DNA BARCODING OF MEDICALLY-IMPORTANT ARTHROPODS INCLUDING MOLECULAR DETECTION OF ASSOCIATED POTENTIAL PATHOGENS IN HEAD LICE

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ABSTRACT

Ectoparasitic infestation of humans is of particular interest because some species can act as vectors of pathogens resulting in wide-ranging pathogenic effects. Identification of ectoparasites using morphological keys is not applicable in some instances, especially if the ectoparasite specimen is damaged or in an immature state of development. DNA barcoding serves as an alternative technique to identify ectoparasite specimens to species by using a fragment of cytochrome c oxidase subunit I (*COI*) mitochondrial gene as an identification key. Similarly, isolation of pathogens from ectoparasites is tedious and time-consuming, therefore a molecular approach is preferred as it offers rapidity, specificity and sensitivity. The main objective of this thesis is to determine the genetic diversity of medically-important ectoparasites and their associated pathogens in welfare homes from two different geographical areas of Peninsular Malaysia. People living in welfare homesare prone to ectoparasitic infestation as they live in densely packed institutions, often with unhygienic practices, therefore identification of ectoparasite species and any pathogens these ectoparasites might harbour is crucial to avoid transmission of diseases among occupants. The identification of arthropod specimens, submitted to the Department of Parasitology,in medical case reports was also attempted and discussed. A total of 900 head lice and 26 bedbugs were collected from 15 welfare homes across Greater Kuala Lumpur/Klang Valley (KL/KV) and 832 head lice were collected from 10 welfare homes across Kedah. Pediculosis infestation rates ranging from 13.0% to 100% and 34.3% to 100% in Greater KL/KV and Kedah, respectively. DNA barcoding identified the head lice, *Pediculus humanus capitis* collected from both areas as belonging to three clades corresponding with three Barcode Index Numbers (BINs) in the Barcode of Life Datasystems (BOLD): Clade A (41%) (=BOLD: AAA1556), Clade B (2%) (= BOLD: AAA1557) and Clade D (57%) (=BOLD:AAW5034). Nine welfare homes in Greater

KL/KV and seven welfare homes in Kedah had head lice from multiple clades. Head lice of Clade B and Clade D were found living on the same human head at Pusat Jagaan Nuri welfare home in Kuala Lumpur. DNA from *Acinetobacter* spp. was detected in 52 (20%) head lice belonging to clade A and D and were identified as *Acinetobacter guillouiae* (8.5%), *Acinetobacter junii* (6.2%), *Acinetobacter baumannii* (3.8%), and *Acinetobacter nosocomialis* (1.5%). In addition, DNA from *Serratia marcescens* was detected in five (1.9%) head lice and DNA from *Staphylococcus aureus* was detected in 20 (7.7%) head lice. DNA barcoding confirmed the bedbugs collected from a single welfare home in Kuala Lumpur as being *Cimex hemipterus* (BOLD ID:BBCH001-16). DNA barcoding identified arthropod specimens presented to the Department of Parasitology in three medical case reports as ticks of the genus *Dermacentor*, larvae of filter fly *Clogmia albipunctatus* and larvae of cigarette beetle *Lasioderma serricorne*. This is the first report on the genetic diversity of head lice in Malaysia through DNA barcoding; as well as the first to provide molecular evidence on the type of bacteria occurring in head lice, suggesting potential transmission of these pathogens to Malaysian populations. The data obtained provide fundamental data so that necessary planning, funding and control measures can be undertaken by the health authorities to prevent the occurrence of head lice infestations in welfare homes. It is anticipated that the DNA barcoding technique used in this study is able to provide rapid and accurate identification of arthropods especially of the medically-important ones.

ABSTRAK

Infestasi ektoparasit mendapat perhatian kerana sesetengah spesies boleh bertindak sebagai vektor kepada patogen yang akan menyebabkan pelbagai kesan patogenik kepada manusia. Identifikasi ektoparasit menggunakan teknik kunci morfologi tidak boleh dipraktikkan dalam situasi tertentu terutamanya apabila spesimen telah rosak atau dalam keadaan pramatang. Pembarkodan DNA berfungsi sebagai teknik alternatif untuk mengenalpasti spesies spesimen ektoparasit dengan menggunakan fragmen gen mitokondria sitokrom c oksidase subunit I (*COI*) sebagai kunci pengenalan. Begitu juga dalam proses pengasingan patogen daripada ektoparasit yang rumit dan memakan masa, pendekatan molekular lebih digemari kerana ia lebih cepat, spesifik dan sensitif. Objektif utama tesis ini adalah untuk menentukan kepelbagaian genetik ektoparasit berkepentingan dalam bidang perubatan dan patogen bawaan ektoparasit di rumah-rumah kebajikan yang terletak di dua kawasan geografi berbeza di Semenanjung Malaysia. Penghuni rumah-rumah kebajikan terdedah kepada infestasi ektoparasit kerana mereka tinggal di kawasan yang padat dan kurang bersih, oleh itu pengenalpastian spesies ektoparasit dan patogen bawaan ektoparasit adalah penting untuk mengelakkan penyebaran penyakit dalam kalangan penghuni. Spesimen artropod daripada kes-kes perubatan yang dihantar ke Jabatan Parasitologi untuk dikenalpasti turut dibincangkan dalam tesis ini. Sejumlah 900 kutu kepala dan 26 pepijat telah dikumpul dari 15 rumah kebajikan di sekitar Kuala Lumpur/Lembah Klang dan 832 kutu kepala dikumpul dari 10 rumah kebajikan di sekitar Kedah. Kadar jangkitan kutu kepala bermula daripada 13.0% hingga 100% di Kuala Lumpur/Lembah Klang dan daripada 34.3% hingga 100% di Kedah. Pembarkodan DNA telah mengenalpasti kutu kepala spesies *Pediculus humanus capitis* daripada kedua-dua kawasan terbahagi kepada tiga klad yang berasal daripada tiga Barcode Index Numbers (BINs) di dalam Barcode

of Life Datasystems (BOLD): klad A (41%) (=BOLD: AAA1556), klad B (2%) (=BOLD: AAA1557) dan klad D (57%) (=BOLD: AAW5034). Sembilan rumah kebajikan di Kuala Lumpur/Lembah Klang dan tujuh di Kedah terdiri daripada pelbagai klad kutu kepala. Kutu kepala daripada klad B dan D dikenalpasti daripada seorang individu di Pusat Jagaan Nuri yang terletak di Kuala Lumpur. DNA *Acinetobacter* spp. telah dikesan di dalam 52 (20%) kutu kepala daripada klad A dan D; dikenalpasti sebagai *Acinetobacter guillouiae* (8.5%), *Acinetobacter junii* (6.2%), *Acinetobacter baumannii* (3.8%) dan *Acinetobacter nosocomialis* (1.5%). Selain itu, DNA *Staphylococcus aureus* juga dikesan dalam 20 (7.7%) kutu kepala dan DNA *Serratia marcescens* dikesan dalam lima (1.9%) kutu kepala. Melalui teknik pembarkodan DNA, pepijat yang dikumpul dari sebuah rumah kebajikan di Kuala Lumpur dikenalpasti sebagai *Cimex hemipterus*(BOLD ID: BBCH001-16). Pembarkodan DNA juga telah mengenalpasti spesimen artropod daripada kes-kes perubatan sebagai sengkenit daripada genus *Dermacentor*, larva lalat *Clogmia albipunctatus* dan lundi kumbang *Lasioderma serricorne*. Ini adalah penemuan pertama bagi diversiti genetik kutu kepala di Malaysia melalui pembarkodan DNA, juga yang pertama menunjukkan bukti molekular tentang jenis-jenis bakteria yang dibawa oleh kutu kepala. Penemuan ini mencadangkan potensi pemindahan patogen-patogen tersebut dalam populasi rakyat Malaysia. Data yang diperoleh akan menjadi penanda aras dan rujukan untuk tindakan susulan oleh pihak berkuasa kesihatan untuk merancang kaedah pengawalan jangkitan kutu kepala di rumah-rumah kebajikan. Teknik pembarkodan DNA yang dipraktikkan dalam kajian ini dijangka dapat mengidentifikasi artropod berkepentingan perubatan secara cepat dan tepat.

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LIST OF SYMBOLS AND ABBREVIATIONS

-	minus
<	less than
%	percent
®	Registered
°C	degree Celsius
µl	microlitre
µM	micromolar
µm	micrometre
ABGD	Automatic Barcode Gap Discovery
BIN	Barcode Index Number
BOLD	Barcode of Life Data Systems
bp	base pair
COI	cytochrome c oxidase subunit 1
DNA	deoxyribonucleic acid
EDTA	ethylene diamine tetraacetic acid
et al.	et alia (Latin), and others
x *g*	times gravity (relative centrifugal force)
gltA	citrate synthase gene
h	hour
HBV	Hepatitis B virus
ICU	intensive care unit
IMR	Institute for Medical Research
ITS	internal transcribed spacer
kdr	knockdown resistance gene

kg	kilogram
K2P	Kimura 2-parameter
LBRF	louse-borne relapsing fever
mg	milligram
mm	millimetre
mmHg	millimetre of mercury
MRSA	methicillin-resistant *Staphylococcus aureus*
NJ	Neigbor-Joining
PCR	polymerase chain reaction
recA	recombinant protein A
ribC	riboflavin C gene
rpoB	rRNA polymerase beta-subunit encoding gene
rRNA	ribosomal ribonucleic acid
SFG	spotted fever group
sp.	species
spp.	species
Taq	*Thermus aquaticus*
TG	typhus group
™	Trade Mark
UPC	Universal Product Code
UV	ultraviolet
V	voltage
VRE	vancomycin-resistant *Enterococcus faecium*
FDA	Food and Drug Administration

CHAPTER 1: INTRODUCTION

1.1 Research Background

DNA barcoding refers to a molecular technique that utilizes a short, standardized sequences of the cytochrome c oxidase subunit I (*COI*) mitochondrial gene termed as 'DNA barcode' (Hebert et al., 2003) to characterize biological specimens, in a same manner a scanner distinguishes commercial products using the Universal Product Code (UPC) labels (Kress & Erickson, 2012). The fact that *COI* has much less variance within species than it does between species (Batovska et al., 2016) makes *COI* DNA barcoding as a prominent tool for species-level identification of medically important arthropods as it can provide better resolution of deeper taxonomic affinities than other molecular markers, thus could potentially provide insights into patterns of molecular evolution and population genetics (Min & Hickey, 2007). Ectoparasites are arthropods or helminths that infest the skin or hair of other animals, from which they derive sustenance and shelter (Maguire & Spielman, 1998). In human medicine, the most prominent medically important arthropods are arachnids (including mites and ticks), insects (including lice, fleas, bedbugs and flies), pentastomes, and leeches (Goddard, 2006; Maguire & Spielman, 1998). Some ectoparasites also act as vectors of protozoa, bacteria, viruses, cestodes and nematodes (Wall, 2007), thus increasing the risk of pathogen transmission to humans. The prevalence of ectoparasitic infestations in humans is determined by various factors; with overcrowding and lack of hygiene playing major roles. Welfare homes, particularly those sheltering children, are susceptible to ectoparasitic infestations such as scabies, body and head lice infestations because they live in densely packed institutions, often with unhygienic practices.

