DEVELOPMENT PATTERNS OF NECROPHAGOUS FLIES INFESTING RABBIT CARCASSES DECOMPOSING IN MOUNT KAPUR CAVE AND ITS SURROUNDING PRIMARY FOREST HABITATS IN KUCHING, SARAWAK

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DEDICATION

This thesis is dedicated to my late father, who taught me that the best kind of knowledge to have is that which is learned for its own sake. It is also dedicated to my cherished mother, who taught me that even the largest task can be accomplished if it is done one step at a time. Last but not least, my dearest husband, who is also my best friend, who was always there with me through thick and thin

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ABSTRACT

Forensic entomological baseline data on the oviposition of necrophagous insects and completion of their life cycles in the Borneo region, as well as in secluded areas like caves remain unreported. Since entomological baseline data can differ from one biogeoclimatic region to another, the lack such data would limit the practical values of applying entomological evidence in estimating minimum postmortem interval (mPMI). Therefore, this present research that investigated the oviposition and completion of life cycles of necrophagous flies infesting rabbit carcasses decomposing in Mount Kapur Cave and its surrounding forest habitat in Kuching, Sarawak merits consideration. In general, 13 taxa of necrophagous flies were identified viz. *Hypopygiopsis violacea*, *Hypopygiopsis. fumipennis*, *Hemipyrellia ligurriens*, *Hemipyrellia tagaliana*, *Chrysomya megacephala*, *Chrysomya villeneuvi*, *Chrysomya rufifacies*, *Chrysomya chani*, *Chrysomya pinguis*, *Chrysomya nigripes*, *Ophyra spinigera* and *Ophyra chalcogaster*, as well as unidentified Sarcophagidae. While all these necrophagous flies were observed infesting carcasses in Mount Kapur Cave, *Hem. ligurriens* and *Hem. tagaliana* were not found infesting carcasses in the surrounding forest habitat. In addition, *Hyp. violacea*, *Hyp. fumipennis* were the two earlier necrophagous flies that oviposited in all rabbit carcasses decomposing in both habitats. Complete life cycles for six and five different necrophagous fly species were successfully observed in Mount Kapur Cave and its surrounding forest habitat, respectively. Significant delay in oviposition, as well as longer durations for completing the life cycles in several necrophagous fly species were observed in Mount Kapur Cave when compared to those of surrounding forest habitat ($p < 0.05$). These data deserve consideration as the first ever forensic empirical baseline data on oviposition and completion of life cycles for necrophagous flies in Sarawak, as well as in a cave habitat, in view of its practical values for estimating mPMI for forensic practical caseworks.

ABSTRAK

Data dasar entomologi forensik berkenaan pengovipositan serangga nekrofaj dan pelengkapan kitar hidupnya di wilayah Borneo serta kawasan terpencil misalnya gua tidak pernah dilaporkan. Memandangkan data dasar entomologi boleh berbeza dari satu wilayah biogeoklimatik dengan satu yang lain, ketiadaan data berkenaan akan menghalang nilai praktikal menggunakan bukti entomologi dalam menganggarkan selang kematian minimum (mPMI). Oleh itu, kajian ini yang mengkaji pengovipositan dan pelengkapan kitar hidup lalat nekrofaj yang menginfestasi bangkai arnab yang mereput di dalam Gua Gunung Kapur dan habitat hutan sekitarnya di Kuching, Sarawak wajar dipertimbangkan. Umumnya, 13 taksa lalat nekrofaj telah dikenalpasti yakni *Hypopygiopsis violacea*, *Hypopygiopsis. fumipennis*, *Hemipyrellia ligurriens*, *Hemipyrellia tagaliana*, *Chrysomya megacephala*, *Chrysomya villeneuvi*, *Chrysomya rufifacies*, *Chrysomya chani*, *Chrysomya pinguis*, *Chrysomya nigripes*, *Ophyra spinigera* dan *Ophyra chalcogaster*, serta Sarcophagidae yang tidak dapat dikenalpasti. Sementara kesemua lalat nekrofaj ini dapat diperhatikan menginfestasi bangkai di dalam Gua Gunung Kapur, *Hem. ligurriens* dan *Hem. tagaliana* tidak dijumpai menginfestasi bangkai di habitat hutan sekitarnya. Tambahan lagi, *Hyp. violacea* dan *Hyp. fumipennis* merupakan dua lalat nekrofaj terawal yang mengoviposit pada kesemua bangkai arnab yang mereput di kedua-dua habitat. Kitar hidup lengkap bagi enam dan lima spesies lalat nekrofaj berjaya diperhatikan masing-masing di dalam Gua Gunung Kapur dan habitat hutan sekitarnya. Penundaan mengovipositan serta pemanjangan tempoh yang signifikan bagi melengkapkan kitar hidup beberapa spesies lalat nekrofaj dapat diperhatikan di dalam Gua Gunung Kapur berbanding dengan habitat hutan sekitarnya ($p < 0.05$). Data ini wajar dipertimbangkan sebagai data dasar empirikal pertama berkenaan pengovipositan dan pelengkapan kitar hidup lalat nekrofaj di Sarawak serta di dalam habitat gua, memandangkan nilai praktikalnya untuk menganggarkan mPMI bagi kerja kes forensik.

