STUDIES OF FORENSICALLY IMPORTANT FLIES OF CALLIPHORIDAE AND SARCOPHAGIDAE IN MALAYSIA: MORPHOLOGICAL TAXONOMY, GEOGRAPHICAL AND ECOLOGICAL DISTRIBUTION, SPECIES SUCCESSION ON CARCASSES, AND DNA-BASED IDENTIFICATION

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Abstract

In forensic entomology, knowledge of insect taxonomy, development, behaviour and ecology is required to allow accurate data interpretation from entomological evidence in investigations. In this thesis, five forensic entomology related studies have been carried out and discussed in two Malaysian forensically important fly families, the Calliphoridae and Sarcophagidae. These studies involve the taxonomy, species checklist, ecological distribution, dipteran succession pattern on pig carcasses in Sarawak and DNA-based species identification analysis.

A detailed taxonomical study of Sarcophagidae encompasses the examination of external morphological characters, and their terminology was constructed. Several important taxonomical characters were identified and their usefulness in establishing taxonomical key was evaluated. Knowledge of basic anatomy is important when inferring relationships among the sarcophagid flies.

The ecological distribution study of Calliphoridae and Sarcophagidae explores whether certain species are more associated with particular ecological habitats (e.g. urban, forest, swamp, etc.). Environmental influences (e.g. habitats and elevations) are discussed with regard to the presence of fly species. Habitat-specific species were also proposed. Understanding the distribution and ecology of these fly species may facilitate criminal investigations, especially in determining the location of the first crime scene. Species checklists of Calliphoridae and Sarcophagidae were also obtained.

Rates and stages of decomposition of two scavenged and clothed pig carcasses were studied in a tropical rainforest in Kuching, Sarawak, Malaysia. Comparisons between these two carcasses revealed that the scavenged carcass decomposed faster than the clothed one. Some important fly species were proposed to serve as the time (e.g. decomposition stage) and location (e.g. habitat) indicators. Diptera species were

significantly different between these two pig carcasses due to differences in physical condition of the carcasses.

The efficacy of DNA-based identification of forensically important fly Calliphoridae species in Malaysia was evaluated using two genetics markers, the *cytochrome c oxidase* subunit I and II as well as 28S ribosomal DNA. Evolution and speciation of *Chrysomya defixa* and *Chrysomya pinguis*, such as incomplete lineage sorting, introgressive hybridisation, and ancestral polymorphism were also discussed. PCR-RFLP analysis of *cytochrome c oxidase* subunit I was also employed to facilitate species identification of *Chrysomya* in Malaysia.

Similar DNA-based study was conducted for Malaysian Sarcophagidae. However, only *cytochrome c oxidase* subunit I and II was included for the DNA barcoding analysis of 49 sarcophagid species. From the phylogeny, almost all species of Sarcophagidae were monophyletic except for *Boettcherisca javanica*, which provide strong evidence for proposal of new combination of genera, such as *Rosellea notabilis, Pseudothyrsocnema borneensis*, *Bellieriomima globovesica* and *Bellieriomima uniseta*.

Abstrak

Dalam bidang entomologi forensik, pengetahuan tentang taksonomi serangga, pembangunan, perilaku dan ekologi diperlukan untuk membenarkan tafsiran data yang tepat daripada bukti berhubung dengan serangga dalam penyiasatan. Dalam tesis ini, lima kajian berkaitan entomologi forensik telah dilakukan dan dibincangkan dengan menggunakan spesies daripada dua famili lalat Malaysia yang memainkan peranan yang penting dalam forensik, iaitu Calliphoridae dan Sarcophagidae. Kajian ini melibatkan taksonomi, senarai spesies, taburan ekologi, corak turutan diptera atas bangkai babi di Sarawak dan analisa pengenalan spesies berasaskan DNA.

Satu kajian taksonomi terperinci tentang Sarcophagidae meliputi pemeriksaan watak morfologi luaran dan terminology telah dilakukan. Beberapa watak taksonomi penting dikenalpasti dan kegunaannya dalam membina kunci taksonomi juga dinilai. Pengetahuan tentang anatomi asas adalah penting untuk menjelaskan dan merumuskan hubungan antara lalat-lalat sarcophagid.

Kajian taburan ekologi Calliphoridae dan Sarcophagidae, menjelajah jenis spesies lalat berkaitan dengan habitat ekologi khusus (seperti bandar, hutan, paya, dan lain-lain). Pengaruh persekitaran (seperti habitat dan altitud) dibahas berkaitan dengan kehadiran jenis lalat. Spesies khusus kepada habitat juga dicadangkan. Dengan memahami taburan dan ekologi lalat dapat memudahkan penyiasatan jenayah, terutama dalam menentukan tempat kejadian pertama bagi sebuah jenayah. Senarai spesies bagi Calliphoridae dan Sarcophagidae juga diperolehi.