Scabies caused by the human itch mite *Sarcoptes scabiei* is one of the most common causes of itching dermatoses throughout the world (Maguire & Spielman, 1998). The wounds may be subjected to secondary infestation or bacterial infection.

Louse-borne diseases can be associated with high incidence of morbidity and mortality, especially epidemic typhus and relapsing fever (Fournier et al., 2002). The body louse, *Pediculus humanus humanus* is a strict human parasite that harbour three pathogenic bacteria: *Borrelia recurrentis*, the agent of relapsing fever;*Bartonella quintana*, the agent of bacillary angiomatosis bacteremia, trench fever, endocarditis, and chronic lymphadenopathy; and *Rickettsia prowazekii*, the agent of epidemic typhus(Raoult & Roux, 1999). Head lice infestation by *Pediculus humanus capitis* is prevalent in all countries, and outbreaks have been described at all levels in society (Hansen, 2004; Raoult & Roux, 1999). Despite the claim that pediculosis capitis is not a major health problem, several studies have reported the presence of *B. recurrentis*(Boutellis et al., 2013a), *B. quintana*(Angelakis et al., 2011b; Bonilla et al., 2009; Sasaki et al., 2006) and *Acinetobacter baumannii*in head lice (Bouvresse et al., 2011; Kempf et al., 2012; Sunantaraporn et al., 2015). Secondary bacterial infection following head lice infestation can also occur and complicate the clinical scenario of pediculosis capitis (Madke & Khopkar, 2012). In addition to lice and mites infestations in humans, bedbug bites by two cosmopolitan species, *Cimex lectularius* in temperate zones and *Cimex hemipterus* in tropical regions, can cause dermatological reactions. These two species were postulated to transmit pathogens to humans, including *Coxiella burnetii*, *Aspergillus* spp., *Trypanosoma cruzi* and *Hepatitis B*virus (HBV) (Delaunay et al., 2011).

In Malaysia, scabies and head lice infestations are the two most reported cases among primary school children and children in welfare homes across states in Malaysia. These include reports by Sinniah et al.(1983) Jamaiah et al. (2000), Bachok et al. (2006), and Muhammad-Zayyid et al. (2010). The prevalence of scabies and head lice has also been reported among students of boarding schools in Sarawak (Yap et al., 2010). Despite numerous reports, data on the genetic diversity of head lice and

molecular evidence of pathogenic bacteria the head lice might harbour have not been investigated.

This study is the first to describe genetic variations of head lice collected from welfare homes in two geographical regions of Malaysia by DNA barcoding; as well as the first to provide molecular evidence of pathogens occurring in head lice. In addition, DNA barcoding is also employed to identify arthropods of medical importance reviewed by the Department of Parasitology, Faculty of Medicine, University of Malaya. These *COI* barcoding-identified specimens; the *Dermacentor* ticks, the larvae of *Clogmia albipunctatus* and *Lasioderma serricorne* had caused intra-aural and ocular infestations, intestinal myiasis and canthariasis, respectively, in humans.

1.2 Objectives

i. To identify the occurrence of medically-important ectoparasites in welfare homes sheltering underprivileged children

ii. To determine the genetic diversity of head lice and bedbugs collected from the welfare homes through DNA barcoding

iii. To investigate the occurrence and prevalence of potential pathogens potentially transmitted by ectoparasites in welfare homes

Figure 1: The incorporation of *COI* DNA barcoding to determine the genetic diversity of ectoparasites collected from welfare homes and identification of unknown arthropod specimens from medical case reports.

CHAPTER 2: LITERATURE REVIEW

2.1 DNAbarcoding

2.1.1 Principles of DNA barcoding

DNA barcoding refers to the technique of sequencing a short fragment (between 400 and 800 base pair long) of the mitochondrial cytochrome c oxidase subunit I (*COI*) gene, termed as "DNA barcode", from a taxonomically unknown specimen and performing comparisons with a reference library of sequences of known species origin which are available in the Barcode of Life Data Systems (BOLD) (Ratnasingham & Hebert, 2007) in order to establish a species-level identification (Wilson, 2010).

DNA barcoding process, first proposed by Hebert et al. (2003), entails two basic steps: (1) taxonomic experts build the DNA barcode library of known species to serve as reference data and (2) users match their generated DNA barcode of the unknown sample against the barcode library in BOLD for identification (Kress & Erickson, 2012). Identification of specimen is made by a strict tree-based assignment model (Wilson et al., 2011) involving sequence alignment algorithm, therefore the accuracy of assignment to species is guaranteed.

2.1.2 DNA barcoding versus conventional morphological identification

Traditionally, identification of biological specimens including arthropods, were performed using morphological keys such as shape, colour and measurements of body parts. For instances, identifications of mosquito, birds and larval fish were made based on their morphological features which were utilised in taxonomic keys by several authors, and has been the gold standard(Chan et al., 2014; Johnsen et al., 2010; Ko et al., 2013). However, to confirm an accurate identification, experienced taxonomist is often needed and the method itself is usually time-consuming (Chan et al., 2014). In some instances, such as when the specimen is damaged, specimens are ecomorphs of

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same species, or specimen is in immature stage of development, existing morphological keys could not be used for identification process thus limit the applicability of this conventional method. Therefore, DNA barcoding serves as an alternative method to overcome these impediments by rapidly and reliably identifying biological specimens of various stages, condition and source.

In DNA barcoding of invertebrates, the 648 bp fragment of *COI* is chosen over other markers due to its high interspecific and low intraspecific variation, thus sufficiently and effectively permitting the discrimination of closely allied species (Hebert et al., 2003). Based on the concept that each species has a unique DNA barcode, DNA barcoding is preferred to identify biological specimens because it speeds up identification by the non-experts as DNA can be recovered non-lethally, routinely, from small tissue samples, especially in the absence of the experienced taxonomists (Chan et al., 2014; Hebert et al., 2003; Pfunder et al., 2004). Without denying the importance of traditional taxonomy, DNA barcoding is essentially complementing conventional morphological identification.

2.1.3 Applications of DNA barcoding

DNA barcoding plays three important roles in science: (1) as a research tool for taxonomists where it assists in identification by expanding the ability to diagnose all stages of a species; (2) as a biodiversity discovery tool where it helps to flag species that are potentially new to science; and (3) as a biological tool where it is being used to address fundamental ecological and evolutionary questions (Kress & Erickson, 2012).

DNA barcodingis proving highly effective in identifying many animal groups. *COI* features high resolution in identifications of Lepidoptera (Hajibabaei et al., 2006), bats (Clare et al., 2011), mosquitoes (Batovska et al., 2016; Chan et al., 2014; Ruiz-Lopez et al., 2012), birds(Johnsen et al., 2010; Kerr et al., 2007), fish (Ko et al., 2013),

spiders (Slowik & Blagoev, 2012), phytoseiid (Li et al., 2012), and eriophyoid mites (Guo et al., 2015).

COI DNA barcoding has becoming increasingly popular as a molecular tool to study animal diversities in Malaysia, a biodiversity hotspot in the heart of Southeast Asia. In marine and coastal studies, Chee and colleagues DNA barcoded the blood cockles, *Tegillarca granosa* (Chee et al., 2011) and neritids (Chan et al., 2014); Song et al. (2013)characterised the genetic diversity of Asian snakehead murrel; Mat-Jaafar et al. (2012) revealed the cryptic diversity within the marine fish Family Carangidae; Mohd-Shamsudin et al. (2011) showed that *COI* enabled the differentiation of Asian Arowana, *Scleropages formosus* from other closely related species within the order Osteoglossidae; and Zierets et al. (2016)recently conducted a comprehensive assessment of Peninsular Malaysia's freshwater mussels through an integrative morphological-*COI* barcoding approach. In insect studies, Sing et al. (2016) evaluated the *COI* diversity of bee in Southeast Asian megacities including Kuala Lumpur; Orr and Dow (2015) identified and described the final stadium larvae of *Onychargia atrocyana* collected from Gunung Mulu National Park, Sarawak through DNA barcoding; Wong et al. (2015) identified insect pollinators of Chinese knotweed and assigned them to 23 species and four orders using DNA barcoding; and Brandon-Mong et al. (2015) coupled DNA barcoding and high-throughput sequencing to evaluate primers and pipelines in identifications of 80 arthropod species representing eleven orders. DNA barcoding also confirmed the identity of the first sighting of the brown widow spider, *Latrodectus geometricus*, in Peninsular Malaysia (Muslimin et al., 2015).

2.2 Medically-important ectoparasites in welfare homes

The Insecta including lice, fleas, bedbugs, flies, bees and ants; and the Arachnida which includes spiders, scorpions, ticks and mites are two arthropod classes of medical importance (Steen et al., 2004) as their bites and stings may induce severe anaphylaxis and transmit diseases (Steen et al., 2004; Wikel, 1982). In impoverished urban and rural communities, particularly children living in welfare homes, ectoparasitic infestations such as scabies (Agrawal et al., 2012; Geoghagen et al., 2004; Kawano et al., 2014; Morsy et al., 2000; Muhammad Zayyid et al., 2010; Pruksachatkunakorn et al., 2003), body lice (Morsy et al., 2000) and head lice infestations (Morsy et al., 2000; Muhammad Zayyid et al., 2010; Pai, 1992; Sawicka et al., 2011) are common because they are usually found in overcrowded premises often with poor domestic and personal hygiene practices (Moretti et al., 2015).

I review at length the head lice as this is the predominant arthropod of medical importance which I have come across at welfare homes. I also review in brief the bedbugs which were found in one welfare home.

