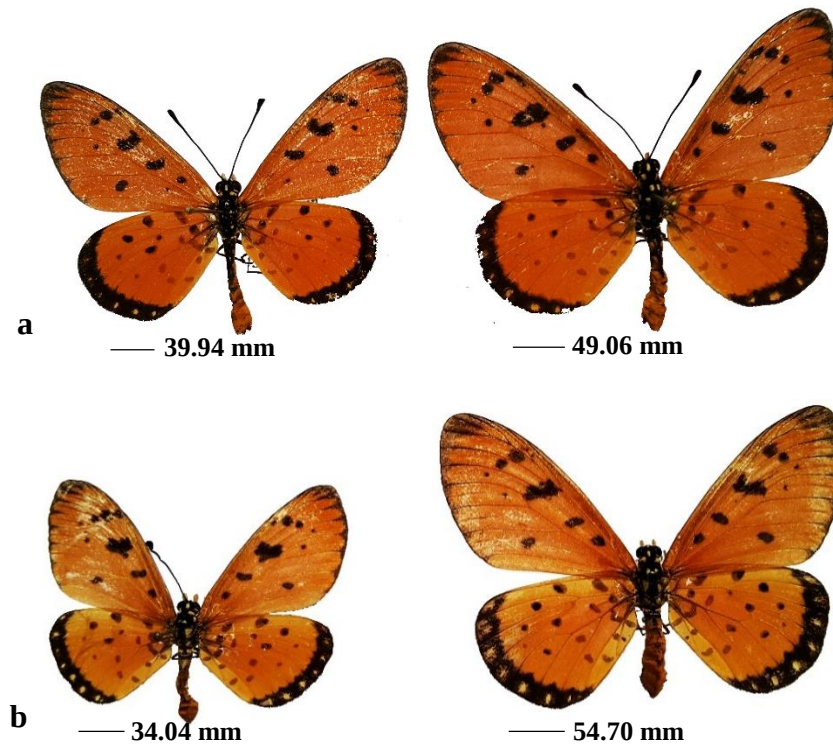


Figure 5.4: Apparent size variations of *A. terpsicore* male (a) and female (b).

5.5 Discussion

In September and October of 2015, the transboundary haze brought deterioration to the air quality of Sarawak (Kota Samarahan). Most of the days the API of the atmosphere was recorded in unhealthy levels. The weather was warm and minimum average precipitation was recorded during that period. Some level of abnormality was observed among *A. terpsicore* adults that included wing faults and the smaller sized adults across the study period. The wing abnormality prevalence was 6% (9/54) and all belonged to the population that emerged in early September 2015, when the API was recorded the highest in Sarawak. During 25th August to 28th September 2015 in Kuching, Samarahan and Sri Aman, the Air Quality Index (AQI) ranged from ~68-~205, with most days in the unhealthy to very unhealthy range (ASMC, 1993). In the other populations wing defects were not recorded such as during November 2015, a period that

recorded lowest API. Apparently, no more individuals suffered defective wings except for the smaller sized adult which was very few in number.

Even though among *A. terpsicore*, both sexes are highly dimorphic but polymorphism of female and male has also been reported at its greatest among *Acraea* species (Eltringham, 1912). This extreme instability and variations have not been reported due to seasonal and geographical conditions. Since the first subjected presence of *A. terpsicore* in Sarawak (Borneo) was reported (Abang et al., 2016), the species has been more or less monitored continuously in the field as well as in laboratory for research purpose. But the defects in wing shape, size and patterns was accounted for the first time during the haze period. The abnormal individuals of both sexes had significant variances between the left and right side wings which in normal individuals are always homogenous and have consistent parallel wing patterns (Nijhout, 2001).

The biological invasions consequences had allowed researchers to study basic evolutionary processes often those that response to the novel abiotic and biotic conditions invaders often evolve rapidly (Sakai et al., 2001). During invasion, phenotypic changes occurs under non-equilibrium demographic conditions and may reflect the influences of evolutionary history, genetic alterations in phenotypic traits, chance events and changes in selection regime that results in the viability of populations (Keller and Taylor, 2008). In addition, historic studies indicate that biological communities are profoundly altered with rapid change in their physical environment and physical factors such as oxygen, air density, humidity and photoperiodic intensities contribute largely in shaping insect communities (Dillon et al., 2006). In insects, oxygen is critically significant for ATP (adenosine triphosphate/ energy) generation and is accountable for the maximum and minimum PO₂ (pressure of oxygen) required to sustain metabolic or growth rate (Harrison et al., 2006). Empirical studies conducted on Hawk moth *Manduca sexta* eggs showed that exposure to low PO₂ resulted in reduced metabolic rates and

greater mortality (Wood and Hill, 2004). In environmental stressful conditions such as coping with reduced atmospheric oxygen levels puts negative effects on survival and growth/ size (Harrison et al., 2013). Assuming the particulate matter (CO, SO₂, NO₂ and O₃) in the atmosphere have been the main cause interrupting the physiological, morphological and tri-trophic interactions of insect's life cycle (Warrington, 1987).

In conclusion, Lepidopterans are profound of quantum changes that characterize their developmental changes through metamorphosis. For instance, the larval form transforms through eclosion into an externally inactive pupa from which the adult butterfly or moth emerges (Smits et al., 2000). Any sudden change in their physical environment or microhabitat can create dire consequences for their development and/ or growth resulting in deformities, with most adverse effects such as increased mortality rates. In this study the smaller body size and wing abnormalities of *A. terpsicore* individuals were attributed as to the interference caused by smoke haze that hit Borneo severely in 2015. More precise morphological and physiological studies with genetic basis need to be incorporated to answer the question of highest polymorphism of *A. terpsicore* species is either due to environmental stress that derive variances or it can be referred as a natural phenomenon.

CHAPTER 6

SPATIAL DISTRIBUTION AND RANGE EXPANSION OF *Acraea terpsicore* (LINNAEUS, 1758) (NYMPHALIDAE: HELICONIINAE) IN BORNEO

6.1 Introduction

Climate change and invasive species are two of the most serious threats to biodiversity and a much more of prime concern is that these threats interact (Bertelmeier et al., 2013). The scientific community widely accepts that anthropogenically induced climate change is occurring (Tett et al., 1999 and Stott, 2003) and its impacts are expressed at various levels. Climate change is the major force on species distribution and species are compelled by their physiological tolerance towards temperature and humidity (Pearson et al., 2004; Ballard et al., 2012). Over half of the species examined during 1970 – 2000 have experienced and responded to the modest level of global warming (Thomas, 2010). This includes alteration of species range limits leading to the range expansion or contraction (Putten et al., 2010). In a wide range of taxa, the impact of climate change has been recorded by their range expansions towards higher altitudes and latitudes (Walther et al., 2002; Parmesan and Yohe, 2003; Hickling et al., 2006; Buckley et al., 2012). About 51 non-migrant butterfly's response to the 20th century climate warming predicted that the role of climate will potentially cause the future distributions of 35 species during a period of 2070-2099 (Hill et al., 2002).

Globally, the impact of climate change on the distribution of organisms may increase the circumstances of extinctions. For the effective protection of biodiversity, the most urgent task is to develop accurate predictions about species responses to global changes (Urban et al., 2016). Therefore, modern research has significantly emphasized on the prediction of biodiversity in response to climate change (Dillon et al., 2010).

The understanding of geographic ranges of species is an important and classical ecological challenge (Brown et al., 1996; Gaston 2003). Species distributional patterns are better understood if one apprehend the ecological requirements and geographical facts which affect the structure and dynamics of the species geographical ranges (Storch et al., 2003). To estimate species range size, the information of its distribution can be assessed either by Extent of Occurrence (EOO) or Area of Occupancy (AOO). Scrutinizing the geographic distribution of species by EOO including discontinuities in habitat or occupancy, can certainly predict information about the conservation status of species (Gaston and Fuller, 2009). In more recent times, rare and endangered species distribution as well as environmental pressures affecting them were monitored and predicted with Species Distribution Models (SDMs) (Silva et al., 2014; Bussion et al., 2010). Theoretically, SDMs aim to determine and map (components) species ecological niche through space and time, as these models have become important tools in pure and applied ecology and evolutionary biology (Kearney and Porter, 2009). SDMs are widely used to forecast range shifts of organisms due to climate change, predict the eventual ranges of invasive species and infer paleoclimate from data on species occurrences (Jeschke and Strayer, 2008). Around the globe, invasive species are signified to intervene both the ecological and economic costs (Mack et al., 2000; Lodge et al., 2006; Lockwood et al., 2013). In recent approaches, many biologists and ecologists have used SDMs to forecast the future ranges of non-native or alien species. To improve the accuracy of predictions, researchers are rapidly developing tools in SDMs, forthcoming to the key biological processes needed for future concerns (policy makers and management actions) (Urban et al., 2016). Thus, these SDMs projections assist to evaluate the risk associated with the establishment of alien or potential pest species (Panetta and Mitchell, 1991) and mark the control of these species via management actions (Peterson, 2003).

Invasive species provide unique opportunities to explore the impacts of recent range-expansion, in particular on life-history traits, making it possible to test for spatial dispersal abilities along the expanding range (Lombaert, 2014). In Southeast Asia invasive alien species are currently outlined by ecologists and it is believed that in the coming decades, the biota of Southeast Asia will be affected far more than the impact of invasive species together with other human disturbances (Peh, 2010). In Borneo, examples of biological invasion in the primary forests include the human-commensal rats, *Rattus rattus* (Wells et al., 2006). Some invasive insects were accidentally introduced into Southeast Asia and are considered as pests to agricultural crops. For example, three species of *Liriomyza* (leaf miners) that are rapidly spreading in Malaysia, Indonesia and the Philippines (Hofsvang et al., 2005). In the case of butterflies, prominent invasion of the Southeastern butterfly *Papilio demoleus* (Lime/citrus Swallowtail) cannot be taken for granted (Cordero, 2001). This species was previously not recorded in Borneo (Cobert and Pendlebury, 1978) but has successfully expanded its range and invaded even the Caribbean and in the adjacent mainland near Florida (Homziak and Homziak, 2006).

The range expansion of *A. terpsicore* (Tawny Coster) and its establishment in Southeast Asia and Australia is anticipated to be as a result of both the climate change and habitat degradation. Thus it colonizes new geographical landscapes that also supplements its larval host plants. *Acraea terpsicore* is native to India and Sri Lanka (Bingham, 1905; Bell, 1910; Talbot, 1947; Wynter-Blyth, 1957) but has extended its range of distribution through the Southeast Asian countries southwards to Australia and Borneo. Until the past couple of decades it was not reported to exist in this region, but it has now become widely distributed and established as reported in Nepal, Thailand, Peninsular Malaysia and Singapore (Smith, 1989; West, 1996; Osada et al., 1999; Arshad et al., 1996 and Khew, 2008). The most recent findings on the

occurrence of *A. terpsicore* were from Indonesia (Matsumoto et al., 2012) and also from Timor and Australia (Braby et al., 2013). It was lately reported for the first time in the Southern foothills of Bhutan (Nidup, 2015). *Acraea terpsicore* has never been reported on Borneo Island until 2013 when it was first sighted in the areas of Kuching, Sarawak (Abang et al., 2016). The presence of *A. terpsicore* in Mandor Landak Regency of Western Kalimantan and East Kalimantan has also been recently documented (Florida et al., 2014; Iqbal et al., 2015).

The aim of this study was to document the occurrence of *A. terpsicore* in Borneo and its areas of establishment across the island. The main approach was to correlate the statistically link spatial data to species distribution records and execute the range margins via maps. This study also aims to predict the potential range of *A. terpsicore* in Southeast Asia and especially in Borneo under current climatic conditions and further future climate change (2050). The Species Distribution Models (SDMs) were used for this purpose. Part of the study is focused on the events during the past couple of decades that may have contributed to its range expansion in Indo-Australian region and above all particularly, to assess its rate of colonization in Borneo as well as its possible approach of entrance into the Island.

6.2 Materials and Methods

6.2.1 Collection of Distributional Data

The distributional data for *A. terpsicore* was obtained mainly from scientific literature and internet sites especially those with photographic proves which also provided the evidence of its presence in the Oriental and Australian region (Appendix II). Most of the occurrence data for India, Bangladesh, Nepal, Bhutan, Thailand, Vietnam, Laos, Malaysia, Indonesia, Lombok, Timor and Australia were obtained from Bingham (1905), Islam et al., (2011), Smith (1989), Nidup (2015), West (1996), Pierre and Bernaud (1997), Osada et al., (1999), Arshad et al., (1996), Matsumoto et al., (2012) and Braby et al., (2013) respectively. The most recent record for West Kalimantan (Borneo) was gathered from Florida et al., (2014) and Iqbal et al., (2015) for Eastern and South Kalimantan. However, in Sarawak (Eastern Malaysia) multiple points of occurrence data were recorded through field observations and species collection. All the presence based records provided a total of 95 occurrence points for Southeast Asia as well as for Borneo was recorded. The spatial precision of these occurrence points varied from 10m to more than 1km. The spatial distribution of *A. terpsicore* was executed by the method of Extent of Occurrence (EOO) through calculating the minimum convex polygon based on the Geographic Positioning System (GPS values) using the ArcGIS (ArcMap version 10.4 software).

The main interest of this study was to extract the broad pattern potential range of *A. terpsicore* in Southeast Asia. Therefore, two model outputs were generated for Southeast Asia and particularly, for Borneo, namely:

- (i) Under current climatic conditions
- (ii) In 2050

The second model output was produced in order to predict the potential future geographic range of *A. terpsicore* in this region.

6.2.2 Environmental Data and Climatic Predictors

The output of species distribution modelling depends specifically on the set of climatic variables selected as a model input. The full set of 19 bioclimatic variables were selected as an input model for species niche modelling and data was obtained from WorldClim database. These bioclimatic variables are commonly used in SDMs which are derived from 1960-1990. These represent the monthly temperature and rainfall values, annual trends (e.g., mean annual temperature, annual precipitation) seasonality (e.g., annual range in temperature and precipitation) and extreme or limiting environmental factors (e.g., temperature of the coldest and warmest month, and precipitation of the wet and dry quarters) (Hijmans et al., 2005; Table 6.1). Environmental data for these bioclimatic variables were selected at a spatial resolution of 10 arcmin (approx. 18.5×18.5 km pixel) (following Bertelsmeier et al., 2013; Braby et al., 2013). Future climate data for 2050 was sourced from the Fifth Assessment Report of IPCC (CMIP5= Coupled Model Intercomparison project phase 5) which was already downscaled and calibrated (bias corrected) using WorldClim 1.4 as baseline “current” climate, in order to compare the projections. For 2050 climate data six GCMs (Global Circulation Models) were selected for bioclimatic variables of 4.5 rcp (RCP; Representative Concentration Pathway). The RCPs are named after a possible range of radiative forcing values in the year 2100 relative to pre-industrial values (Weyant *et al.*, 2009). The global warming increase (°Celsius) and projections for future climate data 2050 is averaged for year 2046-2065. With RCP4.5 the global mean temperature is likely to rise about 1.4°C (0.9 to 2.0) (IPCC AR5 WG1).

Table 6.1: Environmental data of bioclimatic variables with respective codes.