Kadar dan peringkat pereputan dua bangkai babi - memulung dan berpakaian, dikaji di hutan hujan tropika di Kuching, Sarawak, Malaysia. Perbandingan antara kedua-dua bangkai menunjukkan bahawa bangkai memulung reput dengan lebih cepat daripada yang berpakaian. Beberapa spesies lalat yang penting dicadangkan sebagai penunjuk waktu (contohnya peringkat pereputan) dan lokasi (contohnya habitat).

Diptera spesies yang berbeza secara nyata antara dua bangkai babi adalah disebabkan oleh perbezaan dalam keadaan fizikal bangkai.

Keberkesanan teknik berasaskan DNA untuk pengenalan spesies lalat forensik yang penting di Malaysia – Calliphoridae, dianalisis dengan menggunakan dua penanda genetik, *cythochrome oksidase* subunit I dan II serta 28S ribosomal DNA. Evolusi dan spesiasi daripada *Chrysomya defixa* and *Chrysomya pinguis*, seperti "incomplete lineage sorting", "introgressive hybridization" dan "ancestral polymorphism" juga dibincangkan. Analisis PCR-RFLP dalam *cytochrome c oksidase* subunit I juga digunakan untuk memudahkan pengenalan spesies *Chrysomya* di Malaysia.

Kajian berasaskan DNA yang serupa telah dilakukan untuk Sarcophagidae Malaysia. Namun, hanya *cytochrome c oksidase* subunit I dan II dijalankan untuk analisis DNA barcode kepada 49 spesies sarcophagid. Dari filogeni, hampir semua jenis Sarcophagidae spesies adalah monofiletik kecuali *Boettcherisca javanica*, di mana memberikan bukti yang kuat untuk mencadangkan kombinasi baru untuk genus tertentu, seperti *Rosellea notabilis, Pseudothyrsocnema borneensis, Bellieriomima globovesica* and *Bellieriomima uniseta*.

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Abbreviations

A	adenine
AT	adenine and thymine
ACCTRAN	Accelerated transformation
AIC	Akaike Information Criterion
bp	base pair
C	cytosine
CA	California
cm	centimetre
COI	*cytochrome c oxidase* subunit I
COII	*cytochrome c oxidase* subunit II
COI+II	*cytochrome c oxidase* subunits I and II
CR	control region
DNA	deoxyribonucleic acid
dNTP	deoxyribonucleoside triphosphate
Dr.	Doctor
E	east
e.g.	*exempli gratia*
et al.	*et alia*
etc.	*et cetera*
G	gamma distribution, Γ
G	guanine
GmbH	Gesellschaft mit beschränkter Haftung (company with limited liability)
GPS	Global Positioning System
GTR	general time reversible
H	height

I	invariable sites
i.e.	*id est*
ISSR	inter simple sequence repeat
ITS	internal transcribed spacer
ITS1 and 2	internal transcribed spacer 1 and 2
ITS2	internal transcribed spacer 2
kb	kilo base
kg	kilogram
L	length
L.	Linnaeus
LSU	large subunit
m	metre
max	maximum
MEGA	Molecular Evolutionary Genetics Analysis
$MgCl_2$	magnesium chloride
mg/ml	milligram per millilitre
min	minimum
ml	millilitre
mm	millimetre
mM	millimolar
MP	maximum parsimony
mtDNA	mitochondrial DNA
N	north
N/A	not available
ND4	*NADH dehydrogenase* subunits 4
ND5	*NADH dehydrogenase* subunits 5

ng	nanogram
NJ	neighbour-joining
nst	number of substitution type
nuDNA	nuclear DNA
PCR	polymerase chain reaction
PCR-RFLP	polymerase chain reaction-restriction fragment length polymorphism
pers. comm.	personal communication
pH	potential hydrogen
PMI	post-mortem interval
RFLP	restriction fragment length polymorphism
RNA	ribonucleic acid
rDNA	ribosomal DNA
rRNA	ribosomal RNA
Sdn. Bhd.	Sendirian Berhad
sp.	species (in singular)
spp.	species (in plural)
sp. nov.	*species nova*
s. lat.	*sensu lato*
T	thymine
TBE	Tris/Borate/EDTA
TBR	tree bisection–reconnection
™	trademark
tRNA	transfer ribonucleic acid
tRNA-leu	transfer ribonucleic acid leucine
TVM	transversional model
UK	United Kingdom

USA	United States of America
vs	versus
W	width
10^{th}	tenth
13^{th}	thirteenth
17^{th}	seventeenth
20^{th}	twentieth
100^{th}	hundredth
12S	12 Svedberg
18S	18 Svedberg
28S	28 Svedberg
µl	microlitre
µM	micromolar
°C	degree Celsius
%	percent
&	and
=	equal to
>	more than
<	less than
~	approximately
-	to
↓	cleavage site
®	registered
$\times g$	earth’s gravitational acceleration