2.2.1 *Pediculus humanus capitis*

2.2.1.1 General biology

The head louse, *Pediculus humanus capitis* De Geer (Phthiraptera: Pediculidae) is a small, wingless insect and obligate human parasite which resides close to the scalp and lives exclusively on blood (Buxton, 1947). The life cycle of the head louse involves three stages: (i) egg, which takes six to nine days to hatch; (ii) three nymphal stages; and (iii) adult that can live up to 27 days on a person's head("Biology of head lice," 2015; Bonilla et al., 2013).
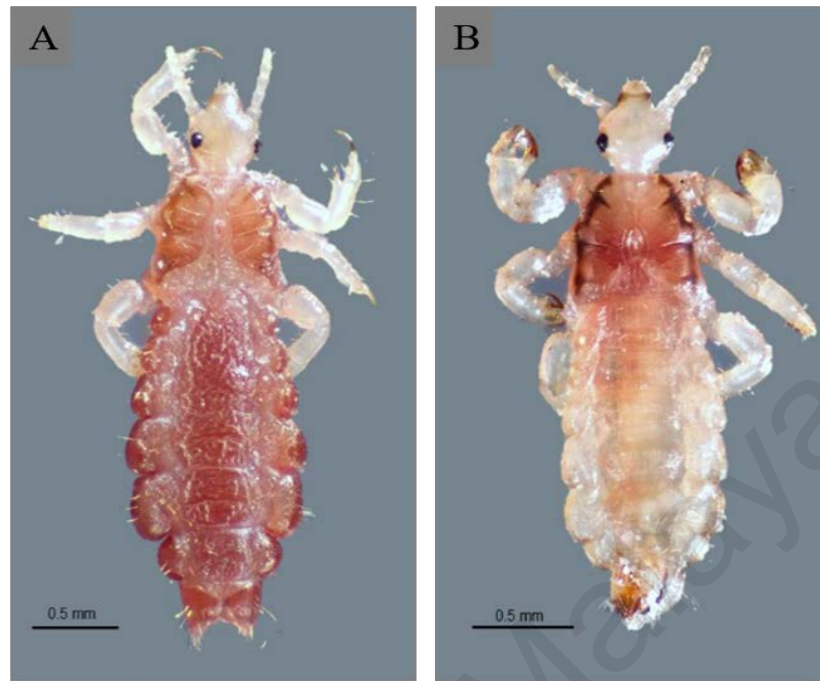
Figure 2.1: The human head lice, *P. h. capitis* (Bonilla et al., 2013). This figure shows the dorsal view of female (left) and male (right) head lice.

The adult female is generally larger (2.4 to 3.3 mm in length) than male (2.1 to 2.6 mm). The female would have a broader abdomen in comparison to the male (Bonilla et al., 2013; Buxton, 1947). Females lay four to five eggs per day at the base of head hairs (Bonilla et al., 2013). Both sexes usually require four to ten blood meals daily (Bonilla et al., 2013), and will die within one to two days off the host ("Biology of head lice," 2015).

2.2.1.2 Taxonomy

The status of head lice as a single species or subspecies of *Pediculus humanus* is controversial. Some scientists argue that the head louse as a distinct species from the body louse, thus the use of the scientific name *Pediculus capitis*, was subsequently employed (Boutellis et al., 2014; Busvine, 1945; Maunder, 1983).

Various morphological, behavioural and molecular evidence have been presented to justify the one- or two-species arguments. The difference seen in size of head and body lice, respectively (body lice tend to be slightly larger and longer) collected from the same individuals led to the conclusion of separate subspecies (Bonilla et al., 2009; Busvine, 1978; Light et al., 2008). The third antennal segment shows considerable differences in proportion and is shorter and wider in head lice compared to body lice, and the abdominal indentations are more prominent in head lice than in body lice (Bonilla et al., 2013; Busvine, 1948).Head lice are usually documented as darker than body lice (Bonilla et al., 2013), and the difference is said to be dependent on the background coloration (skin colour of the host) (Ewing, 1926).However, the colour difference is not constant as grey body lice is found in Ethiopia (Veracx et al., 2012b; Veracx et al., 2012a).

In regard to natural behaviour of lice, head lice aggregate and feed exclusively on human scalp where females oviposit at the base of hair shafts, whereas body lice feed

upon body regions and oviposit on clothing fibres (Bonilla et al., 2013; Light et al., 2008). They do not migrate even in cases of dual infestation (Busvine, 1978). Other differences include a smaller number of eggs laid by female head lice (Bacot, 1916), a higher mortality (Busvine, 1948) and survival rates of body lice compared to head lice (Nuttall, 1919). In addition, head and body lice can interbreed to produce fertile offspring with an intermediate morphology under experimental conditions (Bacot, 1916; Buxton, 1940; Maunder, 1983; Mullen & Durden, 2009; Nuttall, 1919); however, nature inbreeding is still uncleared (Drali et al., 2013; Leo et al., 2005).

Most genetic studies have concluded that these two are ecomorphs of the same species (Leo et al., 2002; Veracx et al., 2012a) with body lice originating from head liceduring instances of low hygiene (Li et al., 2010). These evidence suggested that they are variants of a single species which respond differently to environmental conditions. However, the sequence differences in Phum_PHUM540560 gene that encodes a hypothetical, 69-amino acids protein of unknown function (Drali et al., 2013) supported the conclusion of separate species due to their reproductive isolation, by employing microsatellite DNA evidence from hosts with double infestations (Leo et al., 2005).

Past molecular studies have revealed that *Pediculus humanus* includes three genetically distinct lineages largely based on the studies of two mitochondrial genes, *COI* and cytochrome b (*cytb*) genes (Ashfaq et al., 2015). Clade A lice comprises both head and body lice, whereas clade B and C include only head lice. Recently, two new clades, D and E, have been introduced by Ashfaq et al. (2015) from analysis of sequence variation of available *COI* and *cytb* in *P. humanus* from three countries (Egypt, Pakistan, South Africa). All five clades exhibit geographic differences: A has global distribution (Boutellis et al., 2013b; Veracx & Raoult, 2012); B is found in Australia, Europe, North and Central America, South Africa and Algeria (Ashfaq et al., 2015; Bonilla et al., 2013; Boutellis et al., 2015; Light et al., 2008); C is limited to

Africa (Nepal, Ethiopia, Senegal) (Ashfaq et al., 2015; Bonilla et al., 2013; Light et al., 2008) but recently has been reported in Thailand (Sunantaraporn et al., 2015); D is found in Pakistan, Nepal and Ethiopia; and E is found in Ethiopia (Ashfaq et al., 2015).

In this thesis, head and body lice are considered to be subspecies of *P. humanus*, based on the evidence from mitochondrial and nuclear DNA that supported the single-species argument.

2.2.1.3 Modes of transmission

P. h. capitis is extremely transmissible from person to person either by direct head-to-head contact or occurs via indirect fomite transmission such as hats, jackets, scarves, as well as the shared use of hairbrushes and combs (Bachok et al., 2006; Light et al., 2008). Transmission is enhanced in overcrowded dwellings where direct contact is maximised (Bachok et al., 2006).

2.2.2 *Cimex hemipterus*

C. hemipterus bedbug is an insect of medical interest (together with *C. lectularius*) that belongs to the order Hemiptera of the Cimicidae family (Delaunay et al., 2011). Both species, including the other four species of *Cimex columbarius*, *Cimex pipistrelli*, *Cimex dissimilis*, and *Oeciacus hirundinis* feed on humans (Delaunay et al., 2011). *C. hemipterus* has tropical climate distribution and sometime occurs in temperate zones, compared to *C. lectularius* that is found only in temperate zones (Angelakis et al., 2013; Delaunay et al., 2011).

2.2.2.1 General biology

The life cycle of *C. hemipterus* involves three stages: (i) egg, that hatches into first instar nymph in about four to twelve days; (ii) five nymphal stages that resemble adult but lacking wing buds, and each stagerequires a blood meal to molt into the next

stage before the fifth stage molts into (iii) an adult, which is reddish brown, flat, and wingless oval of approximately four to seven millimetres that can survive up to twelve months without feeding and even up to two years in colder environment ("Biology of bedbugs," 2015; Delaunay et al., 2011). Both sexes are hematophagous and adult female produces 200 to 500 eggs in her lifetime (Delaunay et al., 2011).

2.2.2.2 Medical impact of bedbugs' infestation

Bedbugs hide in any small and dark place, such as bedclothes, seams and edges of mattresses, bed frames, spring and crevices because they fear light (Delaunay et al., 2011). Hosts are usually bitten at night because they are generally active in the dark (Delaunay et al., 2011); and bites are painless and usually unnoticed because bedbug saliva contains anaesthetic compounds (Bernardes et al., 2015; Delaunay et al., 2011). However, in the presence of high infestations, the bites of bedbugs disturb night rest and causing discomfort (Bernardes et al., 2015). Following bites, the clinical manifestations depend on previous exposure to the insect and the degree of immune response of the patient, which can present from cutaneous reactions whereby pruritic erythematous maculopapule with a central haemorrhagic crust at the bite site is the typical skin lesion, to systemic reactions such as urticaria and anaphylaxis (Criado & Criado, 2011; Delaunay et al., 2011; Goddard & deShazo, 2009). These reactions are usually self-limiting and will resolve within one to two weeks (Cleary & Buchanan, 2004). Anti-histamines and topical steroids are beneficial to treat pruritus and inflammation (Cleary & Buchanan, 2004).

Infestations by bedbugs are cosmopolitan as isolated cases, clusters, and epidemics have been reported in all continents (Delaunay et al., 2011). Amongst recorded tropical distribution of *C. hemipterus* includes populous centres and rural areas of Brazil (Nascimento, 2010), overcrowded prisons in Rwanda (Angelakis et al., 2013), hotels in Thailand (Tawatsin et al., 2011), hotels, public accommodations, and

residential premises in Malaysia and Singapore (Ab-Majid & Zahran, 2015; How & Lee, 2010), and neonatal unit of children hospital in India (Bandyopadhyay et al., 2015).

Bedbugs have been implicated to carry over forty microorganisms, particularly in their stomach, faeces, tegument, and saliva (Bandyopadhyay et al., 2015; Delaunay et al., 2011). *C. burnetii*, *T. cruzi*, HBV, *B. quintana*, methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant *Enterococcus faecium*(VRE) are among pathogens reportedly found in bedbugs(Delaunay et al., 2011).