Sr. no.	Code	Description
1	BIO1	Annual Mean Temperature
2	BIO2	Mean Diurnal Range (Mean of monthly (max temp - min temp))
3	BIO3	Isothermality (BIO2/BIO7) (* 100)
4	BIO4	Temperature Seasonality (standard deviation *100)
5	BIO5	Max Temperature of Warmest Month
6	BIO6	Min Temperature of Coldest Month
7	BIO7	Temperature Annual Range (BIO5-BIO6)
8	BIO8	Mean Temperature of Wettest Quarter
9	BIO9	Mean Temperature of Driest Quarter
10	BIO10	Mean Temperature of Warmest Quarter
11	BIO11	Mean Temperature of Coldest Quarter
12	BIO12	Annual Precipitation
13	BIO13	Precipitation of Wettest Month
14	BIO14	Precipitation of Driest Month
15	BIO15	Precipitation Seasonality (Coefficient of Variation)
16	BIO16	Precipitation of Wettest Quarter
17	BIO17	Precipitation of Driest Quarter
18	BIO18	Precipitation of Warmest Quarter
19	BIO19	Precipitation of Coldest Quarter

The six GCMs selected include;

- i. ACCESS1-0 (Australian Community Climate and Earth Simulator System)
- ii. BCC-CSM1-1 (Beijing Climate System-Climate System Model)
- iii. CCSM4 (Community Climate System Model version 4)
- iv. HADGEM2-AO (Hadley Centre Global Environmental Model version 2- Atmosphere)
- v. HADGEM2-ES (Hadley Centre Global Environmental Model version 2- Earth System)
- vi. MRI-GCM3 (Meteorological Research Institute Japan-Global Circulation Model version3).

The environmental data for the Bioclimatic variables were in “.bil” format, thus all the files were converted to “.asc” format using ArcGIS (10.4) software, prior to the projections of bioclimatic niche models.

6.2.3 Model Selection for Ecological Niche Modeling

To generate individual forecasts, six SDMs were selected (see also Bertelsmeier *et al.*, 2013). The distributional data for *A. terpsicore* was presence based only with a total 95 of occurrence points. Hence for presence based SDMs, two models were selected which are as follows:

- i. One-class SVM (Support Vector Machines)
- ii. BioCLIM

For models which required pseudo-absence data, 1000 pseudo-absence points were created randomly using the environmental layer as a baseline file. Therefore, four pseudo-absence models were selected which includes:

- i. Two-class-SVMs (Support Vector Machines)
- ii. BP-ANN (Back propagation-Artificial Neural Networks)
- iii. CTree (Classification trees)
- iv. MaxEnt (Maximum Entropy)

All the models were run using the ModEco Platform version 1.0 software. For BioCLIM and one-class SVM, user specified parameters with optimizing settings of 10% folder cross validation and Kernel= Radial basis Function (RBF) were chosen (see Guo and Liu, 2010 and Bertelsmeier et al., 2013). In case of CTree, the model algorithm was averaged for 10 iterations, windows size=10, and pruning level= 0.25 was selected. Whereas for MaxEnt, there are no parameters in ModEco.

Model Validation

Species Distribution Models are usually evaluated with the value of AUC (Area under the curve), which is a non-parametric threshold-independent measure of accuracy (Manel et al., 1999; Pearce and Ferrier, 2000). For all the models the accuracy assessment was performed with 10% fold cross-validation. AUC values that ranges from 0-1: scores between 0.5 and 0.7 are considered as low, 0.7-0.9 moderate and >0.9 high (Franklin, 2010).

6.3 Data Analysis

Individual sample points of *A. terpsicore*, with the help of Geographical Positioning System (GPS) were used to calculate the rate of colonization via increase expansion rate in area over time (km^2/year).

For the current climatic condition, six individual models were generated, from which consensus current climate map projections were constructed. Similarly, for future climate, 36 individual models forecasts were combined in one projection following Bertelsmeier et al., (2013) and Braby et al., (2013). The reason behind using consensus forecasts was to minimize resultant potential variation and to separate the “signal” from “noise” correlated with errors and uncertainties with individual map outputs (Araújo and New, 2007). The format for modeling techniques can be easily understood with the help of model design as shown Figure 6.1.

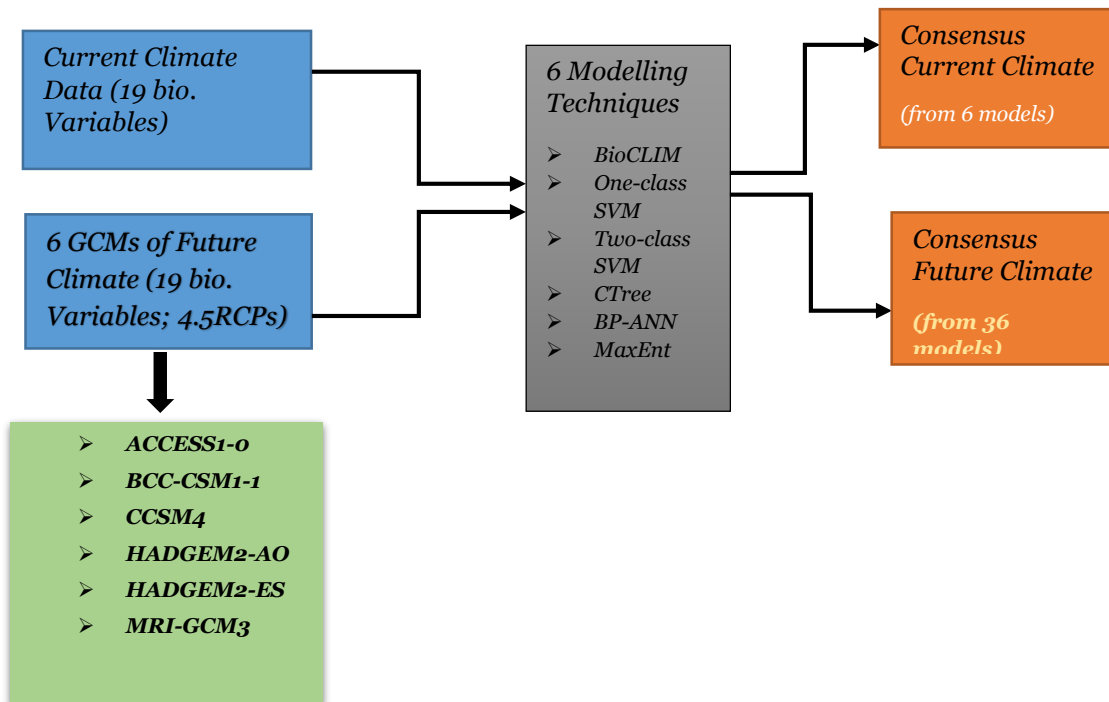


Figure 6.1: Model design for understanding the use of environmental data and models of current and future climate to estimate the suitable occurrence areas for *A. terpsicore*.

6.4 Results

6.4.1 Distribution Records for Southeast Asia

Records of the presence of *A. terpsicore* in Southeast Asia were gathered from information and evidences available on the internet (www.exoticinsects.com; www.ifoundbutterflies.org; lepidoptera.butterflyhouse.com/au; yukuta.it-n.jp/). These sites provide the evidence of *A. terpsicore* in Peninsular Malaysia, Singapore, Sumatra, Java, Flores and Moa Island. However the distributional records for Thailand, Vietnam, Laos and Cambodia were obtained from “Butterflies of Indo-China” (Inayoshi and Saito, 2009) which describes briefly the presence of *A. terpsicore* in these regions (Figure 6.2). There was only one recent published literature of *A. terpsicore* from Lombok by Matsumoto et al., (2012). Appendix II shows the detailed compilation of records on the occurrence of *A. terpsicore* in SEA region. Braby et al., (2013) described the presence of *A. terpsicore*'s in Timor and Australian region.

6.4.2 Distribution Records for Borneo

Acraea terpsicore was firstly recorded from Malaysian Borneo for the first time based on three specimens collected in Samarahan, southwestern Sarawak on 15th March 2013. This indicates that it probably must have arrived in 2012, and hence colonized in this region. Preliminary results of field tracking and observations of the species within the last three years indicated the establishment of *A. terpsicore* in western Borneo and expanded its area of distribution from Samarahan 200 km northwards to Sri Aman including Samariang, Kuching, Kota Samarahan, Asajaya and Serian.

Besides the sample collection from the mentioned areas, the distributional data of *A. terpsicore* was obtained from the very recent published literature of Mandor Landak Regency (West Kalimantan) and East Kalimantan by Florida et al., (2015) and Iqbal et al., (2015) respectively. From Moa Island and Mount Besar (Central Kalimantan) several individuals of *A. terpsicore* were reported (exoticinsects.com). However, the occurrence information for Banjarmasin

(Central Kalimantan) was obtained from an internet site with a photographic evidence in 2014 (see appendix). The summarized distributional data for Borneo is given in Table 6.2.

6.4.3 Rate of colonization

Inayoshi in (1986) recorded nine individuals of *A. terpsicore* from Chang Mai (Inayoshi and Saito, 2009). However, the presence of *A. terpsicore* from Chang Mai in April 1984, by West (1996) is considered to be the earliest record for Thailand. Inayoshi and Saito (2009) mentioned (unpublished literature) the collection of one male individual from Chang Mai in July 1983 that supports West's (1996) documentation. Following that it was recorded in northern Thailand (Chonburi) (Takanishi, 1988; Inayoshi and Saito, 2009) covering about 752 km in four years. Furthermore towards the east of Vietnam it was recorded in 1994 (Inayoshi and Saito, 2009). In 1999, *A. terpsicore* was also traced northwards in Laos (Osada et al., 1999). In Peninsular Malaysia, it was detected the earliest at Perlis in December 1992 (Arshad et al., 1996). Subsequently it was encountered on the Islands of Langkawi and Penang in 1993 (see also Appendix). In Singapore it was traced and recorded in 2006 (Khew, 2008). Hence the migration of *A. terpsicore* all the way from Perlis to Singapore took almost 12 years (560km).

Adjacent to Singapore, *A. terpsicore* was also recorded at Jambi, in 2008 (about 100 km further). Migrating along the path of greater and lesser Sunda Island it took almost four years to reach the Australian Continent, covering almost 3300 km. The above mentioned dates of detection of *A. terpsicore* and the rate at which it is migrating and spreading in South-East Asian region gives an estimate of its rate of colonisation in the region.

Table 6.2: Distribution of *Acraea terpsicore* across Borneo (presence based data).

S. no	Site	Location	Latitude	Longitude	Date	Observer
1.	Desa Ilmu	K. Samarahan	1°27'10.498"N	110°27'26.83"E	March 2013	Noor Amira
2.	Taman BDC	Kuching	1°30'34.164"N	110°21'34.149"E	July 2015	Sabina Noor
3.	Jln. Stampin barat 4	Kuching	1°30'54.037"N	110°20'46.286"E	August 2015	Sabina Noor
4.	Jln. Muara Tabuan	Kuching	1°31'29.474"N	110°23'45.249"E	August 2015	Sabina Noor
5.	Samariang Aman	Samariang	1°36'29.881"N	110°19'35.883"E	November 2013	Noor Amira
6.	Jln Cenderwasih	Petra Jaya	1°35'3.962"N	110°19'28.852"E	September 2014	Fatimah Bt. Abang
7.	Taman Cahaya Damai	Petra Jaya	1°37'44.968"N	110°20'13.827"E	November 2013	Noor Amira
8.	Kampung Semera	Asajaya	1°32'58.031"N	110°40'14.891"E	January 2014	Noor Amira
9.	Kampung Ranchan	Serian	1°8'52.048"N	110°35'3.461"E	May 2016	Alley Majau
10.	Kerangan Petai	Sri Aman	1°19'60"N	111°40'.0"E	March 2014	Tham Vivian
11.	Banjarmasin	South Kalimantan	3°19'6.984"S	114°35'39.762"E	June 2014	Hidayati Rahima
12.	Mount Besar	South Kalimantan	02°42'39"S	115°37'32.999"E	January 2015	Exotic insects
13.	Man. Land Regency	West Kalimantan	0°19'4.778"N	109°25'33.281"E	October 2014	Florida et al.,
14.	Kabupaten Ketapang	West Kalimantan	02°16'35.9"S	110°52'07.6"E	April 2015	Iqbal et al.,
15.	Jelamu Hill	West Kalimantan	02°01'45.3"S	110°47'43.0"E	April 2015	Iqbal et al.,
16.	Sengkuang village	West Kalimantan	02°20'54.1"S	110°46'49.0"E	April 2015	Iqbal et al.,
17.	Perigi village	West Kalimantan	0°42'30.478"N	109°30'43.203"E	April 2015	Iqbal et al.,
18.	Bukit Pelangi	East Kalimantan	0°30'54.5"N	117°36'28.0E	June 2015	Iqbal et al.,

The average rate of colonization from Thailand to Australia was calculated more or less 200 km/ year. The distance between Singapore and Kota Samarahan (Malaysian Borneo) is more than 720km and it took almost five years to reach the mainland. While, in Sarawak *A. terpsicore* was tracked north-west until Sri Aman ($1^{\circ}19'60''\text{N}$, $111^{\circ}40'.0''\text{E}$) covering the distance of about 200km. The rate of colonisation from Singapore to Kota Samarahan was calculated about 150 km/year. While from Kota Samarahan to Sri Aman the rate of colonisation was estimated 100 km/year.

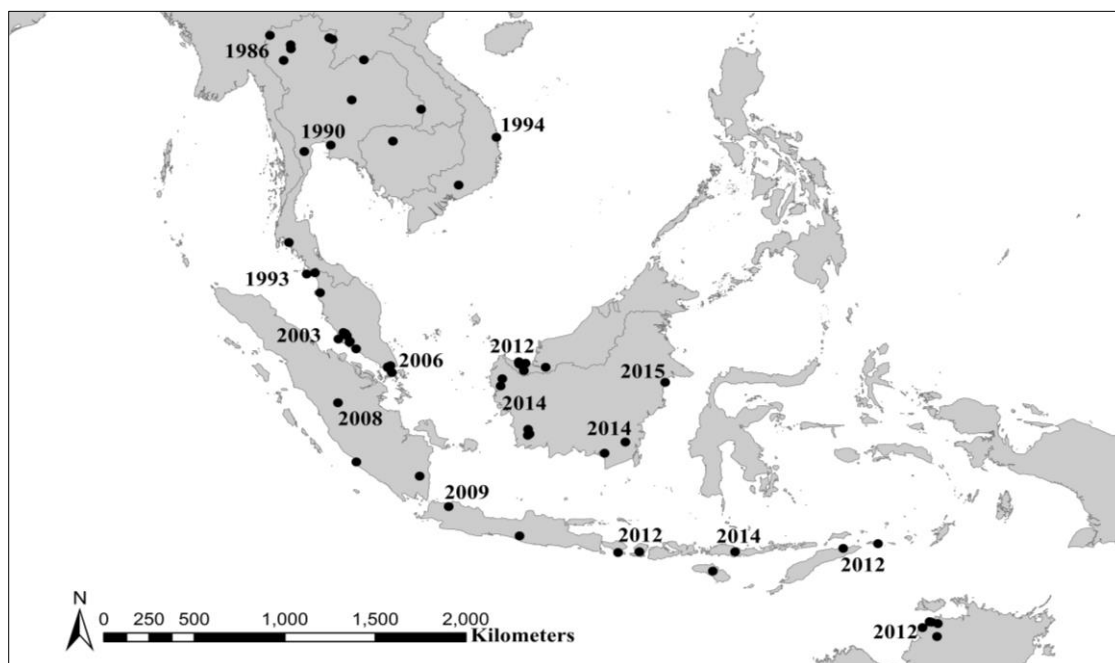


Figure 6.2: Distribution of *A. terpsicore* in South-East Asia (Thailand, Vietnam, Malay Peninsula, Indonesia and Australia) and particularly in Borneo, showing known locations (black circles) and years of detection (see also appendix III, for the localities records and associated data).