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LIST OF ABBREVIATIONS

ADD	-	Accumulated degree days
ADH	-	Accumulated degree hour
ANOVA	-	Analysis of Variance
a.m.	-	*Ante meridiem*
C	-	*Chrysomya*
CGS	-	Crow-Glassman Scale
CO1	-	Cytochrome oxidae 1
CO11	-	Cytochrome oxidase 2
E	-	East
GPS	-	Global positioning system
h	-	Hour
Hyp.	-	*Hypopygiopsis*
Hem.	-	*Hemipyrellia*
IBM	-	International Business Machine
KOH	-	Potassium hydroxide
mPMI	-	Minimum postmortem interval
n	-	Sample size/ replicate
N	-	North
Nov	-	November
O.	-	*Ophyra*
Oct	-	October
p	-	Probability
p.m.	-	*Post meridiem*
PMI	-	Postmortem Interval
Sept	-	September
UTM	-	Universiti Teknologi Malaysia

LIST OF SYMBOLS

°C	-	Degree Celcius
cm	-	centimeter
kg	-	Kilogram
m	-	meter
mL	-	Milliliter
mm	-	Milimeter

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CHAPTER 1

INTRODUCTION

1.1 Problem Background

Forensic entomology deals with insect evidence in the investigation of homicide, suicide and suspicious deaths, as well as urban and stored-products for the purpose of law (Rivers and Dahlem, 2014). Moreover, insect evidence may prove useful in investigating cases of human negligence and animal welfare (Gunn 2009). In addition to its major contribution in the estimation of postmortem interval (PMI), forensic entomological evidence has also been envisaged to answer forensic questions such as the possible cause of death (Gennard, 2012), relocation of human body and secondary disposal (Byrd and Castner, 2010), as well as human identification (Mahat and Jayaprakash, 2013). Pertinently, beyond 72 hours after death, pathological changes (e.g. algor mortis and rigor mortis) might not be able to assist forensic pathologists in determining time of death due to the advanced stages of decomposition (Gennard, 2012). In such an instance, aspects pertaining to forensic entomology may be an available means for answering such an important forensic question (Mahat and Jayaprakash, 2013), proving or disproving one's alibi (Denis et al., 2018).

While estimation of the minimum PMI (mPMI) can be done via assessing the growth pattern of the oldest necrophagous insects (e.g. the thermal summation approach), interpretation of the insect succession and composition data may be more appropriate for bodies recovered at extended period (weeks or months) after death (Rivers and Dahlem, 2014). In this context, it is pertinent to indicate that the interpretation of entomological evidence relies largely on understanding the oviposition and developmental patterns of necrophagous insects (Amendt et al., 2004) which vary according to variations in biogeoclimatic factors (Goff, 2009a) as well as presence of poisons and/or drugs (Mahat et al., 2009; Anderson, 2010). Being poikilotherms, necrophagous insects exhibit holometabolous type of growth with

varying appearances among the different stages of development, dependent on the external source of heat for maintaining the physiological and biochemical processes (Gennard, 2012; Rivers and Dahlem, 2014). Therefore, ambient temperature has been widely reported in the literature as the major factor influencing the duration for completing its life cycle, especially in countries with defined seasonal variations such as summer and winter (Sharanowski et al., 2008; Wells and Lamotte, 2010). Considering that ambient temperature remains largely similar throughout the year in tropical countries like Malaysia, the influence of temperature on insects' development may not be as huge as in temperate countries; other factors such as rainfall (Mahat et al., 2009) may play a bigger role in this forensic context. Therefore, empirical baseline data established for one biogeoclimatic region may not be construed as applicable for estimating PMI in other regions (Wells and Stevens, 2008), emphasizing the importance for replicating forensic entomological studies in various locations and habitats.

Unfortunately, the available forensic entomological data in Malaysia are confined to only several states within Peninsular with limited available data reported from East Malaysia (Sarawak and Sabah) despite being perceived as ecologically richer in fauna (Mahat and Jayaprakash, 2013). The scarcity of forensic entomological data in Sarawak and Sabah would limit the applicability of entomological evidence to be used in death investigation, taking into account that these two states constitute the largest mass of land in Malaysia. Furthermore, because any factors that can mitigate the development of insects have the potential of affecting subsequent insect-based estimates of PMI (Goff and Lord, 2010), continuous efforts to provide empirical evidence on influence of various habitats (e.g. cave and forest) on necrophagous insects appear justifiable.