In 1960, *C. burnetii* was detected in *C. lectularius*, where the Q fever (a worldwide zoonotic disease caused by *C. burnetii*) prevalence was estimated at 29.2% of the population (Daɪ̆ter, 1960). *T. cruzi*, the causative agent of Chagas disease was found infecting *C. lectularius* after feeding on infected mice (Jörg, 1992); and study by Salazar et al. (2015) suggests that *C. lectularius* may be a competent vector of *T. cruzi* and could pose a risk for vector-borne transmission of Chagas disease.HBV surface antigen (HBsAg)has been detected in body parts and faecal material of laboratory and wild-caught *C. hemipterus* (el-Masry & Kotkat, 1990; Jupp et al., 1983; Ogston et al., 1979; Wills et al., 1977); and its partially double-stranded DNA has been detected in bedbugs and their excrement (Silverman et al., 2001), however the mechanical transmission has not been proven. *B. quintana* has been detected in *C. hemipterus* collected from two prisons in Rwanda (Angelakis et al., 2013)and recent study by Leulmi et al. (2015) demonstrated that *C. lectularius* experimentally can acquire, maintain and transmit *B. quintana*, thus suggesting the bedbugs' vector competency under natural conditions. MRSA and VRE have been recovered from bedbugs infesting hospitalised patient in Vancouver, Canada (Lowe & Romney, 2011), however, the role of bedbugs as vector to transmit these pathogens to humans warrants further investigations.

2.3 Pediculosis capitis

Head lice infestation by *P. h capitis* is termed as pediculosis capitis (Nutanson et al, 2008). It is one of the most reported cases of ectoparasitic infestations affecting children between five to eleven years of age (Leung et al., 2005; Nutanson et al., 2008).

Pediculosis capitis has a worldwide distribution and does not discriminate on socioeconomic status grounds (Falagas et al., 2008). Every age stratum is susceptible topediculosis capitis, however children are mostly affected and crowded living conditions is associated with higher prevalence (Nutanson et al., 2008).

2.3.1 Prevalence

Pediculosis capitis is endemic all over the world, both in developed and developing countries, and in tropical and temperate countries Gratz (1997). Prevalence of more than 5% is considered to be an epidemic (Speare & Buettner, 1999). The prevalence remains high and epidemic occurs regularly even in developed countries (Nutanson et al., 2008).

In Europe, the prevalence ranged from 0.48% to 37.4% (Falagas et al., 2008). In the United States (US), pediculosis capitis occurswith an estimated six to twelve million infestations each year ("Head lice," 2015). In Americas, apart from the US, Brazil, Venezuela, Cuba, Chile, Mexico, Peru and Argentina are among countries that recorded high prevalence of pediculosis capitis (Falagas et al., 2008; Lesshafft et al., 2013; Moosazadeh et al., 2015; Omidi et al., 2013).

In Asia, the prevalence rates pediculosis capitis ranged from 4.1% in Korea (Oh et al., 2010), 0.3 to 34.1% in Turkey (Özkan et al., 2015), 7.4% in Iran (Moosazadeh et al., 2015), 26.6% in Jordan (AlBashtawy & Hasna, 2012), 23.3% in Thailand (Rassami & Soonwera, 2012), and 24.7 to 49.0% in Malaysia (Bachok et al., 2006; Muhammad Zayyid et al., 2010; Yap et al., 2010).

In Africa, the prevalence rates ranged from 5.3 to 17.1 % in East Africa, 11.0 % to 66.5 % in North Africa, 0 to 49.0 % in West Africa and 3.7 to 42.1 % in Southern Africa (Abd El Raheem et al., 2015; Govere et al., 2003; Magalhães et al., 2011).

Majority of the above reports involved studies in schoolchildren while the remaining involved refugees, homeless persons, children in orphanages, urban slum residents and the general population.

2.3.2 Clinical presentations of pediculosis capitis

Although most pediculosis capitis are asymptomatic (Mumcuoglu et al., 1991; Nutanson et al., 2008), pruritis of the scalp due to skin sensitisation by louse antigen (introduced during blood meal or lice excreta) is the principal symptom (Madke & Khopkar, 2012; Mumcuoglu et al., 1991; Nutanson et al., 2008). Continuous scratching may lead to loss of skin integrity with secondary bacterial infection, impetiginization and enlarged posterior cervical and auricular nodes (Madke & Khopkar, 2012; Mumcuoglu et al., 1991).Other possible manifestations include excoriations, pyoderma, cervical lymphadenopathy, conjunctivitis, fever, and malaise (Janniger & Kuflik, 1993; Mumcuoglu et al., 1991). Severe pyoderma of the scalp caused by a nephritogenic strain of streptococci may lead to alopecia (Madke & Khopkar, 2012; Mumcuoglu et al., 1991; Nutanson et al., 2008).Chronic, heavy, untreated infestation can lead to anaemia, especially in females who already suffered from iron deficiency anaemia (Madke & Khopkar, 2012). Rarely, plica polonica (scalp is covered with epithelial debris and

crusts) occurs in heavily infested person due to entangled hairs with exudates and predisposing the area to fungal infection (Mumcuoglu et al., 1991; Nutanson et al., 2008).

Apart from physical and clinical symptoms as described above, pediculosis capitis causes psychological stress including social embarrassment, isolation, parental anxiety, peer-criticism, and unnecessary absenteeism from academics (Madke & Khopkar, 2012)because they believe that the occurrence is a result of being dirty (Oh et al., 2010). In addition, intense itching in children may result in sleep disturbances and subsequent concentration difficulties and poor performance in school (Heukelbach & Feldmeier, 2004).

2.3.3 Diagnosis of pediculosis capitis

Identification of a live louse, nymph, or a viable nit is the gold standard for diagnosing pediculosis capitis (Nutanson et al., 2008) and diagnosis is definitive whencrawling lice are seen in the scalp hair or are combed from the scalp (Ko & Elston, 2004). The diagnosis is made by two methods: through visual inspection of hair and scalp with an aid of applicator stick and/or by dry or wet combing using a detection comb (Feldmeier, 2012; Nutanson et al., 2008). In both methods, the hair is systematically combed from the scalp to the ends (Feldmeier, 2012).

Visual inspection is an easy, rapid and optimal method to diagnose historical infestation (Feldmeier, 2010). The inspection is usually confined to predilection sites of left and right temples, behind the ears and the neck (Feldmeier, 2012) because head lice prefer to cement their eggs to hairs shafts in the topographic areas (Nash, 2003).

Visual inspection without combing is difficult because head lice crawl quickly to avoid light (Ko & Elston, 2004; Nutanson et al., 2008). Therefore, direct combing on dry or moistened hair is the optimal method to diagnose active infestation, which is a

fourfold more efficient compared to direct visual inspection (Nutanson et al., 2008) and with a sensitivity of 90% in children with low infestation intensity (Feldmeier, 2012).

2.3.4 Treatment

Treatment for pediculosis capitis should be considered only if live lice or viable nits are observed (Son et al., 1995) and should be directed at killing the lice and the ova (Nutanson et al., 2008). There are three different approaches to eliminate head lice: topical application of pediculicides, wet combing, and oral therapy (Feldmeier, 2012). Mechanical removal of parasitized hair on the scalp by shaving, even though would eradicate head lice, is not recommended and not cosmetically acceptable for most patients (Chosidow, 2000; Magee, 1996).

Malathion is a weak organophosphate cholinesterase inhibitor that causes respiratory paralysis in arthropods (Meinking et al., 2002). It kills lice after five min of exposure, and more than 95% of eggs failed to hatch after 10 min of exposure (Chosidow, 2000) but has been withdrawn from the market for several years due to issue on its safety and commercial failure in 1997. However, application of 0.5% malathion in 78% isopropanol for eight to twelve hours has been approved by the Food and Drug Administration (FDA) but should not be used for children under six months (Chosidow, 2000).

Lindane (1%) is an organochloride that kills lice by causing respiratory paralysis (Ko & Elston, 2004). It is applied to the hair and scalp for not more than four minutes (Madke & Khopkar, 2012). However, the use of lindane is limited due to the reports of

central nervous system toxicity(Chosidow, 2000; Fischer, 1994) and should be avoided for young children, patients with an impaired cutaneous barrier, patients with seizure disorders and also in pregnancy and breastfeeding (Meinking et al., 2002; Nash, 2003).

Permethrin, a synthetic pyrethroid is used as 1% cream (Nash, 2003).Permethrin interferes with sodium transport in the arthropod, leading to depolarisation of neuromembranes and respiratory paralysis (Ko & Elston, 2004). The cream is applied to the scalp and hair for ten minutes after which it should be rinsed off (Madke & Khopkar, 2012).

Pyrethrins have the same mechanism of action as permethrin(Ko & Elston, 2004; Nutanson et al., 2008). It is derived from chrysanthemum extracts and used with piperonyl butoxide to potentiate the effect of the pyrethrin and decrease the development of pyrethrin resistance (Picollo et al., 1998). These agents are available over the counter by pharmacists and used as a 0.33% shampoo or mousse, by applying thoroughly to hair for 10 minutes (Nash, 2003).

Wet combing or known as 'bug-busting' was first introduced in the United Kingdom in response to the concerns about the effectiveness and potential toxicity of pyrethroids and malathion (Ko & Elston, 2004; Roberts et al., 2000). Combing involves combing on wet hair with an added lubricant using a specially designed comb in every three to four days for at least two weeks (Ko & Elston, 2004; Madke & Khopkar, 2012). The hair needs to be wetted to make lice temporarily immobile thus ease the combing process (Ko & Elston, 2004). This method however is time consuming and reports have shown that the cure rate for wet combing was low(Roberts et al., 2000; Vander-Stichele et al., 2002).

Also used as a lotion, ivermectin,a semisynthetic derivative of avermectin that interrupts the γ-aminobutyric acid–induced neurotransmission in invertebrates

(Chosidow et al., 2010), has been administered orally (in tablet form) to cure pediculosis capitis(Chosidow, 2000; Chosidow et al., 2010; Ko & Elston, 2004). A single dose of 200 μg/kg has generally been given and second dose may be given to kill emerging nymphs (Ko & Elston, 2004). A single dose of ivermectin withdiethylcarbamazine, an anti-filarial drug, given to school children for a duration of sixty days has shown a significant reduction in the prevalence of head lice infestation(Munirathinam et al., 2009). Chosidow et al. (2010) showed that two doses of ivermectin of 400 mg each, given eight days apart, had superior efficacy over topical 0.5% malathion lotion in patients with difficult-to-treat head-lice infestation.

Despite the effectiveness of chemical agents in treating pediculosis capitis, resistance of head lice to malathion, lindane, pyrethrins, permethrins, and ivermectin have been documented (Bonilla et al., 2013; Pittendrigh et al., 2006).

2.4 Head louse-borne pathogens

Hitherto, only body lice are the competent and significant vectors for human pathogens, naturally and experimentally (Badiaga & Brouqui, 2012; Bonilla et al., 2013; Light et al., 2008). Body lice are known to transmit three pathogenic bacteria: *Rickettsia prowazekii*, the causative agent of epidemic typhus; *Borrelia recurrentis*, the causative agent of relapsing fever; and *Bartonella quintana*, the causative agent of trench fever, bacillary angiomatosis, endocarditis, chronic bacteremia, and chronic lymphadenopathy(Brouqui, 2011; Jacomo et al., 2002; Raoult & Roux, 1999). Nonetheless, some researchers have reported the presence of *B. recurrentis*(Boutellis et al., 2013a), and *B. quintana*(Angelakis et al., 2011b; Bonilla et al., 2009; Sasaki et al., 2006) in head lice. *Acinetobacter baumannii*, a widespread bacterium capable of causing nosocomial and community acquired infections, has also been detected in head lice(Bouvresse et al., 2011; Kempf et al., 2012; Sunantaraporn et al., 2015).