6.4.4 Range size of *A. terpsicore* in Borneo

The range size of *A. terpsicore* was calculated by means of EOO (Extent of Occurrence) via calculating the minimum convey polygon (using IUCN toolbox in ArcGIS). Within three years (earliest record), *A. terpsicore* has been detected at 18 locations in Borneo (10 locations in

Sarawak and 8 in Kalimantan). An EOO (range size) of *A. terpsicore* was calculated that covers an area of 322,766 km² in Borneo and is displayed in Figure 6.3.

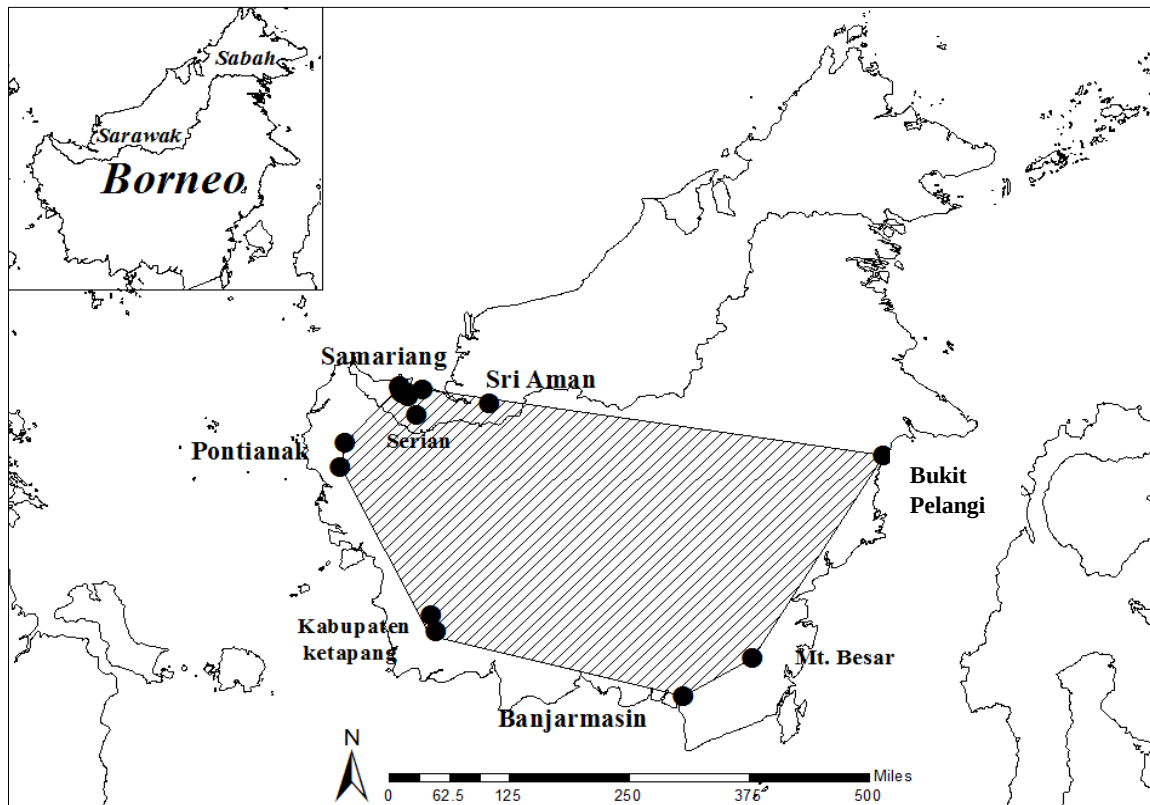


Figure 6.3: Distribution and range size of *A. terpsicore* in Borneo, showing known locations (black circles) and extent of occurrence (EOO) (cross-hatched area) based on the records between 2012 -2016. Inset map of Borneo is shown.

6.4.5 Spatial modelling in South-East Asia and Borneo

Model performances (AUC) with selected parameters values for all the presence based and pseudo-absence models are given in Table 6.3. For all the six models under current climate and future climate of 2050, AUC scores ranged from 0.76-0.96, with a magnitude of moderate to excellent.

The simulation results from bioclimatic niche models under current climate conditions forecasted a much broader potential range compared to the existing geographical range of *A.*

terpsicore. Outputs of SDMs projections for the world and South-East Asia are shown in Figures 6.4 and 6.5, respectively.

Table 6.3: Summary of models performance and parameters range.

Modelling techniques	AUC	Kappa	F-score	True positive rate
BioCLIM	0.83-0.96	-	7.41	-
One-class SVM	0.77-0.92	-	3.24-6.10	-
Two-class SVM	0.89-0.94	0.74-0.79	5.35-6.18	0.93-0.97
CTree	0.76-0.91	0.82-0.96	6.06-9.70	0.96-1.00
BP-ANN	0.91-0.96	0.45-0.86	7.09-9.07	0.76-0.98
MaxEnt	0.86-0.93	0.83-0.88	7.29-9.96	0.93

The consensus forecast of these models pointed out that besides to the well-known range of India, Sri Lanka and Thailand there were promising areas in Northern parts of Philippines, Sulawesi Island as well as in Southeastern China, Taiwan and Papua New Guinea as shown in Figure 6.5. In the consensus current climate forecasts climatically suitable areas of Madagascar, Central Africa, Central and South of America were also indicated as shown in Figure 6.4. Until 2050, the consensus forecasts showed potential range will likely expand further. The expected areas of occupancy in the projections were highlighted as Central Africa and Madagascar as well as in South America which are displayed in Figure 6.6. Furthermore in South-East Asia it will be probably richer in occurrence among areas of Southern Philippines and Northern part of Sulawesi Island. Whereas, moderately favorable climatic areas of Taiwan and Papua New Guinea are susceptible to be conquered by *A. terpsicore* as shown in Figure 6.7. Within Borneo it is predicted to occupy the areas that has been already described under current climate as displayed in Figure 6.8a. While for future climate forecast, the potential range of *A. terpsicore* was predicted to decrease in this region (Figure 6.8b).

6.4.6 Entrance into Borneo

From the bioclimatic niche models, the potential occurrence range of *A. terpsicore* was determined under current and future climate. Given that it can be proposed that *A. terpsicore* entered Borneo

- i. Either from Singapore via nearby islands
- ii. Or via Sumatra /Java and nearby islands into Southern Kalimantan and then established and spread towards the western and Northern Kalimantan regions.

The second proposed mode of entry via Sumatra is more acceptable because this species was not encountered in the western tip of Eastern Malaysia (Lundu), suggesting it might have spread from western Kalimantan (Pontianak) into Sarawak via Bau and then established here.

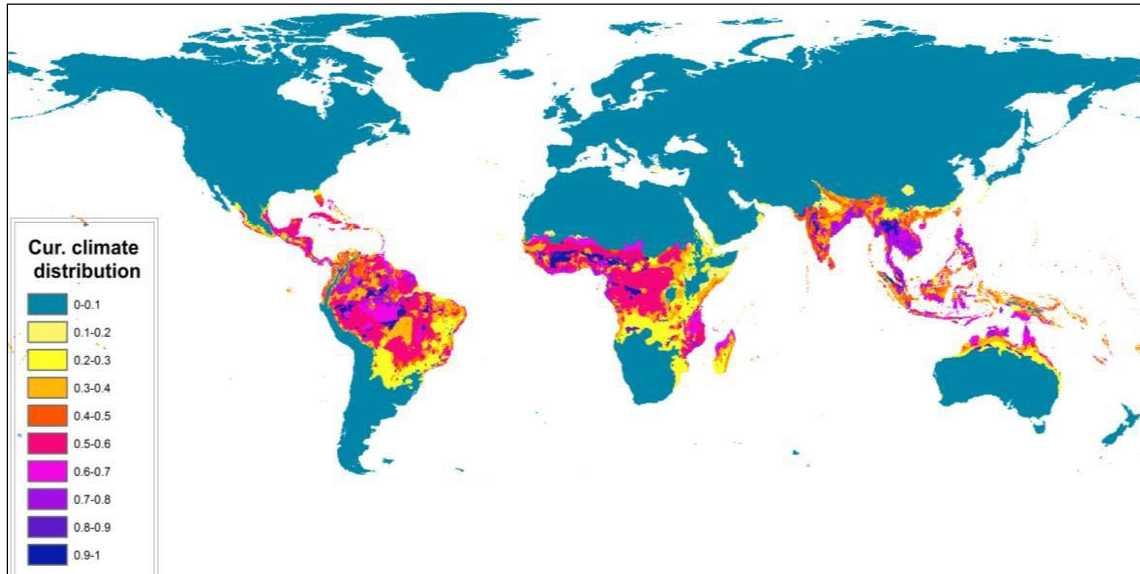


Figure 6.4: Potential range of distribution for *A. terpsicore* across the world, predicted under current climatic conditions are shown (blue, purple and pink shades). Likelihood of occurrence for the given pixel (from 0= unlikely to 1= very likely) is specified in the legend. The consensus forecast for current climatic conditions is based on six individual models.

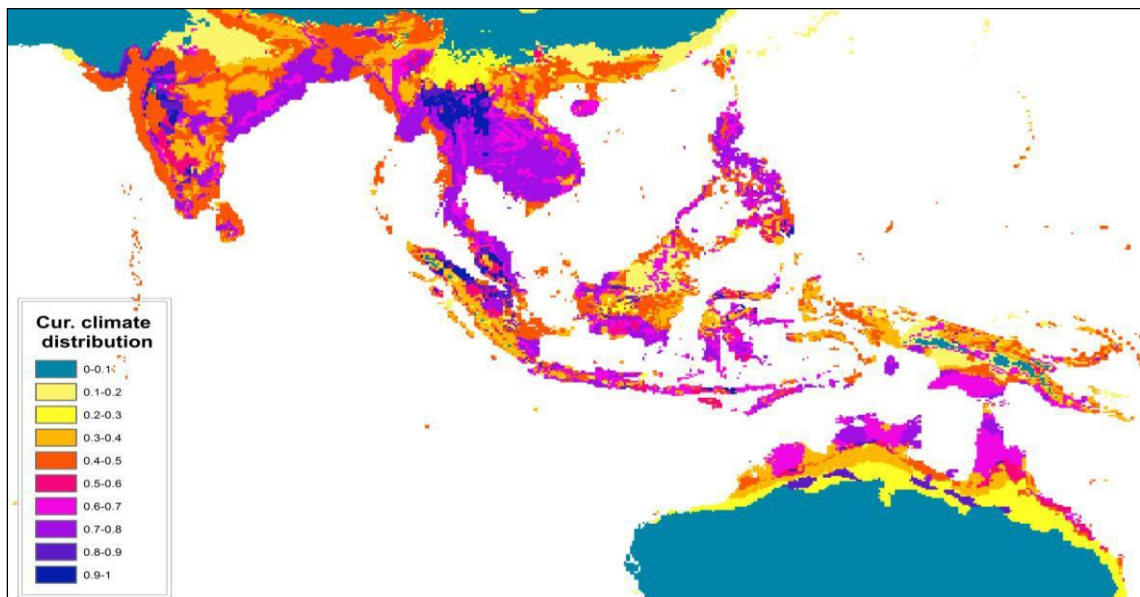


Figure 6.5: Potential range of distribution for *A. terpsicore* in South-East Asia, predicted under current climatic conditions are shown (blue, purple and pink shades). Likelihood of occurrence for the given pixel (from 0= unlikely to 1= very likely) is specified in the legend. The consensus forecast for current climatic conditions is based on six individual models

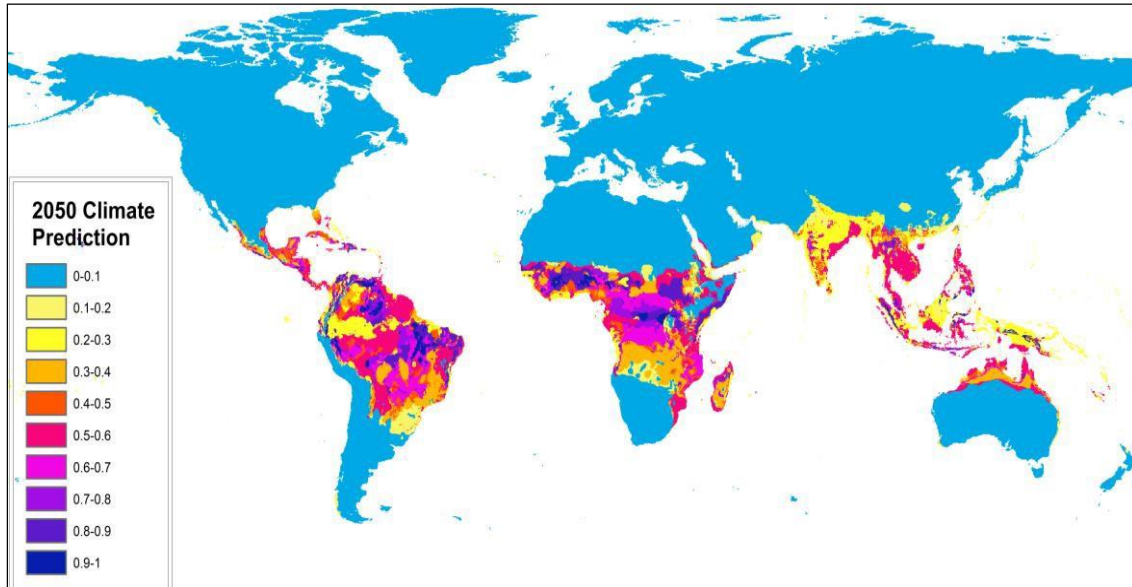


Figure 6.6: Potential range of distribution for *A. terpsicore* across the world, predicted for future 2050, climatic conditions are shown (blue, purple and pink shades). Likelihood of occurrence for the given pixel (from 0= unlikely to 1= very likely) is specified in the legend. The consensus forecast for future 2050, climatic conditions is based on 36 individual models.