1.2 Problem Statement

In Malaysia, application of entomological evidence is rapidly gaining popularity for forensic practice (Syamsa et al., 2015). Despite the substantial efforts put forth in forensic entomological studies within Peninsular Malaysia, it is evident

that such efforts remain geographically imbalanced with no mention of data originating from East Malaysia (Sarawak and Sabah) (Mahat and Jayaprakash, 2013). Considering that Sarawak and Sabah are (a) geographically separated by the South China Sea (Figure 1.1), (b) consisted of a larger land area than that of Peninsular Malaysia and (c) perceived as more diverse ecologically in its fauna; forensic entomological studies in that region remain generally unexplored (Mahat and Jayaprakash, 2013). In view of the indication made by Anderson (2010) that data generated in one biogeoclimatic zone should not be regarded as the same for a different region, specific study focusing on developing empirical baseline data for Sarawak acquires forensic significance. Therefore, this present study focusing on the developmental and successional patterns of necrophagous flies infesting rabbit carcasses in two different habitats (*viz.* cave and the surrounding primary forest) in Kuching, Sarawak would pave the way for scientific application of forensic entomology parameters for estimating PMI in that region.

Figure 1.1 Distribution of forensic entomological studies in Malaysia (1991–2015)[1]

[1] Image modified from Google Maps.

1.3 Objectives and Research Hypothesis

Using rabbit carcasses as animal models decomposing in a cave and the surrounding primary forest habitats in Kuching, Sarawak, this present study was designed to:

1. Investigate species of necrophagous flies in both habitats.

2. Compare the durations for completing life cycles for necrophagous flies in both habitats.

It is hypothesized that statistically significant differences would be observed in species compositions and durations for completing life cycles for necrophagous flies infesting rabbit carcasses in both habitats. Marked variations in compositions and durations for completing life cycles were also expected from those reported in Peninsular Malaysia, triggering the needs for establishing separate baseline data for estimating PMI in that region.

1.4 Scopes of Study

This present study focused on species composition and durations for completing life cycles for necrophagous flies infesting rabbit carcasses decomposing in Mount Kapur Cave (1° 22’ 50.88” N, 110° 7’ 10.46” E) in Kuching Division, Sarawak and its surrounding primary forest (1° 22’ 56.44” N, 110° 6’ 57.42” E). Documentation of the ambient and carcass surface temperatures, relative humidity, total daily rainfall, light intensity (in cave only), presence of adult and immature of necrophagous insects as well as the decomposition process per se will also be made. Putting this research scope in forensic perspective for Malaysia, the theoretical framework of this present research is presented in Figure 1.2.

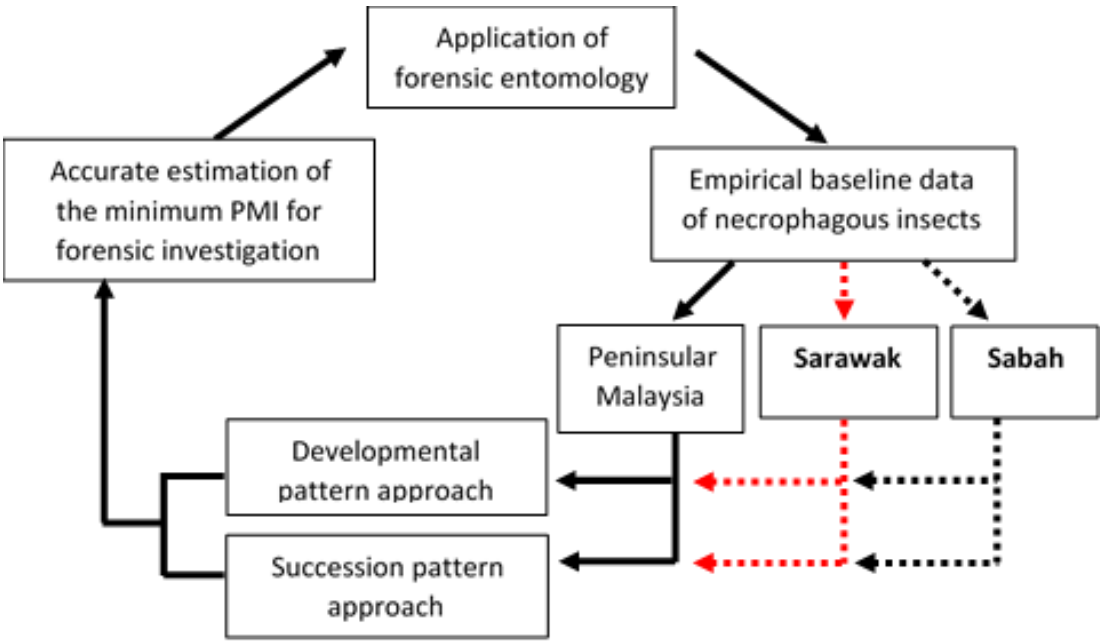

Figure 1.2 Research theoretical framework[2]

1.5 Significance of Study

The data gathered here would elucidate for the first time, the species of necrophagous flies infesting rabbit carcasses decomposing in different habitats in Kuching, Sarawak as well as completion of their life cycles, in view of its practical values for estimating PMI as well as paving the way for scientific application of forensic entomology parameters in Borneo region.

[2] Dotted lines represent the incomplete forensic entomology empirical baseline data in Malaysia with red ones indicating in this present research.

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