2.4.1 *Borrelia recurrentis*

B. recurrentis is a motile, human restricted spirochaeta and is transmitted from person to person primarily by the body louse, *P. h. humanus*(Antinori et al., 2016; Badiaga & Brouqui, 2012). Infection with *B. recurrentis* can cause acute febrile illness known as louse-borne relapsing fever (LBRF). LBRF manifests when a louse is accidentally ruptured by scratching and subsequent inoculation of the spirochaetes into the patient's eyes or mouth (Boutellis et al., 2013a; Colomba et al., 2016). Clinical manifestations of LBRF include variable periods of apyrexia between the febrile episodes, accompanied by non-specific symptoms as headache, arthralgias and myalgias that resemble other serious diseases such as malaria, leptospirosis, tick-borne recurrentis fever, and typhoid fever(Colomba et al., 2016). Patients suffering from LBRF are usually successfully treated by penicillin, tetracycline, or doxycycline (Cutler, 2015), however infection can be severe and death occurs in the absence of appropriate treatment(Colomba et al., 2016).

Major outbreaks of LBRF initially occurredin Eastern Europe, the Balkans, former Soviet Union and Africa during World Wars I and II(Antinori et al., 2016; Colomba et al., 2016), however its geographical distribution has reduced due to improvements in living standards (Colomba et al., 2016). Currently, LBRF is endemic in Eastern Africa (Ethiopia, Eritrea, Somalia, and Sudan)(Colomba et al., 2016; Cutler et al., 2009; Elbir et al., 2013; Yimer et al., 2014). In addition, LBRF has been reported recently in refugee camps in Europe, including Italy(Ciervo et al., 2016; Colomba et al., 2016; Lucchini et al., 2016), Switzerland (Goldenberger et al., 2015), Netherlands(Wilting et al., 2015), and Germany (Hoch et al., 2015) that shelter East African asylum seekers.

Despite the fact that body louse is the principal vector that transmits *B. recurrentis*, DNA of *B. recurrentis* has been found in head lice from patients with

double infestations of head and body lice, and also in patients infested with head lice only (Boutellis et al., 2013a). However, the status of head lice as a vector remains unknown.

2.4.2 *Bartonella quintana*

B. quintana is a facultative Gram-negative bacillus that causes trench fever in human (Badiaga & Brouqui, 2012). Humans are the natural reservoir in which *B. quintana* persists in erythrocytes and erythroblasts(Rolain et al., 2003). *B. quintana*is transmitted primarily by the human body louse(Foucault et al., 2006). The presence of *B. quintana* has been reported in body lice collected from the homeless in France, US, Netherlands, Ethiopia, Japan, Russia, and Mexico; and body lice collected from refugees, prisoners, and rural populations in Burundi, Rwanda, Zimbabwe, and Peru (Fournier et al., 2002).

Nevertheless, human head and pubic lice have also been found to be competent vectors in the laboratory settings (Badiaga & Brouqui, 2012). Sasaki et al. (2006) was the first to detect the presence of *B. quintana* DNA in head lice collected from Nepalese slum children. In addition, *B. quintana* has also been found in head lice from homeless adults without concurrent body lice infestation in the US (San Francisco) (Bonilla et al., 2009), in head louse nits from a homeless man in Marseilles, France (Angelakis et al., 2011a), head lice of clade C in Gibarku and Tikemit Eshet of Ethiopia (Angelakis et al., 2011b). These evidence suggest the potential role head lice in the transmission of *B.*

quintana to humans. The role of macaque monkeys and their lice, *Pedicinus obtusus*, as potential reservoir and vector, respectively, have also been implicated by Li et al. (2013). In addition, *B. quintana* was also detected in *Ctenocephalides felis* cat fleas (Rolain et al., 2003)and *Ixodes pacificus* ticks (Chang et al., 2001).

Transmission to humans occurs when the infected lice excrete *B. quintana* onto the skin while feeding, and the bacteria are either scratched into the skin or rubbed into mucous membranes (Bonilla et al., 2013). Trench fever is characterized by an acute onset of a high-grade fever that last for one to three days, and are associated with headache, shin pain, and dizziness(Badiaga & Brouqui, 2012; Foucault et al., 2006). Occasionally, the first fever episode is followed by a relapse every four to five days (Badiaga & Brouqui, 2012). The current treatment for trench fever is gentamicin for two weeks, followed by doxycycline for four weeks (Angelakis & Raoult, 2014).

Other reported clinical presentations resulted from the infection of *B. quintana* include chronic bacteremia (Badiaga & Brouqui, 2012), bacillary angiomatosis (Santos et al., 2000) including vulval bacillary angiomatosis(Ramdial et al., 2000), chronic lymphadenopathy(Raoult et al., 1994) and endocarditis (Foucault et al., 2006).

2.4.3 *Acinetobater baumannii*

A. baumannii, an aerobic Gram-negative coccobacillus is a frequent skin and oropharyngeal commensal (Rosenthal, 1974). Infections caused by *A. baumannii* have become a critical problem for hospitalised patients, worldwide (Anudit et al., 2016), predominantly in patients with endotracheal intubation, prolonged mechanical ventilation, underlying lung diseases, prior broad spectrum antibiotic treatment, recent major surgery, enteric feeding, or who are being treated in an intensive care unit (ICU)(Chen et al., 2001). It has also been reported as a cause of severe community-

acquired infections such as pneumonia, endocarditis and meningitis, especially in alcoholic patients (Chen et al., 2001).

Houhamdi and Raoult (2006) have documented that *A. baumannii* is capable of causing persistent and life-long infection in the human body lice. Moreover, body lice excreted living *A. baumannii* in their faeces(Houhamdi & Raoult, 2006). *A. baumannii* has been detected in body lice of homeless individuals in Marseille, France (La Scola et al., 2001; La Scola & Raoult, 2004), in head lice of elementary schoolchildren in Paris (Bouvresse et al., 2011), in head and body lice of healthy individuals in Ethiopia (Kempf et al., 2012), and in head lice of primary schoolchildren in Thailand(Sunantaraporn et al., 2015).

2.5 Study areas: Greater Kuala Lumpur / Klang Valley and state of Kedah

Malaysia is a multicultural country in the Asia Pacific region with an estimated population of 31.7 million in year 2016 according to Department of Statistics Malaysia. Malaysia consists of eleven states and two federal territories located in the peninsula, collectively referred to as Peninsular Malaysia; and two states and one federal territory located on the island of Borneo, collectively known as East Malaysia or Malaysian Borneo (Figure 2.2).

Greater Kuala Lumpur/Klang Valley (KL/KV) region is an area comprising Kuala Lumpur (the capital of Malaysia), Federal Territory of Putrajaya and adjoining cities and towns in the state of Selangor with the exception of Kuala Langat, Kuala Selangor, Sabak Bernam and Hulu Selangor districts ("Greater kuala lumpur & klang valley," 2012). Greater KL/KV is covered by ten municipalities, each governed under jurisdiction of local authorities of two states (Federal Territory of Kuala Lumpur and state of Selangor. Being the Malaysia's financial centre, population of Greater KL/KV

is estimated at 7.2 million as of year 2016 with the highest median monthly household income of Ringgit Malaysia (RM) 7115 in year 2014("Report of household income and basic amenities survey 2014," 2015).

State of Kedah is located in the north-western region of Peninsular Malaysia (Figure 2.2). Kedah is divided into twelve administrative districts; and Alor Star which is located in Kota Setar district is the capital (Figure 2.2). With a population estimated at 2.12 million in year 2016, Kedah recorded the median monthly household income below the national level: RM 3451 in year 2014 ("Report of household income and basic amenities survey 2014," 2015).

2.5.1 Children welfare institutions in Malaysia

Children welfare institution is defined as a safe home devoted to the care, protection and rehabilitation of children; referred under the Malaysian Section 54, Child Act 2001. In Malaysia, these institutions are often classified into three types; governmental, semi-governmental and non-governmental organisations. To date, there are thirteen governmental children welfare homes managed by the Department of Social Welfare, Ministry of Women, Family and Community Development; located across states of Malaysia.

Being the most commonest (Saim et al., 2013), the non-governmental institutions often rely on public donations to sustain their operations. Insuffiecient funds, unavoidable bureaucracy to obtain licences to run the institution, and many other challenges facing by these institutions have hampered the efforts of the administrations in providing adequate care for the children. In this study, non-governmental welfare

institutions that shelter underprivileged childrenwhere the transmission of ectoparasites and their potential pathogens is likely to occur, were chosen to represent the appropriate host population. The contributing factors that may have lead to the higher prevalence of head lice and their potential pathogens are further discussed in Chapter 5.
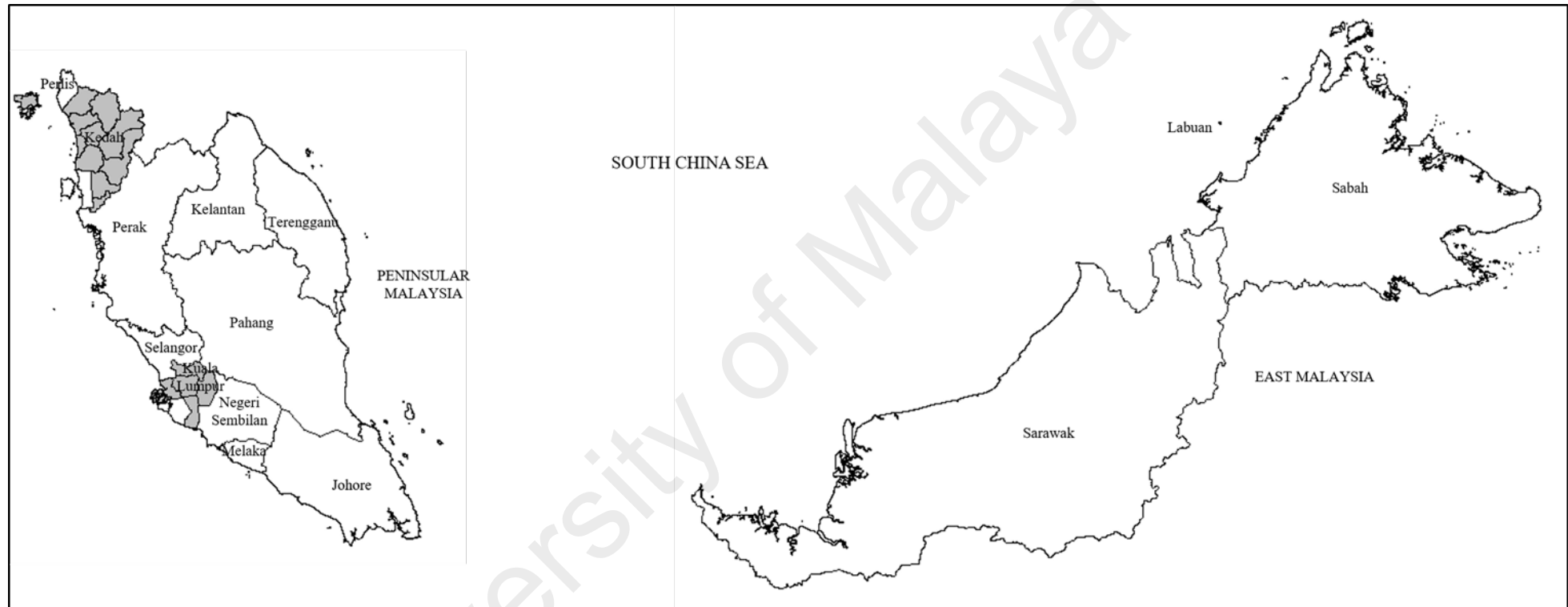
Figure 2.2: Map of Malaysia. Greater KL/KV and state of Kedah are marked in grey.