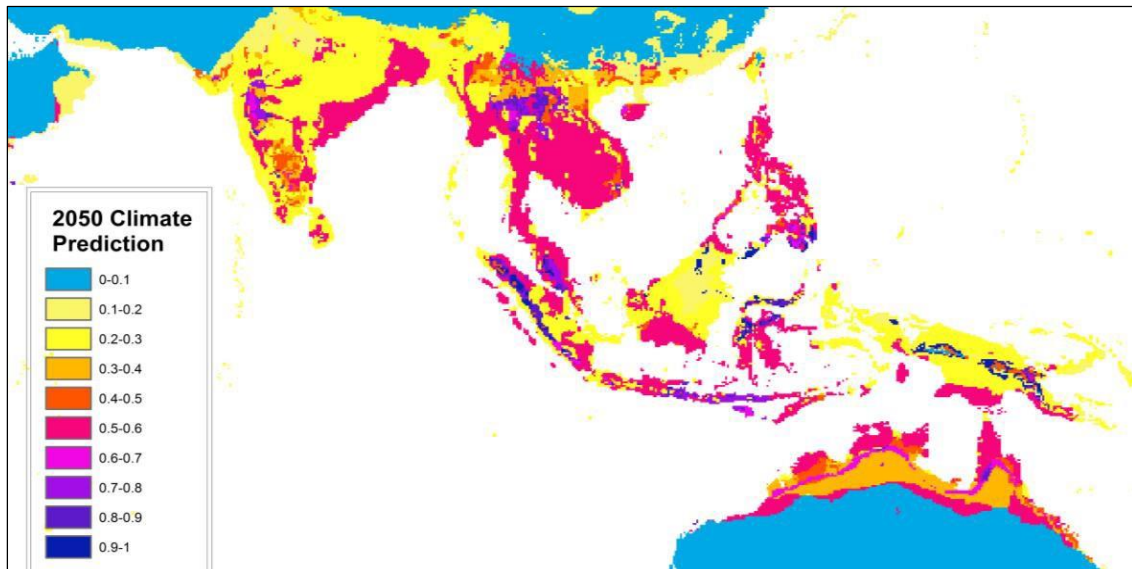


Figure 6.7: Potential range of distribution for *A. terpsicore* in South-East Asia, predicted for future 2050, climatic conditions are shown (blue, purple and pink shades). Likelihood of occurrence for the given pixel (from 0= unlikely to 1= very likely) is specified in the legend. The consensus forecast for future 2050, climatic conditions is based on 36 individual models.

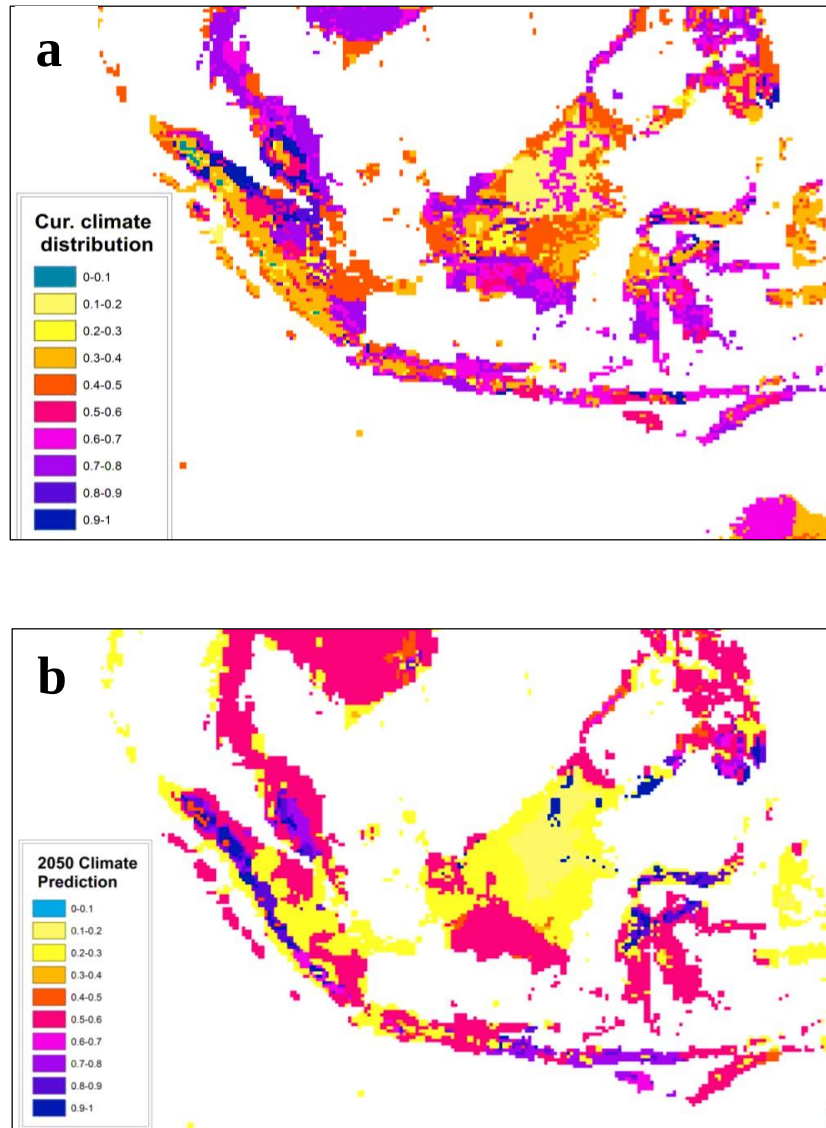


Figure 6.8: Potential range of distribution for *A. terpsicore* in Borneo, predicted under (a) current climatic and (b) future 2050 climatic conditions are shown (blue, purple and pink shades). Likelihood of occurrence for the given pixel (from 0= unlikely to 1= very likely) is specified in the legend. The consensus forecast for current climatic conditions is based on six and for future from 36 individual models.

6.5 Discussion

India and Sri Lanka are known to be the native lands of *A. terpsicore* but in the past couple of decades this tiny Nymphalid has explored and established prosperously in the region of South-East Asia, which includes particularly Thailand, Vietnam, Laos, Cambodia, Peninsular Malaysia and Singapore (Takanishi, 1988; Inayoshi and Saito, 2009; West, 1996; Osada et al., 1999; Arshad et al., 1996 and Khew, 2008). With the help of internet sites and of photographic evidence the detailed distribution of *A. terpsicore* was compiled. After Arshad et al., (1996), the detailed distribution of this species for India, Sri Lanka, Thailand and Vietnam was provided by Pierre and Bernaud, (1997). Following that the detailed distributional map of *A. terpsicore*'s occurrence for the above mentioned region was executed. Matsumoto et al., (2012) documented the presence of *A. terpsicore* as a species from Indonesia (Lombok) which provided its latest record in this region. However, when tracked there was plenty of photographic evidence from different online sites (particularly for Lepidoptera) that provided the establishment this butterfly in the Indo-Australian region. Later on it was reported to be encountered from Timor and Australia (Braby et al., 2013). As a matter of fact, in recent times it has also been encountered in the Southern foothills of Bhutan (Nidup, 2015) and potentially denies the statement from Saji et al., (2014) that this species is rarely found in northern and north-eastern areas of Southern India. In Borneo Island it was collected for the first time in Kota Samarahan, Sarawak in 2013 (Abang et al., 2016). While in Western Kalimantan the latest literature was published by Florida et al., (2014) from Mandor Landak Regency and they did not mentioned its first presence in Kalimantan (Indonesia), although Iqbal et al., (2015) described this species from five locations of East and West Kalimantan.

The migration and establishment of *A. terpsicore* in the South-East Asian region is still unclear, but on the other hand the hypothesis proposed by Braby et al., (2013) were quite supportive about its presence and concludes that:

- i. The species was accidentally and recently introduced into Indo-China from India/ Sri Lanka.
- ii. The species naturally expanded its range and colonized in Thailand via Myanmar (Burma).
- iii. The species always existed in the South-east Asian region but become abundant due to the modification of the environment (climate change) and forest degradation.

With the detailed investigation the second and third hypothesis are much more acceptable due to the bottom line of alteration in environment (anthropogenic climate change) and forest degradation, clearing of lands for agricultural purposes alternatively provides the suitable habitat for the growth of its larval host-plant and establishment (in turn)(Braby et al., 2013 and Iqbal et al., 2015).

Throughout Southeast Asia, forests are rapidly being logged, particularly in Malaysia (Whitmore, 1984; Hill, 1999). In Borneo, through the course of past decades, the forest area has been transformed on a larger scale into palm-oil or *Acacia* plantations (Koh, 2008) creating open habitats, half open habitats and short grass areas, which are intensely habitable for *A. terpsicore* (Iqbal et al., 2015). This species was enormously encountered feeding on *P. foetida* in Borneo. *P. foetida* is an exotic wild plant, chiefly found in areas of pronounced wet season and irrepressibly grows on upland rice paddies and other crops. It is more common in forest clearings, plantations, rough pastures, roadsides and wastelands. Sometimes, it is cultivated in coconut plantations as soil cover with the intention to control grass or erosion, chiefly in Borneo and Philippines (Waterhouse, 1994). One of the main reason, for the rapid spread of *A.*

terpsicore within the region is the availability of suitable habitat and cannot be taken for granted.

The invading capacity of a species is simply a reflection of the width of their fundamental niche (Beaumont et al., 2009) and working with niche-based models calibrated in the native range by relating species observations to the climatic variables are commonly used to predict the potential spatial extent of species invasion (Franklin, 2010; Bertelsmeier et al., 2013). Range expansion studies, with regards to the butterfly species is more dominant in the European and Western parts of the world, suggesting the results (range expansion) as influences of climate change as well as habitat loss. Hence, due to the wide distributional history and ecology, butterflies are tracked for their range expansion towards higher altitudes and latitudes (Thomas et al., 2004; Parmesan and Yohe, 2003; Parmesan et al., 1999 and Hill et al., 1999).

To estimate the range size (EOO) the occurrence record of *A. terpsicore* was utilized and resulted to be covering an area of about 322,766 km² in Borneo. SDMs projections forecasted the current and future's climatically suitable range margins for *A. terpsicore* in Borneo and adjacent regions. The consensus projections of SDMs showed that the range expansion of *A. terpsicore* will likely to increase in the Indo-Australian region with the warning that this species has the potential of becoming an invasive species (Braby et al., 2013). The nearest areas predicted to be habitable with suitable climate were predominantly Sulawesi Island and the Philippines. At least a decade before in the Indo-Pacific region, the genus *Acraea* was weakly observed with four to five species inhabiting in India, Burma and China. In addition to that the butterfly fauna of Sulawesi Island was also characterized by only two species of *Acraea*, with no supportive information of *A. terpsicore* to reside the island (Vane-Wright and Jong, 2003) (with supplementary information to include here is at that time the following genus was suddenly lacking from Peninsular Malaysia, Borneo and Philippines).

Currently *A. terpsicore* is still in the phase of expanding its range in Borneo. Studies have indicated that invasive species conserve their climatic niche in the invaded ranges profoundly relies on the assumptions of climate matching approach (Borennimann, 2007). But it must also be considered that climate change may create opportunities for niche differentiation as well as adaptation of evolution for non-native species in invaded regions (Ackerly, 2003). Hence assuming that *A. terpsicore* in Borneo has a successful distribution range covering mostly eastern and western parts of the island and will follow the trend of range expansion via dispersal to the nearby adjacent areas. Here concluding to the fact that *A. terpsicore* being an invasive species, it has to choose establishment by colonizing the habitable range margins in non-native regions rather than becoming an invasive species at the first place. Also it has the potential to invade further the nearby regions that comprehends plenty of resources of its larval host plant and achieving a foothold in dispersal and establishment in Southeast Asian region.

CHAPTER 7

MITOCHONDRIAL DNA VARIATION (*COI*) AMONG *Acraea terpsicore* (LINNAEUS, 1758) (NYMPHALIDAE: HELICONIINAE) FROM BORNEO AND ADJACENT AREAS

7.1 Introduction

In the recent approaches within the systematics of the family Nymphalidae, *Acraea terpsicore* is classified in the subfamily Heliconiinae under tribe Acraeini (Penz and Peggie, 2003). The genus *Acraea* is widely distributed over Africa and Asia and about 250 species inhabit in Africa, while India has only two native species, which are *A. terpsicore* and *A. issoria* (Kunte and Gadgil, 2001; Larsen, 2005). The native Indian nymphalid butterfly *A. terpsicore*, in the past three decades has invaded most regions of the Southeast Asia and Australia including Peninsular Malaysia (Arshad et al., 1996). After its first documentation in Peninsular Malaysia, this exotic butterfly through the course of migration took almost two decades to invade Sarawak, Borneo (Abang et al., 2016). Its distribution has since then extended to most of the areas in Borneo.

Exotic species in their novel range are reported to be as a significant threat to the native biodiversity (Vitousek et al., 1997 and Wilcove et al., 1998) and once introduced, it is difficult to interpret the introduction phenomena (Eastwood et al., 2007). Exotic species in their initial phases of introduction and establishment are often foreseeing the loss of genetic variability due to smaller population size (first few generations) (Dlugosch and Parker, 2008). In contrast, invasive populations from several genetically segregated source populations may contribute to greater genetic variance (Facon et al., 2003). Several contemporary genetic studies displays that invasive species rapid adaptation to their novel environments can occur within twenty

generations or less (Prentis et al., 2008). However, during invasion changes in phenotypic traits occurs under non-equilibrium demographic conditions and may reflect the influences of prior evolutionary history and chance events, as well as selection (Keller and Tylor, 2008). Analyses of the genetic variance of the invading populations provide a great insight of the historical demography for the invasion phases (introduction and establishment) (Ciosi et al., 2008). Indeed, genetic variance of molecular and quantitative traits of invading populations depends on the history and demographic features of their population of origin (Estoup and Guillemaud, 2010). Several studies on the population genetic variation and demographic history has been reported in butterflies through analyzing the mitochondrial (*COI*) data set (Pfeiler et al., 2016). Mitochondrial DNA (mtDNA) are often assessed for historical patterns and gene flow and is considered one of the essential marker (Harrison, 1989). Mitochondrial markers are uniparental and haploid, which makes them frequent targets for genetic variance in population level studies (Avise, 2004).

Given the reason that invasive populations have to deal with genetic variation during the course of invasion and adaptation to their novel environment. This study aims

- a) to examine the mitochondrial cytochrome *c* oxidase subunit I, sequence variation of *A. terpsicore* from Borneo and adjacent areas.

7.2 Materials and Methods

7.2.1 Sample Collection

The adults of *A. terpsicore* were collected from seven locations of Sarawak, one each from Kalimantan and Moa Island. Whereas, five of the sample sequences were obtained from Genbank and Bold, one of which was deposited from Peninsular Malaysia and four from India. A total number of 14 samples were utilized including one species *A. issoria* as outgroup in this study and their localities description is given in Table 7.1 and Figure 7.1.

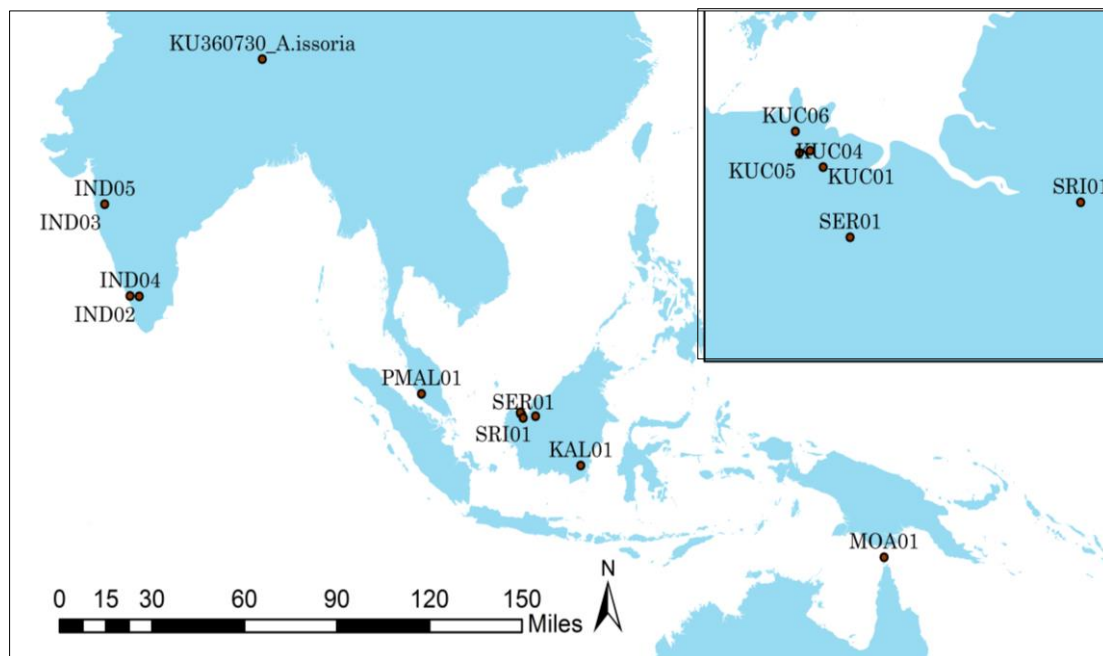


Figure 7.1: Sample collection localities including those that were accessed from Genbank with their respective field ID's. The Sarawak samples are enlarged in the inset map. (Source ArcMAP 10.4)

Table 7.1: Samples of *Acraea terpsicore* analyzed for DNA (*COI*) sequence variation.

S #	Species	Locality	GPS reading	Field number	Genbank accession no.
1	<i>A. terpsicore</i>	Desa Ilmu Sarawak	1°27'10.498"N 110°27'26.83"E	KUC01	
2	<i>A. terpsicore</i>	Sama Jaya Sarawak	1°31'29.474"N 110 °23'45.249"E	KUC03	
3	<i>A. terpsicore</i>	Desa Ilmu (2013) Sarawak	1°27'10.498"N 110°27'26.83"E	KUC04	
4	<i>A. terpsicore</i>	Jln Stampin barat Sarawak	1°30'54.037"N 110°20'46.286"E	KUC05	
5	<i>A. terpsicore</i>	Samariang Sarawak	1°36'29.881"N 110°19'35.883"E	KUC06	
6	<i>A. terpsicore</i>	Sri Aman Sarawak	1°19'60"N 111°40'.0"E	SRI01	
7	<i>A. terpsicore</i>	Serian Sarawak	1°8'52.048"N 110°35'3.461"E	SER01	
8	<i>A. terpsicore</i>	Moa Island Australia	10°10'14.027"S 142°15'17.899" E	MOA01	
9	<i>A. terpsicore</i>	Mount Besar Kalimantan	02°42'39"S 115°37'32.999"E	KAL01	
10	<i>A. terpsicore</i>	Tamilnadu, India	11°2'22.093" N 76°52'43.867" E	IND02	JX226067.1
11	<i>A. terpsicore</i>	Maharashtra, India	18°31'33.639" N 73°50'43.755" E	IND03	KJ459777.1
12	<i>A. terpsicore</i>	Kerala, India	11°4'23.455" N 76°4'26.399" E	IND04	KT880658.1
13	<i>A. terpsicore</i>	Maharashtra, India	18°31'33.639" N 73°50'43.755" E	IND05	KJ459773
14	<i>A. terpsicore</i>	UMKL, Peninsular Malaysia	3°7'12.384" N 101°39'16.377" E	PMAL01	KF226260.1
15	<i>Acraea issoria</i>	Uttarkand, India	30°20'1.868"N 78°1'34.801"E		KU360730

7.2.2 DNA Extraction

Among all the samples collected, total genomic DNA was extracted from museum dried specimens using the thorax of *A. terpsicore* individuals and following the modified Cetyl trimethyl ammonium bromide (CTAB) protocol of Alwanie (2012).

Liquid nitrogen was utilized to break down the chitin exoskeleton of the samples and allows to maximize the tissues exposure to the lysis mixture. The samples (thorax) were then grinded in 1.5 mL micro-centrifuge tube, after which 800 µl of CTAB was added. Into the tube fifteen µl of Proteinase K was added into the tube and incubated in a water bath at 60°C until the mixture was seen to be clear (overnight). After incubation, 700 µl of Chloroform Isoamyl-Alcohol (CIA) was added into mixture and centrifuged at 13,000 rpm for 20 minutes. The supernatant (upper layer) was transferred it into a new micro-centrifuge tube equal volume of cold absolute ethanol was added and centrifuged at 13,000 rpm for 20 minutes. After that, the rest of the ethanol was discarded leaving the white DNA pellet at the bottom of the tube. Following that an equal volume (~600 µl) of 70% ethanol and 25 µl of 3M NaCl were added into the tube and centrifuged again for 20 minutes at 13,000 rpm. DNA pellet was obtained at the bottom of the tube, while ethanol was discarded carefully. Pellet was dried at room temperature for 25-30 minutes. The pellet was resuspended by adding 25 µl of ddH₂O and stored at -20°C of temperature.

7.2.3 Polymerase Chain Reaction (PCR Amplification) and Sequencing

Double-stranded DNA samples were amplified for the cytochrome oxidase subunit I (*COI*) gene of *A. terpsicore* through polymerase chain reaction (PCR) Biometra Machine (Table 7.2 and 7.3). The standard PCR protocol was followed to prepare the master mix of 25 µl using (specific) primers of *COI* gene, which included Forward primer C1-J-1751 (5'-

GGATCACCTGATATAGCATTCCC-3') and Reverse primer C1-N-2191 (5'-CCCGGTAAAATTAAAATATAAACTTC-3') (obtained from First base Laboratories).

The PCR products were visualized on 1% agarose gel containing ethidium bromide and run at 90V for 30 minutes. The amplified PCR products were send for further purification and sequencing to the First Base Laboratories Sdn Bhd, Kuala Lumpur.

Table 7.2: Master mixture components and volume of PCR amplification for one reaction.

Component	Volume 1 reaction (µl)
5 X reaction buffer	5.0
MgCl ₂	1.5
dNTP mixed	0.5
Forward primer (<i>COI</i>)	1.0
Reverse primer (<i>COI</i>)	1.0
ddH ₂ O	13.8
DNA template	2
Taq polymerase	0.2
Total volume of reaction mixture	25.0

Table 7.3: Amplification parameters for PCR.

Steps	Temperature (°C)	Duration	Number of cycles
Pre-denturation	95	5 minutes	1
Denaturation	95	1 minute	30
Annealing	50	1 minute	30
Extension	72	1 minute	30
Final extension	72	7 minutes	1
Soak	4	∞	

7.3 Data Analysis

The resultant sequences were analyzed through Chromas Programme (2.24) by McCarthy (2004). The sequences were aligned with Clustal X (Thompson et al., 1997) and translated to amino acid sequences in MEGA (7.0) (Kumar, Stecher, and Tamura 2015) for comparative phylogenetic and distance analyses following Zainudin et al., (2010). For all the comparative analysis the *COI* subunit sequence of *Acraea issoria* (Genbank KU360730) was selected as outgroup. Results of the Maximum likelihood (ML), Neighbour-Joining (NJ) and Minimum Evolution was obtained via MEGA and PUAP 4.0b10 (Swofford 2001). The intra and inter nucleotide divergence was calculated using the Kimura-2-parameter. Maximum likelihood analyses was based on the substitution model GTR+I using the AIC criterion. The ML tree was constructed on 1000 replicates with model GTR+I based on due to its lowest Akaike's Information Score (AIC) from best fit models. While NJ tree was constructed based on the genetic distance determined by Kimura-2-Parameter from 1000 replication and bootstrap values were indicated at the nodes. The acceptable bootstrap values for the phylogenetic trees is more than 80 percent (>80 %).

7.4 Results

DNA extraction of *A. terpsicore* was obtained from ten samples which yielded single bright color DNA products at approximately 500bp (Figure 7.2). However, for phylogenetic analysis only nine of the sample sequences were evaluated and sample KUC02 was omitted from the sequencing data file due to gaps (missing data). Altogether among 14 samples of *A. terpsicore*, a total of 356 bp of partial *COI* gene sequence with one outgroup *A. issoria* were successfully aligned.

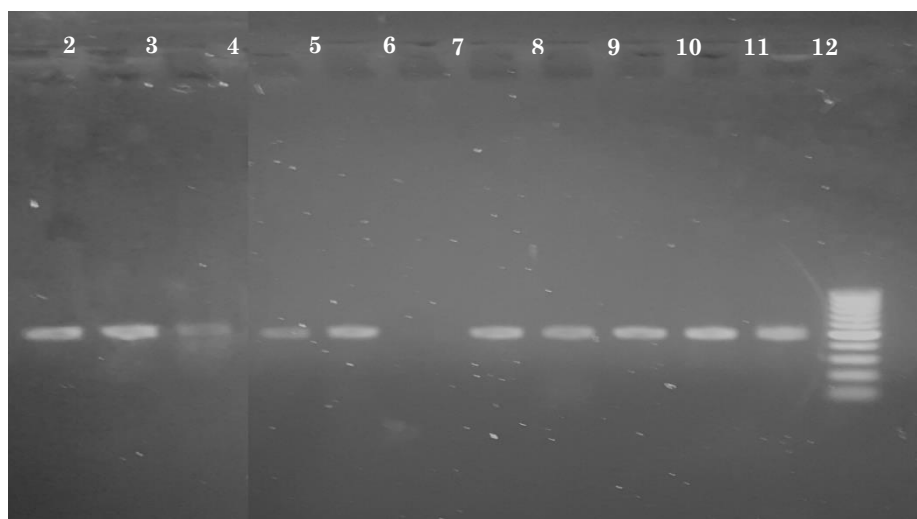


Figure 7.2: Gel electrophoresis using 2% Agarose gel and PCR products amplified at mtDNA *COI* in *A.terpsicore* samples; **Lane 1:** KUC01; **Lane 2:** KUC02; **Lane 3:** KUC03; **Lane 4:** KUC04; **Lane 5:** KUC05; **Lane 6:** negative control; **Lane 7:** KUC06; **Lane 8:** SRI01; **Lane 9:** SER01; **Lane 10:** MOA01; **Lane 11:** KAL01; **Lane 12:** 100 bp DNA ladder.

The overall nucleotide frequency distribution at the 1st, 2nd and 3rd codon position were as follows: A= 29.4%, 13.4% and 42.4%; C= 16.0%, 29.4% and 11.0%; G= 24.4%, 14.3% and 0%; T= 30%, 43% and 47%. Of the 356bp of the partial *COI* gene sequence 203 are conserved sites and 47 variable sites with 46 singleton sites leaving only one parsimonally site (of outgroup). The highest number of conserved sites infers to *COI* genetic marker to be highly conserved and is least suitable for the analyses of genetic variance among the population of *A. terpsicore*. (see Appendix III)

7.4.1 Pairwise Genetic Distance

All the population of *A. terpsicore* that were included in this study didn't showed any pairwise genetic variance except for one sample sequence (IND05) which showed a genetic distance of 0.006%. The highest genetic distance value of 0.142 % was obtained for *A. issoria*, whereas the rest of the samples showed 0.00 value (Table 7.4).

7.4.2 Phylogenetic Analysis

The phylogenetic trees of *A. terpsicore* were constructed which includes Maximum likelihood (ML), Neighbour-Joining (NJ) and Minimum Evolution (Figure 7.3, 7.4 and 7.5). The bootstrap value obtained in the ML and NJ trees was relatively small and hence the trees were considered unresolved. All the phlogenetic analysis produced the same topography with no resolve on the relationship as reflected by low bootstrap value (64%). However, all the samples are monophylatic with respect to the outgroup.

Table 7.4: Pairwise genetic distance of *Acraea terpsicore* using Kimura-2-Parameter

No.	Individuals	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	KUC_01															
2	KUC_03	0.00														
3	KUC_04	0.000	0.00													
4	KUC_05	0.00	0.00	0.00												
5	KUC_06	0.00	0.00	0.00	0.00											
6	SER_01	0.00	0.00	0.00	0.00	0.00										
7	SRI_01	0.00	0.00	0.00	0.00	0.00	0.00									
8	KT880658_IND_04	0.00	0.00	0.00	0.00	0.00	0.00	0.00								
9	KF226260_PMAL_01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00							
10	KJ459777_IND_03	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00						
11	JX226067_IND_02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00					
12	KAL_01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00				
13	MOA_01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00			
14	KJ459773_IND_05	0.006	0.006	0.006	0.006	0.006	0.006	0.006	0.006	0.006	0.006	0.006	0.006	0.006		
15	KU360730_A_ISSORIA	0.142	0.142	0.142	0.142	0.142	0.142	0.142	0.142	0.142	0.142	0.142	0.142	0.142	0.142	0.142

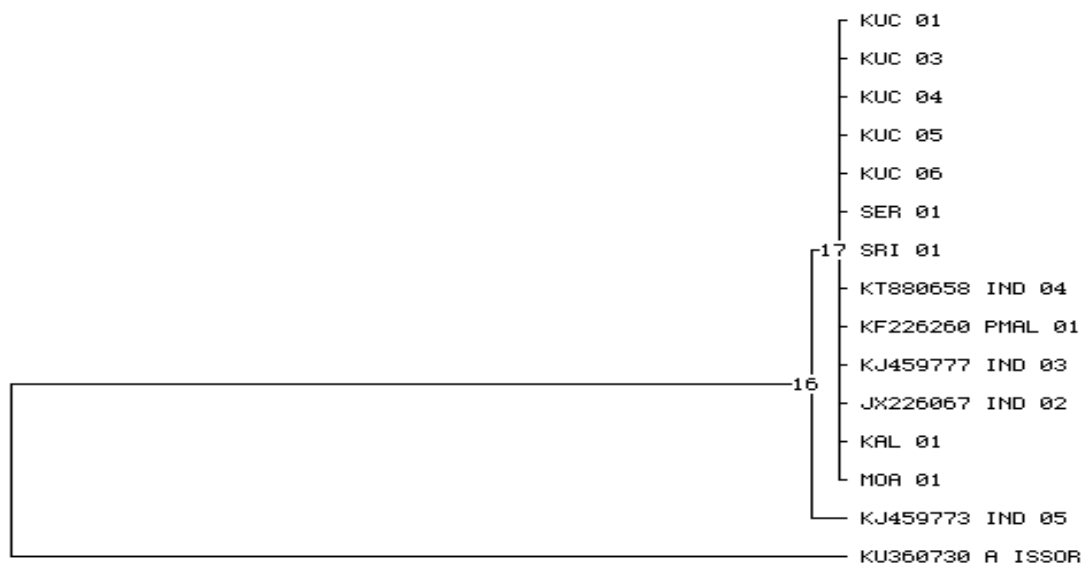


Figure 7.3: Maximum Likelihood (ML) tree of *Acraea terpsicore*, constructed from partial *COI* gene and based on 1000 replications.