2.6 Human ectoparasitic infestations in Malaysia

Being a fast developing country, ectoparasitic infestations such as scabies, pediculosis capitis and bedbug infestations however are still occurring in the populations, affecting especially the urban and rural poor.

A considerable number of studies investigating the occurrence of scabies and pediculosis capitis have been conducted in the country since 1980s. The majority of studies were conducted among school children primarily to assess the prevalence and therapeutic effectiveness. These include reports by Sinniah et al. (1984; 1981, 1983), Sinniah and Sinniah (1982), Bachok et al. (2006), and Jamaiah et al. (2000) that focused on the areas in Peninsular Malaysia; and study by Yap et al. (2010) is the last published report on scabies and head lice infestation among students of secondary boarding schools in Sarawak, Malaysia. Only two publications by Wan-Omar et al. (1993) and Oothuman et al. (2007) deal with the occurrence of the crab louse *Pthirus pubis* (pubic lice) in Malaysia. Infestation of the body louse, hitherto, has not been reported to occur in Malaysia.

In addition to scabies, head and pubic lice infestations, active infestations of *C. hemipterus* is at alarming state, and abundantly affecting premises including residential houses, flats and hotels(Zahran et al., 2016). Surveys conducted by How and Lee (2010) between year 2005 to 2008 showed active infestations occurred in two geographical areas of Peninsular Malaysia (Pulau Pinang and Kuala Lumpur). Lee et al. (2006) reported that *C. hemipterus* were among arthropod specimens received in a large number by the Medical Entomology Unit, Institute for Medical Research (IMR). A survey by Ab-Majid and Zahran (2015) further reported that premises in Kuala Lumpur and Selangor have the highest infestation of *C. hemipterus* compared to other states in Peninsular Malaysia.

Despite above reports, data on what pathogens the ectoparasites might carry and their genetic diversity are lacking. Therefore, studies focusing on ectoparasite-borne pathogens in populations at risk, such as children residing in welfare institutions that could provide further insight into these aspects are much needed in order to prevent plausible disease transmission.

2.7 DNA barcoding of arthropods in medical case reports

From time to time, the Department of Parasitology, Faculty of Medicine, University of Malaya receives arthropod specimens of various species for identification. Specimens from three medical case reports identified through DNA barcoding were documented in this thesis.

2.7.1 Case report 1: Recurrent tick infestation of humans in Pekan, Malaysia

Ticks of the family Ixodidae are blood-sucking ectoparasites that can infest a variety of vertebrate hosts, including humans. Although ticks are usually encountered attached to the host's external surface, the preferred sites of tick infestation on a host's body vary depending on the species of tick and its life-stage (Kar et al., 2013). Ticks have been implicated as vectors of a number of human pathogens that can cause serious illnesses, such as Lyme disease(Juckett, 2013; Overstreet, 2013; Wu et al., 2013), Rocky Mountain spotted fever (Graham et al., 2011; Minniear & Buckingham, 2009), tick-borne encephalitis (Kunze, 2015; Lani et al., 2014), tularemia(Gürcan, 2014; Weber et al., 2012), Crimean-Congo hemorrhagic fever (Bente et al., 2013)and Q fever (Keklikçi et al., 2009).

In Malaysia, despite numerous reports on the distribution of tick species(Ahamad et al., 2013; Hoogstraal et al., 1972; Hoogstraal & Wassef, 1984, 1985; Hoogstraal & Wassef, 1988; Kohls, 1957; Madinah et al., 2011; Madinah et al., 2013; Mariana et al., 2011; Mariana et al., 2008a; Mariana et al., 2005, 2008b; Nursyazana et

al., 2013; Paramasvaran et al., 2009), particularly their infestations on small mammals and avifauna, the epidemiology and prevalence of human infestation is poorly understood, although there now exists a growing number of reports(Abdul-Rahim et al., 2013; Lazim et al., 2012; Shibghatullah et al., 2012; Srinovianti & Raja-Ahmad, 2003; Zamzil-Amin et al., 2007).

2.7.2 Case report 2: Intestinal myiasis in a patient from urban area

Clogmia albipunctatus is a cosmopolitan fly belonging to the family Psychodidae and is one of the medically-important insects associated with urban environments (Smith & Thomas, 1979). The psychodid larvae can cause myiasis in humans through infestation of healthy or traumatized tissues (Hall & Smith, 1993). Human myiasis can be presented in various forms with cutaneous myiasis the most common form (Tu et al., 2007). Other infestation sites include nasal, aural, lung, ophthalmic cavities, body cavities, and the gastrointestinal tract and urogenital system (El-Badry et al., 2014; Tu et al., 2007). Intestinal myiasis may be due to accidental ingestion of larvae. Subsequently, it presents symptoms such as nausea, vomiting, abdominal pain and distention, loss of appetite, weight loss and episodic diarrhoea (Ramana, 2012).

This is the second reported case of human intestinal myiasis in Malaysia caused by larvae of *C. albipunctatus*. Microscopic examination revealed the structure of the larvae and DNA barcoding established the species identity.

2.7.3 Case report 3: Canthariasis in an infant

Infection of the gastrointestinal tract is common in infancy (Purssell, 2009) and viruses (rotavirus, norovirus and enteric adenoviruses) account for the majority of cases (Iturriza-Gómara et al., 2008). Bacterial infectionsincluding *Salmonella* and *Campylobacte*r spp. infections are significantly less common (Davies et al.,

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2001).Occasionally, insects of the order Diptera have been reported to cause gastrointestinal infections (referred to as intestinal myiasis) in children (Francesconi & Lupi, 2012; Kandi et al., 2013). Infestation by beetle larvae is termed canthariasis and even rarer. Enteric infestation by the cigarette beetle, *Lasioderma serricorne*, has never been reported. To date, only two cases of canthariasis in infants attributed to ingestion of dermestid beetle larvae have been reported by Okumura (1967). *L. serricorne*is a cosmopolitan pest of stored tobacco(Ashworth, 1993). *L. serricorne* also infests a wide range of other stored commodities such as grains, rice, pasta and beans and is of considerable economic importance (Blanc et al., 2006).

An unusual cause of gastrointestinal infection occurring in a one-year-old infant patient who was brought to a public hospital in Kuala Lumpur, Malaysia is confirmed through DNA barcoding and documented in this thesis.

CHAPTER 3: MATERIALS AND METHODS

3.1 Medically-important ectoparasites from welfare homes

The methodologies and experiments performed in order to provide information on the types of ectoparasites occurring in the welfare homes, and their genetic diversity are explained further below.

3.1.1 Ethics statement

The University of Malaya Medical Centre Ethics Committee (MEC Reference Number: 201312-0608) approved our research protocols involving human subjects in welfare homes (Appendix A).

3.1.2 Specimen collection

Sampling of ectoparasites were conducted in two different geographical regions of Malaysia: (i) Greater Kuala Lumpur/Klang Valley (KL/KV) representing the urban population, and (ii) the State of Kedah representing the rural population from May 2013 to December 2015. Welfare organisations that shelter orphans and neglected children were randomly selected to represent each major city in the Greater KL/KV (Table 3.1) and each district in the state of Kedah (Table 3.2). In total, fifteen welfare homes in the Greater KL/KV and ten welfare homes in Kedah were included in this study (Figure 3.1). Due to unforeseen circumstances (as the welfare homes were randomly selected), majority of the subjects were girls and boys were under-represented.

Table 3.1: List of welfare homes in the Greater KL/KV included in this study

Municipal Council	City	Welfare homes	GPS Coordinates (Decimal degrees)	
			x	y
DBKL	Kuala Lumpur	Rumah Titian Kaseh	101.7035	3.1790
MP Klang	Ampang	Rumah BAKTI Datuk Harun	101.7638	3.1945
MP Selayang	Rawang	Baitul Aini Selangor	101.5302	3.2429
MP Selayang	Rawang	Pusat Tahfiz Anak Yatim Nur Ikhlas	101.5231	3.2559
MP Kajang	Bandar Baru Bangi	Rumah Bakti Al-Kausar	101.7808	2.9656
DBKL	Kuala Lumpur	Pusat Jagaan Kasih Murni	101.7461	3.1725
MP Sepang	Sepang	Baitul Barokah Wal Mahabbah	101.7298	2.7933
MP Petaling Jaya	Petaling Jaya	Pusat Jagaan Rumah Kesayangan	101.6505	3.0880
MP Kajang	Kajang	Rumah Nur Hikmah	101.7782	3.0160
MP Subang Jaya	Puchong	Rumah Amal Limpahan Kasih	101.5903	3.0020
MP Shah Alam	Shah Alam	Rumah Amal Al-Firdaus	101.5172	3.1550
DBKL	Setapak	Pusat Jagaan Nuri	101.7131	3.1908
MP Kajang	Cheras	Rumah Jalinan Kasih	101.7697	3.0534
MP Klang	Klang	Pertubuhan Kebajikan Anak-Anak Yatim Miskin Sungai Pinang	101.4480	3.0511
MP Kuala Langat	Banting	Rumah Anak Yatim & Asnaf As-Sholihin	101.4731	2.7936