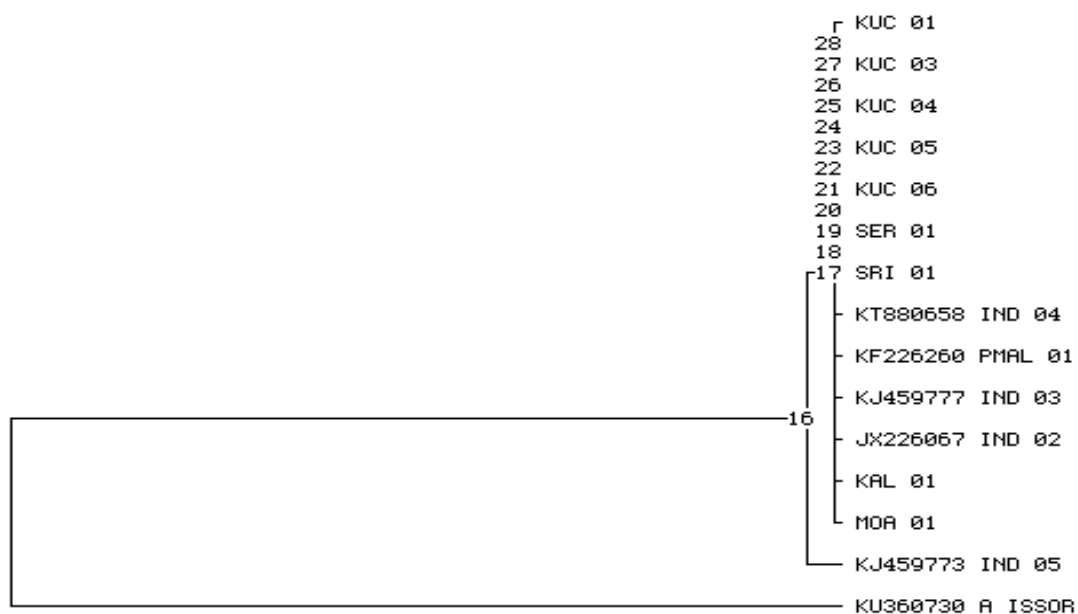


Figure 7.4: Neighbour-Joining (NJ) tree of *Acraea terpsicore*, constructed from partial *COI* gene and based on 1000 replications.

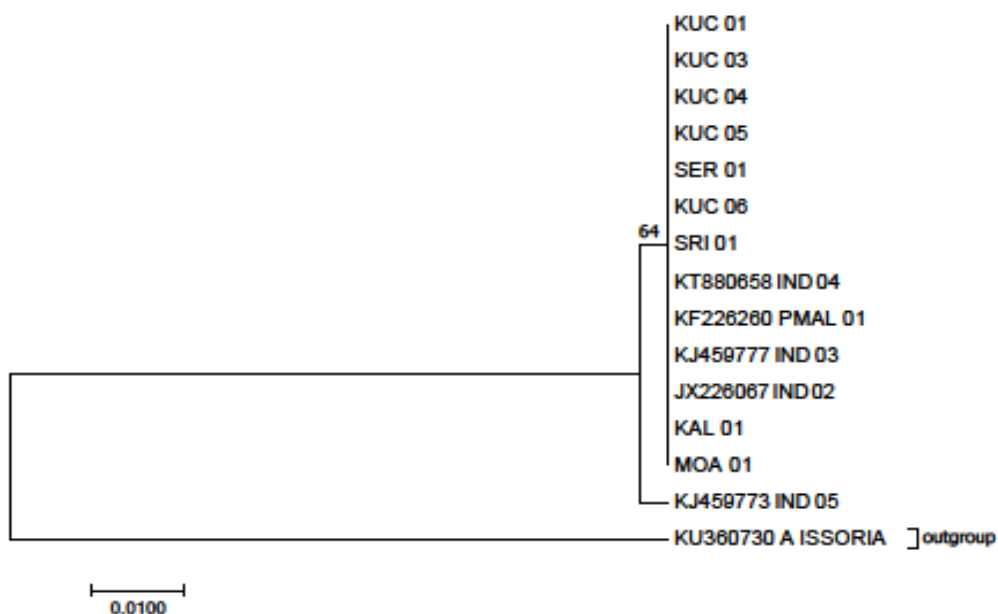


Figure 7.5: Minimum evolution tree for *Acraea terpsicore*, constructed from partial *COI* gene.

7.5 Discussion

The two distinct species of Acraeini tribe are *A. terpsicore* and *A. issoria*, which are native to India (Kunte and Gadgil, 2001). *A. terpsicore* has been on the route of migration and invasion southwards in Southeast Asian region up to Australia and was recently recorded in Sarawak, Borneo (Abang et al., 2016). While *A. issoria* also has a wide distribution range in Indo-China region and Taiwan (Emmel and Heppner, 1990).

Systematic studies in insects have been revolutionized by the development of PCR and even from tiniest samples the phylogenetic problems through the nucleotide sequence data comparison have been resolved successfully (Dillon et al., 1996). Most of the times lepidopteran legs are the main source used from morphological vouchers for molecular studies but the available amount of tissue yields very low fragmented DNA. For excellent DNA preservation the specimens should be preserved in 100% ethanol and stored at -80°C for higher

quality of total genomic DNA extraction (Dillon et al., 1996 and Cho et al., 2016). In this study the DNA extraction of *A. terpsicore* was one of the trickiest part due to the museum dried samples have been reported to yield smaller fragments of genomic DNA. The DNA extraction was conducted with CTAB protocol modified from Alwanie (2012). The incubation period was the crucial part which was repeated for longer time (every three hours) and finally was selected overnight to allow the sample tissue for maximum lysis.

The partial mitochondrial (*COI*) gene utilized in this study yielded 356bp sequence. Pairwise genetic distance executed with Kimura-2-Parameter showed almost zero genetic variance among the population of *A. terpsicore*. This is due to the relationship is not resolved and extensive gene flow cause none genetic variation among the population.

In the current study, the phylogenetic analysis with the ML, NJ and Minimum Evolution phylogenies resulted in same topography and revealed that the relationship is not resolved due to extensive gene flow among the population studied. All the samples included in this study are assumed to evolve at a single clade and share the same branch except for the IND05 sample which had a divergence rate of 0.006%. This was considered as a result of mutation at 95 and 246 sites of the 356bp mtDNA (*COI*). Analyzing the genetic relationships between *A. terpsicore* sampled from its invaded range in Borneo (Sarawak and Kalimantan) and Moa Island as well as Peninsular Malaysia (Genbank) might indicate that how many populations of *A. terpsicore* have migrated across the equator, become dispersed and established in the Southern Hemisphere. The results of phylogenetic analysis suggested there was no genetic variance among the population of *A. terpsicore* when compared from different geographic landscapes. These results were very consistent as reported by Vadivalagan et al., (2014) for *A. terpsicore* when compared from India and Peninsular Malaysia and by Burg et al., (2014) describing the same phenomena in an exotic butterfly *Dyras iulia*. Invasive alien species are traditionally

linked with reduced or none genetic variation relative to their source population. This is due to their founder effects associated with the phases of introduction and establishment connected with small population size (Handley et al., 2011) in varying geographic landscapes.

As a conclusion of this study, it was anticipated that partial mitochondrial (*COI*) marker is not suggested as a good marker to detect any genetic variation among the population of *A. terpsicore*. Multiple molecular genes should be utilized such as control region to implement accurate phylogenetic analysis. Also, a large number of haplotypes should be included from a single locality in genetic variance studies conducted for exotic/ non-indigenous species, which can yield maximum accurate results.

CHAPTER 8

GENERAL SUMMARY

The new exotic butterfly *Acraea terpsicore* (Linnaeus, 1758) has been recently reported from Sarawak, Borneo and this brings an addition to its geographic distributional range in Southeast Asia. This species has been reported in the past three decades to invade regions of Southeast Asia and Australia but very recently it has expanded southwards and invaded Borneo. The establishment of this exotic butterfly was assessed in Sarawak, Borneo by studying its comparative biology relative to three habitat types based on urbanization levels. The result inferred that *A. terpsicore* has five larval instars and the immatures possess the same characteristics as reported by several authors. In conclusion, no major discrimination was obtained in the comparative biology studies. The host plant preference studies suggested that this species feeds insatiably on *Passiflora foetida* in this novel range. Experimental host plants association studies resulted in the failure (at present) of *A. terpsicore* to adopting a new larval plant and remained unsusceptible towards Cucurbitaceae. Therefore, this species at this stage does not poses a crucial threat of becoming an economical pest, as Passifloraceae still remains its primarily choice of host plant in this region. In 2015, the smaller size, defects of wing patterns and wing shape of *A. terpsicore* individuals were detected and anticipated as a result of their exposure to smoke haze that hit Borneo severely. It is important to scrutinize at molecular basis the dynamic relationship of environmental stress conditions causing negative impacts and deformities in butterfly morphology via physiology.

The spatial distribution and range expansion of *A. terpsicore* evaluated with ArcGIS and SDMs forecasted a much broader climatically suitable range of *A. terpsicore* in Southeast Asia and Australia including Borneo. The SDMs also predicted the climatically suitable future

range of this species and suggested that it will continue to expand further north and southwards to Taiwan, Philippines and the Sulawesi Island. In general, *A. terpsicore* has already been reported from several locations of Borneo and its future distribution to the nearby regions will likely to happen. This might create a chance of overlapping the geographical range and increases the chance of competition for the native biodiversity.

The genetic variance studies resulted in no genetic variance among the population of *A. terpsicore*, in pairwise genetic distance and phylogenetic analysis. This suggested that the species may be too recent with higher gene flow among the populations and is still in the phase of establishment of invasion. These results were consistent with the other studies which state that invasive species are traditionally linked with reduced genetic variation due to their association in founder effects of the phases of introduction and establishment inversely linked to small population size. In future, multiple molecular genes should be utilized in order to implement accurate phylogenetic analysis as partial mitochondrial DNA (*COI*) marker might not be a good one to detect any genetic variation among the population of *A. terpsicore*. Also large number of haplotypes from single locality of exotic species will outcome in more accurate findings of genetic variance.

In this era, the biodiversity of the earth is under serious threats due to human activities as well as biological invasions and Biodiversity conservation management of any region can have positive impacts if precise and accountable information of an invasive species is available in real time. Therefore, it is recommended that *A. terpsicore* should be closely monitored in future as this species is still in the initial phase of invasiveness and can be a threat at any point in future for becoming a pest of economic importance.

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APPENDICES

Appendix I

(Chapter 3): Developmental cycle data for *Acraea terpsicore* body length (mm)

Stages	no. eggs	Larva					Pre- Pupa	Pupa	Adult		
		L1	L2	L3	L4	L5			Length	Wingspan	Sex
Batch # 1	6	3.5	9.5	14	19	28	25	22	16	49	♂
		3	10	15.5	19.5	30	27	23	15	44	♀
		3	11	15	20	31	28	22	15.5	46	♀
		3.5	11	16	21	30.5	27	23	14	43	♀
		3	9.5	14.5	18	29	25	21	14.5	47	♀
		3	9	14	19	28	24	20	14	44	♂
Batch # 2	6	3	9	14.5	20	29	24	22	15	46	♀
		3.5	9.5	14.5	21	30	25	23	16	41	♀
		3	9	15	21.5	31	28	22	16	49	♂
		3	10	16							
		3.5	10	15.5							
		3	9	14	19	29	25	21	15	48	♀
Batch # 3	6	3	10	15	20						
		3	9	14	19	29	25	21	14	48	♂
		3.5	10	14.5							
		3.5	10	16	20	30	26	22	14.5	44	♂
		3.5	9	14.5	21	29	25	21	15	45	♀
		3	9	15	21.5	31	27	23	16	48	♂
Batch # 4	6	3	10	14.5	20						
		3.5	10	15	19.5	29	24	21	15	51	♂
		3	9	15	20	31	26	22	16	48	♀
		3.5	11	15.5	21	31	27	21.5	15	46	♀
		3.5	11	14	22	30	26	22	16	47	♀
		3	10.5	15.5	20	30	25	21	15.5	44	♂
Batch # 5	6	3	9	13	20	29	25	21	14.5	44	♀
		3	10	14	20.5	28	24.5	21	14	46	♀
		3.5	11	15.5	21	30.5	27	22	16	43	♂
		3	9	14	21	31	28	23	16	48	♀
		3.5	10	16	20.5	31	28.5	23	15	46	♂
		3.5	11	15	21	28	24	21	14	47	♀
Batch # 6	6	3	9	14	21	29	25	21	14	43	♂
		3.5	9.5	15	22	30	27	22	15	47	♂
		3.5	10	14.5	20	30	26	21	14.5	41	♂
		3	8	13	21	31	28	21.5	16	47	♂
		3	9	14	21	31	28	22	16.5	51	♀
		3	9	14	20	28	25	21	15.5	49	♀
Batch # 7	6	3	9	15	21	29	25	21	14	41	♀
		3.5	10	16	22	30	28	23	16.5	47	♀
		3	9	14.5	21	31	28	23	16.5	49	♀
		3	9	14	20	28	24	20.5	15	39	♂

Stages	no. eggs	L1	L2	L3	L4	L5	Pre- Pupa	Pupa	Length	Wingspan	Sex
Batch # 8	6	3	9.5	15							
		3.5	11	16	22	29	25.5	21	14	40	♂
		3	9	14	19	28	24.5	20	14	41	♀
		3.5	10	15	20	30	27	22	16	49	♂
		3.5	11	15	21	31	28	23	16.5	44	♀
		3	10	15.5	20	30	26	21	15	47	♂
Batch # 9	6	3	10	15	18.5	29	25	20	14	44	♂
		3	9	14.5	19	30	26.5	21	16	43	♀
		3	10	15	20	29	24.5	21	14	46	♀
		3	9	15.5							
		3.5	10	16	21	29	24	20	14.5	49	♀
		3.5	9	14.5	19	29	25	21	16	47	♂
Batch # 10	6	3.5	10	15	20	30	27	22	15	41	♂
		3.5	10	16	22	31	28	23	16	44	♀
		3	9	14	19	28	24	21	15	43	♀
		3.5	10	15	19.5	30	26	22	16	47	♂
		3.5	10	15.5	20	31	27	23	16.5	43	♂
		3.5	11	16	21	30	27	23	16	41	♂
Batch # 11	6	3	9	15	20	29	26	22	15	44	♂
		3.5	10	15.5	21	30	26	21	14	41	♀
		3	9	15	19						
		3.5	10	15.5	20	29	25	21	15	49	♀
		3	10	14.5	19	31	27	22	15.5	51	♀
		3	9	15	20	30	26.5	21.5	16	53	♂
Batch # 12	6	3.5	10	16	21	31	28	23	16	54	♀
		3	9	15.5	21	31	27	21	14	39	♂
		3	9	15	20	29	24	20	14.5	43	♂
		3	9	14.5	19	30	27	21	15	46	♂
		3.5	10	16	21	31	28.5	23	16	48	♂
		3.5	9	15	21	31	27.5	22	15.5	41	♂
Batch # 13	6	3.5	10	15.5	20	30.5	27	22.5	14.5	41	♀
		3.5	10	14.5	21	30	26	21	15	47	♀
		3	9	15	20	29	24	20	14.5	39	♂
		3	10	14							
		3.5	9.5	16							
		3.5	10	15.5	20	29	24.5	20	15	41	♀
Batch # 14	6	3.5	9	14.5	20.5	30	27	22	15	38	♀
		3.5	9.5	16	21	31	27.5	22	16	44	♂
		3	9	14.5	20	29.5	25	20.5	14.5	39	♂
		3.5	10	15	20	31	28	21	16	38	♀
		3.5	11	16	21	30	27.5	22	15.5	43	♀
		3.5	11	16	22	30.5	27	21.5	15	41	♂
		3	10	15.5	21	31	27	21	15	48	♂

Stages	no. eggs	L1	L2	L3	L4	L5	Pre- Pupa	Pupa	Length	Wingspan	Sex
Batch # 15	6	3	9.5	15	21	30.5	26.5	21.5	14.5	40.5	♀
		3.5	10	16	20	29	24	20	15	39	♂
		3.5	10	15.5	21	30	27	22	16	41	♀
		3	9.5								
		3	9.5	14.5	19	28	23	19.5	15	40.5	♀
Batch # 16	6	3.5	9.5	15	20	31	28	23	16	43.5	♂
		3	9	15.5	21	31	28	23	15.5	44	♂
		3	10	14	20						
		3	10	15	21	29	24	20	15	43	♂
		3	9	14	21	31	28	22	16	44	♀
Batch # 17	6	3	9	14	20	31	28.5	23	16	46	♀
		3.5	9.5	16	22	30	27	22	16	41	♀
		3.5	10	15.5	21	29	24.5	21	15	41.5	♂
		3	9	14	20	29.5	24	20	16	39	♂
		3	9	14	20	30	27	21	15	40	♂
Batch # 18	6	3	9	15	21	30	26	21	16	38.5	♂
		3.5	10	16	22	31	27	22	15	39	♂
		3	9	14.5	21	30.5	26	21.5	16	43	♀
		3	9	14.5	22	31	27	21.5	16	44	♀
		3	9	14	20	29	24	20.5	14	38	♂
Batch # 19	6	3	9	15	21	30	27	22	16	40.5	♂
		3.5	10	15	22	29	24	21	16	44	♀
		3.5	11	16	21	30	26	21	14	39	♂
		3.5	11	16.5	22.5	31	27	22	16	45	♂
		3	10	16.5	23	33	28	23	16	48	♀
Batch # 20	6	3	9	14	20	29	24.5	20	15	43	♀
		3	10	15	21	30	26	20.5	16	41.5	♀
		3.5	10	15.5	22	31	27	22	15	46	♀
		3	9	14.5	21	30.5	28	23	16	41	♀
		3.5	10.5	15	20	30	26	20	16	44	♀
Batch # 21	6	3	9	14	21	29.5	25	21	14.5	38	♂
		3	9	14	20	29	25	20	16.5	41.5	♀
		3.5	10	15	21.5	30	26	21	15	40	♂
		3	9.5	15	22	31	28	22	14.5	39.5	♂
		3.5	9.5	15.5	21	30.5	27	21	14.5	39.5	♂
Batch # 21	6	3.5	10	16	22	31	27.5	20.5	16	38	♀
		3	9	14.5	21	30.5	26.5	21	15.5	41	♂
		3	9	14	20	29	25.5	20	14	38	♂
		3	9.5	15	20.5	30	26	21	15.5	41	♂
		3.5	9.5	15	21	31	27	22	16	39.5	♂
Batch # 21	6	3.5	10	15.5	22	30	26.5	20.5	14.5	41.5	♀
		3	9.5	15.5	21.5	30.5	27	22	14.5	44	♂

Stages	no. eggs	L1	L2	L3	L4	L5	Pre-Pupa	Pupa	Length	Wingspan	Sex
Batch # 22	6	3.5	9	14	21	29	24.5	21	14	38.5	♂
		3.5	10	14.5	20	30	26.5	21	16	39.5	♀
		3.5	10	14	20.5	29	25	20.5	16	44.5	♀
		3.5	9.5	15	21	31	27	22	16.5	41.5	♀
		3	9.5	14.5	20	30	26	20	15	43	♂
		3	9	14	20.5	31.5	27	21	14.5	40.5	♀
		3.5	9.5	15	21	30	26.5	21.5	15	41	♂

(Chapter 3): Developmental duration of each stage of *A. terpsicore* in days.

Stages	Eggs	L1	L2	L3	L4	L5	Pre-Pupal	Pupa	Adult
Batch #1	2.5	3	3	4.5	4	4	1	5	♂
	2.5	3	3	4.5	3.5	4.5	1	6	♀
	2.5	3	3	4.5	3	4.5	1	6	♀
	2.5	3	3	4.5	3.5	5	0.5	6	♀
	2.5	3	3	4	3.5	4.5	1	6	♀
	2.5	3	3	4.5	3.5	4.5	1	5	♂
Batch # 2	3	3	3	4	4	4	1	6	♀
	3	3	3	4	4	4.5	1	5	♀
	2.5	3.5	3	4.5	3.5	5	1	5	♂
	2.5	3.5	3	4					
	3	3	3	4					
Batch # 3	3	3	3	4.5	3.5	4.5	1	6	♀
	3	3	3	4	4				
	3	3	3	4	3.5	4.5	1.5	5	♂
	3	3	3	4					
	3	3	3	4.5	3.5	5	1	6	♂
	3	3	3	4.5	3.5	4	1	6	♀
Batch # 4	3	3	3	4	4	4.5	1	5	♂
	2.5		3	3	3.5	4			
	2.5	3	3	4	4	5	1	5	♂
	2.5	3	3	4	3.5	4	1	6	♀
	2.5	3	3	4	4	4	1	6	♀
	3	3	3	4.5	4	4	1	6	♀
Batch # 5	3	3	3	4	4	5	1	5	♂
	2.5	3	3	4.5	4	4.5	1	6	♀
	2.5	3	3	4	4	5	1	5	♂
	2.5	3	3	4	4	4	1	6	♀
	2.5	3	3	4	4	4	1	6	♀
	3	3	3	4.5	4	4	1	6	♀

Stages	Eggs	L1	L2	L3	L4	L5	Pre-Pupal	Pupa	Adult
Batch# 6	2.5	3	3	4	3.5	4	1	5	♀
	2.5	3	3	4	4	4.5	0.5	5	♂
	2.5	3	3	4.5	3.5	4.5	0.5	6	♀
	2.5	3	3	4	3.5	5	1	5	♂
	2.5	3	3	4	4	4	1	6	♀
	2.5	3	3	4	4	4	1	5	♂
	2.5	3	3.5	3.5	4	4	1	5	♂
	2.5	3	3	3.5	4	4.5	0.5	4.5	♂
	2.5	3	3	4	4	5	1	5	♂
	2.5	3	3.5	4	4	4.5	1.5	6.5	♀
Batch # 7	2.5	3	3	4	4	4	1.5	6	♀
	3	3	3.5	3.5	4	4	1	6	♀
	3	3	3	4	3.5	5	0.5	6	♀
	3	3	3	4	4	4	1	6	♀
	3	3	3.5	4	4	4	1	5	♂
	3	3	3.5	3.5					
	3	3	3	4	4	4.5	1.5	5	♂
Batch # 8	3	3.5	2.5	4	4	4	1	6	♀
	3	3	3	3.5	4.5	4	1	4.5	♂
	3	3	3	4	4	4	1.5	5	♀
	3	3.5	2.5	4	4	4	1.5	6	♂
	3	3	3	4	4	4	1	5	♂
Batch # 9	3	3	3	3.5	4.5	4	1.5	6	♀
	2.5	3	3	4	4	4	1	6.5	♀
	2.5	3	3	4					
	2.5	3	3	4	4.5	4.5	0.5	6	♀
	2.5	3	3	4	4	4	1	5	♂
Batch # 10	2.5	3	3	4	4	4	1	5	♂
	2.5	3	3	4	4.5	4.5	0.5	5.5	♀
	3	3	3	4	5	4	1	5	♀
	3	3	3	4	5	4	1	5	♂
	3	3	3	4	4	4	1	6	♂
Batch # 11	3	3	3	4	4	4	1	5	♂
	3	3	3	4	5	4	1	6	♀
	2.5	3	3	4	4				
	2.5	3	3	4	4	4	1	6	♀
	2.5	3	3.5	3.5	4	4	1	5.5	♀

Stages	Eggs	L1	L2	L3	L4	L5	Pre-Pupal	Pupa	Adult
Batch # 12	2.5	3	3.5	3.5	4	4	1	5	♂
	2.5	3	3	3	4	4	1	6	♀
	2.5	3	3.5	3.5	4	4	1	5	♂
	2.5	3	3	4	4	4	1	5	♂
	2.5	3	3	4	4.5	4.5	0.5	4.5	♂
	2.5	3	3	4	4	4	1	5	♂
	0.5	3	3	4	4	4	1	5	♂
Batch # 13	2.5	4	3	4	4.5	4.5	0.5	6	♀
	2.5	3	3	4	4	4	1	6	♀
	2.5	3	3	4	4	5	0.5	5	♂
	2.5	3	3	4.5					
	2.5	3	3	4.5					
	2.5	3	3	3	4	4	1	6	♀
	2.5	3	3	3	4	4	1	6	♀
Batch # 14	2.5	3	3	4.5	3.5	4	1	6	♂
	2.5	3	3	4	4	5	0.5	5	♂
	2.5	3	3	4	4	5	0.5	6	♀
	2.5	3	3	4	4	4	1	6	♀
	2.5	3	3	4	4	4.5	1.5	6	♂
	2.5	3	3	4	4	5	1	5	♂
	2.5	3	3	4	4	4	1	6	♀
Batch # 15	3	3	3	4	4.5	4.5	1	5	♂
	3	3	3	4	4	5	0.5	6	♀
	3	3	3	4	4	5	1	5	♀
	3	3	3	4	4.5	4.5	0.5	4.5	♂
	3	3	3	4	4	4	1	5	♂
	3	3	3	4	4	4	1	5	♂
	3	3	3.5	4.5	4				
Batch # 16	3	3	3.5	4.5	4	5	0.5	4.5	♂
	3	3	3.5	4.5	4	4	1	5	♀
	3	3	3.5	4.5	4	4	1	6	♀
	3	3	3.5	4.5	4	4	1	6	♀
	3	3	3.5	4.5	4	4	1	5	♂
	3	3	3	4	4	4	1	5	♂
	3	3	3.5	4.5	4	4	1	5	♂
Batch # 17	3	3	3	4	4	4	1	5	♂
	3	3	3	4	4	4	1	5	♂
	3	3	3	4	4	4	1	5	♂
	3	3	3	4	4	4	1	6	♀
	3	3	3.5	3.5	4.5	4	1	6	♀
	3	3	3	4	4	4	1	6	♀
	3	3	3	4	4	4	1	6	♀

Stages	Eggs	L1	L2	L3	L4	L5	Pre-Pupal	Pupa	Adult
Batch # 18	2.5	3.5	3	4.5	3.5	4	1	5	♂
	2.5	3.5	3	4.5	3.5	5	0.5	5	♂
	2.5	3.5	3	4	4	4	1	5	♀
	2.5	3.5	3	4.5	3.5	4	1	6	♂
	2.5	3.5	3	4	4	4	1	5	♂
	2.5	3.5	3	4	4	4	1	6	♀
Batch # 19	3	3.5	3.5	4	4	5.5	0.5	6	♀
	3	3.5	3.5	4	4	5	1	7	♀
	3	3.5	3.5	4	4	5	1	7	♀
	3	3.5	3.5	4	4	5	1	6	♀
	3	3.5	3.5	4	4	5	1	6	♀
	3	3.5	3.5	4	4	5	1	5	♂
Batch # 20	3	3.5	3.5	4.5	4	4.5	0.5	7	♀
	3	3.5	3.5	4	5	5	1	6	♂
	3	3.5	3.5	4	5	5	1	5	♂
	3	3.5	3.5	4	5	5	1	5	♂
	3	3.5	3.5	4.5	4	5	1	6	♀
	3	3.5	3.5	4	5	4.5	0.5	5	♂
Batch # 21	3	4	3	4.5	4.5	5	1	6	♂
	3	4	3	4	5	5	1	6	♂
	3	4	3	4.5	4.5	5	1	5	♂
	3	4	3	4	5	5	1	6	♀
	3	4	3	4	5	5	1	5	♂
	3	4	3	4.5	4.5	5	1	6	♂
Batch # 22	3	5	4	4	5	5	1	7	♀
	3	5	4	4.5	4.5	5	1	7	♀
	3	5	4	4.5	4.5	5	1	7	♀
	3	5	4	4	4	5	1	6	♂
	3	5	4	4.5	4.5	5	1	6	♀
	3	5	4	4	4	5	1	6	♂

(Chapter 3): Survival ratio of *A. terpsicore* at each stage of developmental timeline.

Cohorts	No. of eggs	eggs turned black	eggs hatched	L1	L2	L3	L4	L5	Pre- Pupa	Pupa	Adult	♂	♀
1	43	43	43	43	43	43	41	41	41	41	41	24	17
2	53	53	49	49	49	45	45	45	45	45	45	21	24
3	46	46	39	39	39	34	31	30	30	30	30	17	13
4	48	45	45	44	44	44	44	43	30	43	43	17	26
5	68	68	68	68	61	61	53	49	46	41	41	26	15
6	83	69	69	69	69	62	62	61	59	59	57	31	26
7	57	57	57	57	57	57	55	51	51	51	51	31	20
8	72	70	70	70	67	67	67	64	64	64	64	37	27
9	53	53	53	53	51	51	36	31	31	31	29	14	15
10	32	32	32	32	32	32	32	32	32	32	32	14	18
11	62	62	62	62	62	62	51	50	47	47	46	19	27
12	48	48	48	48	48	48	39	31	30	27	23	12	11
13	83	83	83	83	76	71	66	63	61	60	59	35	24
14	67	67	61	61	58	58	41	37	33	32	32	18	14
15	46	46	46	46	43	43	39	39	38	38	38	18	20
16	59	53	53	53	53	51	41	33	30	30	28	13	15
17	64	64	64	64	64	64	59	51	50	49	49	28	21
18	51	51	51	51	50	47	41	41	41	41	39	16	23
19	37	37	37	37	37	32	28	28	27	27	25	14	11
20	69	69	65	65	61	61	52	48	44	41	41	19	22
21	39	39	39	39	39	31	30	30	29	27	27	16	11
22	49	49	49	49	49	49	45	45	45	43	43	16	27
Total	1229	1204	1183	1182	1152	1113	998	943	904	899	883	456	427

Appendix II

(Chapter 6): Distributional data of *Acraea terpsicore* in Indo-Australian region (published and unpublished record)