Table 3.2: List of welfare homes in state of Kedah included in this study

District	Town/City	Welfare homes	GPS Coordinates (Decimal Degrees)	
			x	y
Padang Terap	Kuala Nerang	Pusat Jagaan Baitul Mahabbah Al-Hashimi	100.5991	6.2825
Pendang	Pendang	Rumah Anak Yatim Amal Solehah	100.4761	5.9567
Kota Setar	Alor Star	PERKIM Bahagian Negeri Kedah	100.3786	6.1165
Baling	Baling	Persatuan Kebajikan Anak Yatim & Miskin Nur Hidayah	100.8472	5.6147
Sik	Sik	Pertubuhan Asuhan dan Didikan Anak-Anak Yatim Islam Daerah Sik	100.7478	5.8127
Kuala Muda	Gurun	Persatuan Kebajikan Anak Yatim dan Miskin Al-Munirah	100.4940	5.8557
Yan	Yan	Rumah Budi Kedah	100.3760	5.8004
Langkawi	Langkawi	Rumah Nur Kasih	99.7630	6.3512
Kulim	Kulim	Pertubuhan Pembangunan Anak-Anak Yatim Bekas Parajurit Malaysia Daerah Kulim	100.5495	5.4089
Kubang Pasu	Jitra	Pusat Jagaan IHMK	100.4390	6.2335
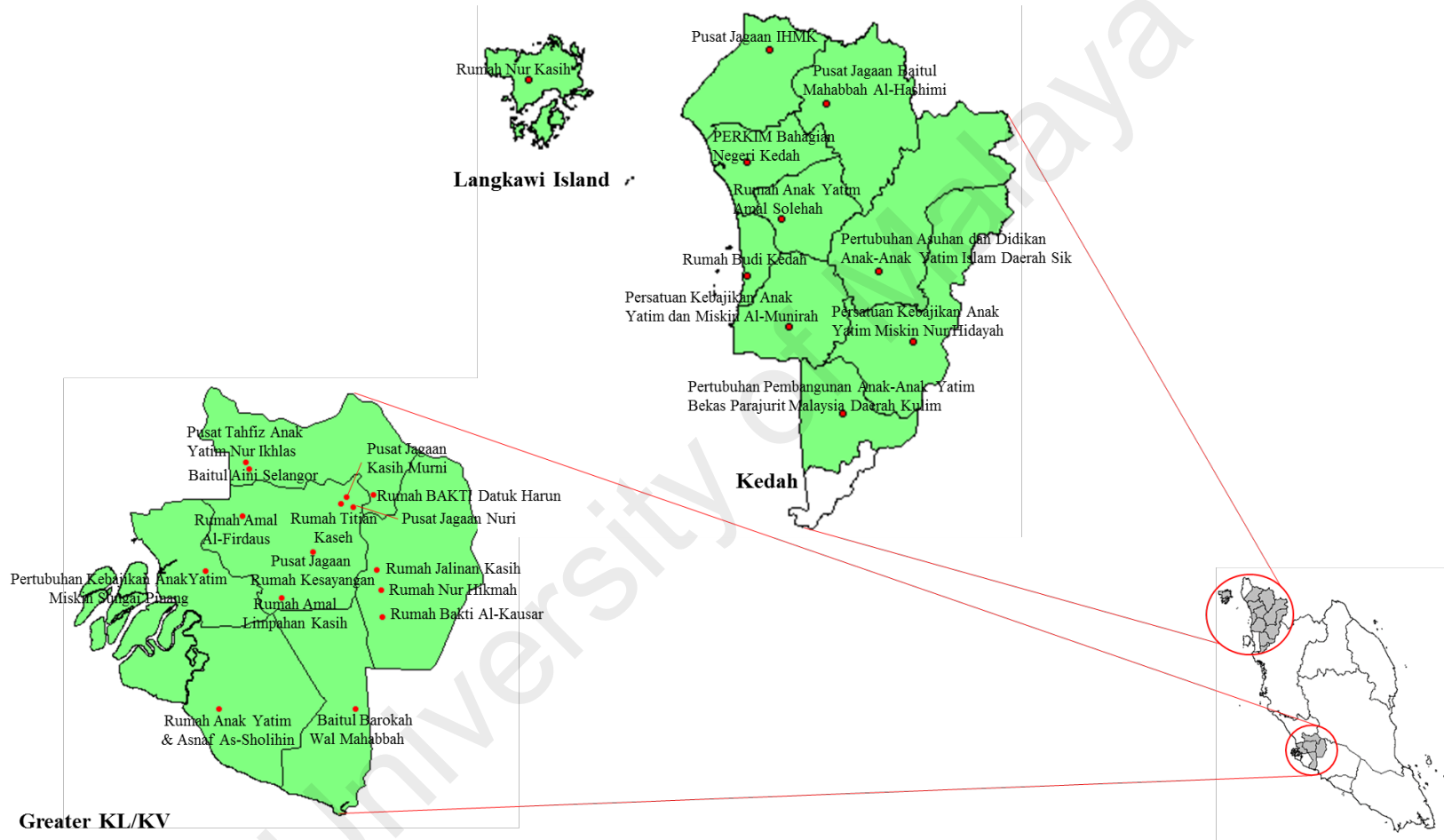
Figure 3.1: Map of ectoparasites sampling in welfare homes in two geographical regions of Peninsular Malaysia. Fifteen welfare homes from thirteen cities in Greater KL/KV and ten welfare homes from ten districts in the state of Kedah were included in this study. Red dot shows the GIS location of welfare homes.

Each occupant was examined for the presence of head lice and/or nits on hair, neck and areas behind the ears. Pediculosis capitis is confirmed when living adults, nymphs or viable nits is detected. The head lice were removed from the hair of infested occupants using a fine-tooth comb. The occupants were provided with a comb each, as sharing of comb was not permitted to prevent lice transmission. Furthermore, the seams and edges of mattresses and pillows were thoroughly examined for the presence of bedbugs. All collected specimens were kept in Ziploc® bags. The specimens were then transferred to the laboratory and were individually preserved in microcentrifuge tubes containing 70% ethanol and stored at -20 °C prior to downstream experiments.

The images of the ectoparasites were viewed and captured by a high-resolution stereomicroscope (Leica Microsystems, Germany). Occupants with head lice infestation were treated with anti-head lice shampoo (Figure 3.3) to eliminate the infestation.

Figure 3.2: Sampling of head lice.This figure shows the sampling process conducted in welfare homes in Greater KL/KV:(A) Baitul Barokah Wal Mahabbah, (B) Pusat Jagaan Nuri, (C) Pusat Jagaan Rumah Kesayangan and (D)Pertubuhan Kebajikan Anak-Anak Yatim & Miskin Sungai Pinang; and the state of Kedah: (E) Pusat Jagaan Baitul Mahabbah Al-Hashimi and (F) Pusat Jagaan IHMK.

Figure 3.3: Anti-head lice products.Dimethicone shampoos and fine-toothed combswere provided to occupants with pediculosis capitis.

3.1.3 Prevalence of pediculosis capitis in the Greater KL/KV and state of Kedah

GraphPad Prism Version 7.01 software was used for data comparison. Un-paired t-test was selected to compare the prevalence rates between the Greater KL/KV and Kedah. A p-value of <0.05 was considered significant.

3.1.4 DNA extraction

One louse per individual was randomly chosen as a representative sample. In total, 260 head lice collected from 260 infested persons and 26 bedbugs were subjected for DNA extraction.All specimens were treated individually. Taken from the 70 % alcohol vial, ectoparasites were first rinsed in distilled water and dried on a sterile filter paper.DNA was extracted from the whole specimen using the NucleoSpin® Tissue kit (Macherey-Nagel, Germany). An additional step was added to crush specimens with pipette tips prior to DNA extraction.Briefly, 180 μl Buffer T1 (pre-lysis buffer) and 25 μl Proteinase K solution were added to the homogenised specimens and mixed thoroughly by pulse-vortexing for 15 seconds. The homogenate was incubated at 56 °C for at least 1 hour. Next, 200 μl Buffer B3 (lysis buffer) was added, vigorously vortexed, and incubated at 70 °C for 10 minutes. As much as 210 μl absolute ethanol was then added, and mixed. The mixture was carefully applied to the spin column and centrifuged at 11,000 x g for 1 minute. Collection tube with flow-through was discarded and the column was placed in a new collection tube. Then, 500 μl Buffer BW (wash buffer) was added and the column was again centrifuged at 11,000 x g for 1 minute. The flow-through was discarded and the column was placed back into the collection tube. Next, 600 μl Buffer B5 (wash buffer) was added and the column was centrifuged at 11,000 x g for 1 minute. Again, the flow-through was discarded and the column was placed back into the collection tube. Next, the column was centrifugedat 11,000 x g for 1 minute to remove residual ethanol. To elute highly pure DNA, the column was placed

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in a sterile 1.5 ml microcentrifuge tube and DNA was eluted using 100 µl Buffer BE (elution buffer). Eluted DNA was stored at -20 °C prior to PCR amplification.

3.1.5 DNA barcoding of head lice and bedbugs

COI barcoding technique was employed to study the genetic diversity of head lice and bedbugs collected from the welfare homes.

3.1.5.1 Amplification of mitochondrial cytochrome c oxidase subunit I (*COI*) gene

All PCRs (20 µl) were performed in a SuperCycler thermal cycler (Kyratec, Australia) by adding 1 µl DNA template to 7 µl sterile distilled water, 1 µl of each primer (10 µM) and 10 µl 2X ExPrime™ Taq Premix (GeNet Bio, Korea) (Table 3.3).

The *COI* partial sequences in head lice were amplified using forward primer 5'-GGTACTGGCTGGACTRTTTATCC-3', and reverse primer 5'-CTAAARACTTTYACTCCCGTTGG-3' as described by Sunantaraporn et al. (2015). PCR amplification conditions include initial denaturation at 95 °C for 3 minutes, followed by 40 cycles of denaturation at 95 °C for 1 minute, annealing at 50°C for 1 minute and extension at 72 °C for 1 minute; and the final extension at 72°C for 7 minutes.

The *COI* partial sequences of bedbugs were amplified using the "Lep" primer combinations: LepF1 (5'-ATTCAACCAATCATAAAGATATTGG-3') and LepR1 (5'-TAAACTTCTGGATGTCCAAAAAATCA-3') as described by Wilson (2012). One cycle of initial denaturation at 94 °C for 1 minute was followed by six cycles of denaturation at 95 °C for 1 minute, annealing at 45 °C for 1 minute 30 seconds and extension at 72 °C for 1 minute 15 seconds; 36 cycles of denaturation at 94 °C for 1 minute, annealing at 51°C for 1 minute 30 seconds and extension at 72 °C for 1 minute 15 seconds. A final extension at 72 °C for 5 minutes was then performed and reactions were held at 4 °.