No.	Location	Country	Author(s)/ Source	Year	Latitude	Longitude
1	Bangalore Nehru Garden,	India	Shashikumar and Venkatesha	2008	12° 58' 17.7564" N	77° 35' 40.4268" E
2	Nasik	India	Shodhganga	2007	19° 59' 50.8308" N	73° 47' 23.2872" E
3	N.W. Ghats	India	Padhye et al., Sharma and Ahmed	2013	25° 31' 57.864" N	76° 4' 25.8888" E
4	Gujrat Madhya	India	Tiple	2012	22° 58' 24.3228" N	78° 39' 24.8184" E
5	Pardesh Andhera	India	Gupta et al., Rane and Ranade	2012	15° 54' 46.44" N	79° 44' 23.9532" E
6	Pardesh	India	Arun	2004	19° 45' 5.328" N	75° 42' 49.9968" E
7	Maharashtra	India	Chrefreearth	2010	8° 26' 29.3964" N	72° 52' 39.5616" E
8	Mumbai	India	Aneesh et al.,	2012	10° 51' 1.8576" N	76° 59' 39.3972" E
9	kerala	India	Larsen	2013	21° 33' 58.9716" N	76° 16' 15.8988" E
10	Kerala	India	Ramana	2005	17° 41' 12.5376" N	73° 13' 13.8252" E
11	Dehli	India	Ramana	2010	18° 6' 34.83" N	83° 13' 6.5352" E
12	Veshakapatnam	India	Ramana	2010	18° 11' 48.7428" N	83° 5' 37.8384" E
13	Punyagiri	India	Ramana	2010	16° 59' 24.774" N	82° 59' 52.3068" E
14	Ananthagiri	India	Ramana	2010	20° 57' 5.9976" N	73° 18' 43.2828" E
15	Ratnagiri	India	Mishra et al., Alagumurugan et al.,	2010	11° 7' 37.6428" N	85° 5' 54.6864" E
16	Orrisa	India	Bawri Beas	2011	26° 12' 2.1744" N	78° 39' 24.8184" E
17	Tamilnadu	India	Chakraborty	2014	23° 35' 14.3268" N	92° 56' 15.2664" E
18	Assam Dhobigat	India	Rohit	2014	12° 44' 49.3116" N	87° 17' 32.2548" E
19	Durgapore	India	NLBIF	2013	13° 4' 57.648" N	78° 19' 58.8576" E
20	Kuppam	India	Soubadra devi	1972	11° 19' 8.5656" N	80° 16' 14.5848" E
21	Kakachi	India	Vijay Anand Vidvalagan et al.,	1999	22° 39' 57.4272" N	76° 44' 24.522" E
22	Jharkand	India	J. M. Grag	2013	11° 25' 40.9656" N	85° 37' 52.3344" E
23	Western Ghats Andhra	India	D. Tilakartana	2008	13° 26' 31.4088" N	79° 49' 20.0316" E
24	Pradesh	India	Talbot	1985	7° 8' 31.0452" N	79° 53' 39.318" E
25	Randoluwa	Sri Lanka	Nidup	1947	7° 52' 22.9944" N	80° 46' 18.4692" E
26	Gonawela	Sri lanka	Islam et al.,	2015	26° 56' 14.2944" N	90° 29' 16.7712" E
27	Sarpang Distt.	Bhutan				
28	Dhaka	Bangladesh				
29						

No.	Location	Country	Author(s)/ Source	Year	Latitude	Longitude
30	Kumaon	Nepal	Smith	1989	29° 16' 45.6636" N	79° 28' 13.4004" E
31	Mai Hong Son	Thailand	West	1996	19° 18' 7.308" N	97° 57' 55.5732" E
32	Krabi	Thailand	Olli Vesikko Y.Inayoshi	2004	8° 5' 10.68" N	98° 54' 22.6188" E
33	Phu Kha	Thailand	and K.Saito	1991	19° 5' 22.9956" N	101° 4' 2.6976" E
34	Chonburi	Thailand	Y.Shiraishi Y.Inayoshi	1990	13° 21' 40.1148" N	100° 59' 4.8192" E
35	Chang Mai	Thailand	and K.Saito	1986	18° 47' 15.8892" N	98° 59' 35.2608" E
36	Chaiyaphum	Thailand	Pinratana Yamaguchi	1996	15° 48' 24.5412" N	102° 1' 53.4108" E
37	Kaeng Tana	Thailand	and Aoki	2002	15° 17' 58.3692" N	105° 28' 36.5916" E
38	Doi Tao	Thailand	Nishimura	1994	17° 56' 39.1992" N	98° 38' 36.8124" E
39	Pua	Thailand	Nishimura	1994	19° 9' 54.4752" N	100° 54' 26.1072" E
40	Phu Sawan	Thailand	Nishimura	1994	13° 0' 48.9996" N	99° 40' 31.0008" E
41	Lamphun	Thailand	Nishimura	1994	18° 34' 28.0596" N	99° 0' 31.3992" E
42	Qui Nhon	Vietnam	Nishimura	1994	13° 46' 58.6812" N	109° 13' 10.7868" E
43	Dinh Quan	Vietnam	Nishimura	1994	11° 11' 37.554" N	107° 20' 36.5712" E
44	Phnum Kulen	Combodia	Onodera	2009	13° 34' 32.9988" N	104° 4' 30" E
45	Vientiane	Laos	Osada et al.,	1999	17° 58' 32.5416" N	102° 37' 59.1744" E
46	Perlis	Malaysia	Arshad et al.,	1996	6° 26' 41.6868" N	100° 12' 17.1684" E
47	Langkawi Island	Malaysia	Wong Tet Seng	1993	6° 22' 41.4984" N	99° 47' 24.6408" E
48	Pennag	Malaysia	Wong Tet Seng	1993	5° 21' 55.6488" N	100° 27' 32.4324" E
49	Negri Sembilan	Malaysia	Wong Tet Seng	2003	2° 43' 32.9016" N	101° 56' 32.5608" E
50	F.T, KL	Malaysia	Narong Jaturas (BOLD)	2015	3° 9' 25.0164" N	101° 42' 44.2908" E
51	Selangor	Malaysia	M. Sufian Azirun	2005	2° 51' 37.7064" N	101° 21' 56.88" E
52	Selangor	Malaysia	Gerald Lee Zhang Yang	2015	3° 2' 30.4908" N	101° 47' 35.07" E
53	Malacca Negari	Malaysia	See Saw Pang	2015	2° 19' 39.2556" N	102° 14' 57.8472" E
54	Sambilan	Malaysia	Lee See Mun NG Say	2015	2° 41' 19.2552" N	101° 54' 52.0488" E
55	Aman puri Punngol	Malaysia	chong	2015	3° 12' 49.8204" N	101° 36' 54.4824" E
56	wasteland Pulau	Singapore	Federick ho	2009	1° 21' 7.4988" N	103° 49' 11.4096" E
57	Serangoon	Singapore	Kerry Pereira	2015	1° 21' 19.2852" N	103° 52' 4.3356" E
58	Pulau Ubin	Singapore	Khew	2008	1° 24' 45.3312" N	103° 57' 28.4688" E
59	Botanic garden Kota	Singapore	Khew	2009	1° 18' 49.824" N	103° 48' 57.2904" E
60	Samarahan	Malaysia	Noor Amira	2013	1°27'10.498"N	110°27'26.83"E
61	Kuching	Malaysia	Sabina Noor	2015	1°30'34.164"N	110°21'34.149"E

No.	Location	Country	Author(s)/ Source	Year	Latitude	Longitude
62	Kuching	Malaysia	Sabina Noor	2015	1°30'54.037"N	110°20'46.286"E
63	Kuching	Malaysia	Sabina Noor	2015	131°29.474"N	11023°45.249"E
64	Samariang	Malaysia	Noor Amira	2013	1°36'29.881"N	110°19'35.883"E
65	Samariang	Malaysia	Abang. F	2014	1°35'3.962"N	110°19'28.852"E
66	Samariang	Malaysia	Noor Amira	2013	1°37'44.968"N	110°20'13.827"E
67	Assajaya	Malaysia	Noor Amira	2014	1°32'58.031"N	110°40'14.891"E
68	Serian	Malaysia	Alley Majau	2016	1°8'52.048"N	110°35'3.461"E
69	Sri Aman	Malaysia	Tham Vivian Subaraj	2014	1°19'60"N	111°40'.0"E
70	Batam	Indonesia	Rajathuri	2006	1° 2' 44.2536" N	104° 1' 49.6344" E
71	Lombok	Indonesia	Matsomoto	2012	8° 39' 3.5244" S	116° 19' 29.7984" E
72	Wagait Beach	Australia	Braby et al.,	2013	12° 26' 8.0016" S	130° 44' 53.0016" E
73	Dundee Beach	Australia	Braby et al.,	2013	12° 45' 27.1512" S	130° 23' 34.2528" E
74	Darwin	Australia	Braby et al.,	2013	12° 27' 48.384" S	130° 50' 44.3112" E
75	Herbert	Australia	Braby et al.,	2013	12° 32' 32.3484" S	131° 9' 25.8804" E
76	Adelaide River	Australia	Braby et al., King and	2013	13° 14' 30.7644" S	131° 6' 49.788" E
77	Kununurra	Australia	Daniel Hidayati	2013	15° 46' 42.7476" S	128° 44' 30.066" E
78	Banjarmasin	Indonesia	Rahima	2014	3°19'6.984"S	114°35'39.762"E
79	Mount Besar M. Land.	Indonesia	Exotic insects	2014	02°42'39"S	115°37'32.999"E
80	Regency	Indonesia	Florida et. al	2015	0°19'4.778"N	109°25'33.281"E
81	Kab. Ketapang	Indonesia	Iqbal et. al	2015	02°16'35.9"S	110°52'07.6"E
82	Kab. ketapang	Indonesia	Iqbal et. al	2015	02°01'45.3"S	110°47'43.0"E
83	Sengkuang village	Indonesia	Iqbal et. al	2015	02°20'54.1"S	110°46'49.0"E
84	Perigi village	Indonesia	Iqbal et. al	2015	0°42'30.478"N	109°30'43.203"E
85	Bukit Pelagi	Indonesia	Iqbal et. al	2015	0°30'54.5"N	117°36'28.0E
86	Jambi	Indonesia	Geoff Welch Nurul Iman	2008	0° 35' 23.0064" S	101° 20' 35.1816" E
87	Bengkulu	Indonesia	Supardi	2012	3° 47' 34.242" S	102° 15' 38.7504" E
88	Bali	Indonesia	LEPIDIGI Gita Persada	2014	8° 41' 41.0532" S	115° 15' 46.8504" E
89	Lampung	Indonesia	Bt. Park	2009	4° 33' 30.906" S	105° 24' 24.5088" E
90	Flores	Indonesia	Exotic insects	2014	8° 39' 26.5752" S	121° 4' 45.732" E
91	Moa Island	Indonesia	Exotic insects	2014	8° 13' 0.0012" S	128° 10' 59.9988" E
92	Bogor	Indonesia	Braby et al.,	2009	6° 12' 31.5468" S	106° 50' 44.1564" E
93	Baucau	Timor	Braby et al.,	2012	8° 28' 23.1636" S	126° 27' 19.3428" E
94	Sumba	Indonesia	Braby et al.,	2012	9° 41' 57.6384" S	119° 58' 26.5908" E
95	Yogyakarta, Java	Indonesia	A.Z's Lepidoptera	2012	7° 47' 44.088" S	110° 22' 10.164" E

Appendix III

(Chapter 7): Multiple sequence comparison of *COI* gene sequence between the populations of *A. terpsicore* from different geographical regions.

KUC_01	AGTTTTTGAC TTTTACCCCC CTCATTAATT TTATTAATTT CTAGAAGAAT	[50]
KUC_03		[50]
KUC_04		[50]
KUC_05		[50]
KUC_06		[50]
SER_01		[50]
SRI_01		[50]
MOA_01		[50]
KAL_01		[50]
JX226067_IND_02		[50]
KJ459777_IND_03		[50]
KT880658_IND_04		[50]
KJ459773_IND_05		[50]
KF226260_PMAL_01		[50]
KU360730_A_ISSORIA	C.T.....	[50]

KUC_01	TGTTGAAAAT GGAGCTGGTA CAGGATGAAC AGTTTACCCC CCACTCTCAT	[100]
KUC_03		[100]
KUC_04		[100]
KUC_05		[100]
KUC_06		[100]
SER_01		[100]
SRI_01		[100]
MOA_01		[100]
KAL_01		[100]
JX226067_IND_02		[100]
KJ459777_IND_03		[100]
KT880658_IND_04		[100]
KJ459773_IND_05	T....	[100]
KF226260_PMAL_01		[100]
KU360730_A_ISSORIA	TA..A. G.. C..T..C.	[100]

KUC_01	CTAACATTGC TCATGGTGGA TCATCTGTTG ATTTAGCCAT TTTTTCACCT	[150]
KUC_03		[150]
KUC_04		[150]
KUC_05		[150]
KUC_06		[150]
SER_01		[150]
SRI_01		[150]
MOA_01		[150]
KAL_01		[150]
JX226067_IND_02		[150]
KJ459777_IND_03		[150]
KT880658_IND_04		[150]
KJ459773_IND_05		[150]
KF226260_PMAL_01		[150]
KU360730_A_ISSORIA	T.... C...A.A..C ..T..A..A. T.. TT.A	[150]

KUC_01	CATTTAGCCG GAATTTTCATC TATTTTAGGA GCAATTAATT TCATTACAAC	[200]
KUC_03		[200]
KUC_04		[200]
KUC_05		[200]
KUC_06		[200]
SER_01		[200]
SRI_01		[200]
MOA_01		[200]
KAL_01		[200]
JX226067_IND_02		[200]
KJ459777_IND_03		[200]
KT880658_IND_04		[200]
KJ459773_IND_05		[200]
KF226260_PMAL_01		[200]
KU360730_A_ISSORIA	T. .T.....T.. C. .T.....	[200]

KUC_01	AATTATTAAT ATACGAGTAA ATAATCTATC TTTTGATCAA ATACCCCTTAT	[250]
KUC_03		[250]
KUC_04		[250]
KUC_05		[250]
KUC_06		[250]
SER_01		[250]
SRI_01		[250]
MOA_01		[250]
KAL_01		[250]
JX226067_IND_02		[250]
KJ459777_IND_03		[250]
KT880658_IND_04		[250]
KJ459773_IND_05	C...	[250]
KF226260_PMAL_01		[250]
KU360730_A_ISSORIA	A.T. T.... T....	[250]

KUC_01	TTGTTTGAGC AGTTGGTATC ACAGCTCTTC TTTTACTTCT ATCTTTACCA	[300]
KUC_03		[300]
KUC_04		[300]
KUC_05		[300]
KUC_06		[300]
SER_01		[300]
SRI_01		[300]
MOA_01		[300]
KAL_01		[300]
JX226067_IND_02		[300]
KJ459777_IND_03		[300]
KT880658_IND_04		[300]
KJ459773_IND_05		[300]
KF226260_PMAL_01		[300]
KU360730_A_ISSORIA	A.....T C...T .AC.C...T. ...CC....T	[300]

KUC_01	GTTTTAGCAG GAGCTATTAC TATATTATTA ACCGATCGTA ACTTAAATAC	[350]
KUC_03		[350]
KUC_04		[350]
KUC_05		[350]
KUC_06		[350]
SER_01		[350]
SRI_01		[350]
MOA_01		[350]
KAL_01		[350]
JX226067_IND_02		[350]
KJ459777_IND_03		[350]
KT880658_IND_04		[350]
KJ459773_IND_05		[350]
KF226260_PMAL_01		[350]
KU360730_A_ISSORIA	..A..... ..A.....A. .T.....	[350]

KUC_01	TTCATT	[356]
KUC_03		[356]
KUC_04		[356]
KUC_05		[356]
KUC_06		[356]
SER_01		[356]
SRI_01		[356]
MOA_01		[356]
KAL_01		[356]
JX226067_IND_02		[356]
KJ459777_IND_03		[356]
KT880658_IND_04		[356]
KJ459773_IND_05		[356]
KF226260_PMAL_01		[356]
KU360730_A_ISSORIA	...C..	[356]