Table 3.3: PCR reaction mixture used amplification of *COI* partial sequences

Components	Concentration	Volume (µl per reaction)
DNA template	~50 ng/µl	1
ExPrime™ Taq Premix	2X	10
Forward Primer	10 µM	1
Reverse Primer	10 µM	1
Sterile distilled water		7
Total		20

The amplified products of approximately 650 bp were analyzed by agarose gel electrophoresis. 3 µl of 100 bp DNA ladder (GeneDirex, Taiwan) was electrophoresised in parallel with 5 µl PCR products. The amplified product was electrophoresed in 1 % agarose gel with Tris-Acetate-EDTA (TAE) buffer and stained with ethidium bromide at a constant voltage of 90 V for 60 minutes. After completion of electrophoresis, gels were visualized and documented using a UV transilluminator (Nyx Technik Inc, Taiwan). Positive amplification results were indicated by the presence of amplified products with expected sizes.

3.1.5.2 *COI* sequence analyses

The purified PCR products were sequenced by MyTACG Bioscience Enterprise (Selangor, Malaysia) using forward or reverse primers of the respective amplification reaction. The sequences obtained were aligned by ClustalW algorithm in BioEdit Sequence Alignment Editor Software version 7.2.5(Hall, 1999). Each sequence alignment was examined for sequencing quality. The resulting contigs were trimmed and edited, if required. The *COI* DNA barcodes werethen submitted to the BOLD(Ratnasingham & Hebert, 2007), making the data publicly available. The DNA barcodes were assigned into Barcode Index Numbers (BINs) by BOLD (Ratnasingham & Hebert, 2013). BOLD identification tree is produced by a full database sequence identification request in the BOLD website (http://v4.boldsystems.org/).

Pairwise genetic distances for *COI* were computed with the Kimura 2-parameter method (K2P). Neighbour-joining (NJ) phylogenetic trees were constructed in MEGA 6.06 (Tamura et al., 2013),and distance histograms were generated with the online version of automatic barcode gap discovery (ABGD)(Puillandre et al., 2012). Genetic diversity indices includinghaplotype diversity (Hd) and nucleotide diversity (π), and

neutrality test (Tajima's *D*) was generated by using DnaSP version 5.10.01(Rozas, 2009).

3.2 Molecular detection of potential associated pathogens

All PCRs (20 µl) were performed in a SuperCycler thermal cycler (Kyratec, Australia) by adding 5 µl DNA template to 3 µl sterile distilled water, 1 µl of each primer (10 µM) and 10 µl 2X ExPrime™ Taq Premix (GeNet Bio, Korea) as shown in Table 3.4.

3.2.1 Amplification of *Rickettsia* spp. DNA

Amplification of a 401 bp of rickettsial *gltA*in head lice and bedbugs was performed using forward primer CS-78 (5'-GCAAGTATCGGTGAGGATGTAAT-3') and reverse primer CS-323 (5'-GCTTCCTTAAAATTCAATAAATCAGGAT-3')(Labruna et al., 2004). PCR thermal cycling conditions were as follows: one cycle of initial denaturation at 94 °C for 10 minutes, 35 cycles of 15 seconds at 95 °C, 30 seconds at 55 °C, and 30 seconds at 72 °C, and one final step at 72 °C for 7 minutes (Capelli et al., 2009). In every experiment, sterile distilled water was used as a negative control. I also used DNA of *Rickettsia honei* (kindly provided by Prof. Tay Sun Tee from Department of Medical Microbiology, Faculty of Medicine, University of Malaya) as a positive control.

3.2.2 Amplification of *B. quintana*DNA

Primers BhCS781p (5'-GGGGACCAGCTCATGGTGG-3') and BhCS1137n (5'- AATGCAAAAAGAACAGTAAACA-3') (Norman et al., 1995)which amplify a 379 bp *gltA* fragment of *Bartonella* spp. were used to detect *B. quintana* DNA in the head lice and bedbugs. PCR amplification profiles for this primer set consisted of 35 cycles of denaturation at 95 °C for 20 seconds, annealing at 51 °C for 30 seconds, and extension at 72 °C for 2 minutes. Sterile distilled water as a negative control and DNA

of *Bartonella tribocorum* (kindly provided by Prof. Tay Sun Tee from Department of Medical Microbiology, Faculty of Medicine, University of Malaya) as a positive control were included in every experiment.

Table 3.4: PCR reaction mixture used for PCR amplifications of associated pathogens

Components	Concentration	Volume (µl per reaction)
DNA template	~50 ng/µl	5
ExPrime™ Taq Premix	2X	10
Forward Primer	10 µM	1
Reverse Primer	10 µM	1
Sterile distilled water		3
Total		20

A primer pair of PBH-L1 (5'-GATATCGGTTGTGTTGAAGA-3') and PBQ-R1 (5'-AAAGGGCGTGAATTTTG-3') (Bereswill et al., 1999) which is specific to *B. quintana* was used to amplify a 390 bp riboflavin gene (*ribC*). PCR was performed for 30 cycles with denaturation at 94 °C for 1 minute, annealing at 60 °C for 2 minutes, and extension at 72 °C for 3 minutes. A final extension at 72 °C for 10 minutes was then performed and reactions were held at 4 °C.

3.2.3 Amplification of *Acinetobacter* spp. DNA

Head lice were subjected to PCR assays targeting RNA polymerase β-subunit (*rpoB*) and recombinant protein A (*recA*) genes of *Acinetobacter* spp.

Amplification of a 238 bp of *AcinetobacterrpoB* was performed using primer pair AcropBF (5'-TACTCATATACCGAAAAGAAACGG-3') and AcropBR (5'-GGYTTACCAAGRCTATACTCAAC-3') as described by Kempf et al. (2012). PCR amplification conditions were as follows: initial denaturation at 95 °C for 3 minutes,followed by 40 cycles of denaturation at 95 °C for 1 minute, annealing at 62 °C for 1 minute and extension at 72 °C for 1 minute; and the final extension at 72°C for 7 minutes. Sterile distilled water was used as a negative control in every experiment.

The 424 bp of *recA*which is was shown to be specific to *A. baumannii*was amplified using primers rA1F (5'-CCTGAATCTTCTGGTAAAAC-3') and rA2R (5' GTTTCTGGGCTGCCAAACATTAC-3') as described by Krawczyk et al. (2002). PCR was performed with 35 cycles consisting of denaturation at 94 °C for 1 minute, annealing at 57 °C for 1 minute and extension at 72 °C for 2 minutes.Sterile distilled water was used as a negative control in every experiment.

3.2.4 Amplification of *Serratia marcescens* DNA

Primers YV1 (5'-GGGAGCTTGCTCCCCGG-3') and YV4 (5'-AACGTCAATTGATGAACGTATTAAGT-3') (Melcher et al., 1999) were used to amplify a 409 bp of the 16S *rRNA* region of *S. marcescens*. PCR amplification conditions consisted of initial denaturation at 95 °C for 3 minutes; followed by 45 cycles of denaturation at 95 °C for 30 seconds, annealing at 55 °C for 30 seconds, and extension at 72 °C for 2 minutes; and a final extension at 72 °C for 10 minutes.

3.2.5 Amplification of *Staphylococcus aureus* DNA

Amplification of the 279 bp of *S. aureus* thermostable nuclease (*nucA*) gene was performed using the following primer pair: 5'-GCGATTGATGGTGATACGGTT-3' and 5'-AGCCAAGCCTTGACGAACTAAAGC-3' (Brakstad et al., 1992). The PCR for 533 bp *mecA* structural gene of MRSA was performed using primer pair Mec-A1 (5'-AAAATCGATGGTAAAGGTTGGC-3') and Mec-A2 (5'-AGTTCTGCAGTACCGGATTTGC-3') (Kobayashi et al., 1994). The PCR amplifications for both genes were performed with 25 cycles of denaturation at 94 °C for 1 minute, annealing at 57 °C for 1 minute and extension at 72 °C for 2 minutes.

3.2.6 Amplification of fungal DNA

Primers ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') were used to amplify the internal transcribed spacer (*ITS*) of fungal *rRNA* (White et al., 1990). The PCR cycling protocol consisted of the following: 32 cycles of denaturation at 94 °C for 1 minute, annealing at 58 °C for 1 minute, and extension at 72 °C for 1 minute.

3.2.7 Phylogenetic analyses of potential associated pathogens

The purified PCR products were sequenced by MyTACG Bioscience Enterprise (Selangor, Malaysia) using forward or reverse primers of the respective amplification reaction. The sequences obtained were aligned by ClustalW algorithm with the corresponding sequences of other related species available in the GenBank database usingBioEdit Sequence Alignment Editor Software version 7.2.5(Hall, 1999). Each sequence alignment was examined for sequencing quality. The resulting contigs were trimmed and edited, if required.

Phylogenetic analyses were conducted using MEGA version 6 (Tamura et al., 2013).Phylogeny reconstruction was performed using Neighbor-joining (NJ) method and Bootstrap method with 1000 replications was used to determine the confidence values for individual branches of the resulting tree. Kimura 2-parameter model was used to calculate pairwise distances between nucleotide sequences.All positions containing gaps and missing data were eliminated. The subtree for each clade of lice was collapsed with the "compress/ expand subtree" function.

3.3 Arthropods in medical case reports

Arthropod specimens from three medical case reports consisted of larvae of flies and beetles causing gastrointestinal upsets and ticks of aural and ocular infestations were sent to the Department of Parasitology, Faculty of Medicine, and the Museum of Zoology, University of Malaya, Kuala Lumpur.

3.3.1 Ethics statement

The patient and parents of medical case report 1 (aural and ocular tick infestations) verbally consented for the documentation of the cases. Patient in case report 2 (intestinal myiasis) provided written consent for this study (Appendix A). The specimens in case report 3 (canthariasis) were submitted to the Department of Parasitology by the parent himself who is the co-author of the published article (Appendix B) in regard to the case.

3.3.2 DNA extraction

DNA was extracted from the whole specimens using the NucleoSpin® Tissue kit (Macherey-Nagel, Germany) as described in section 3.1.3.

3.4.3 DNA barcoding

The specimens were subjected to molecular identification through DNA barcoding. The *COI* partial sequences were amplified using the "Lep" primer combinations as described in section 3.1.5.1 and subjected to analyses as described in section 3.1.5.2.

CHAPTER 4: RESULTS

4.1 Prevalence of medically-important ectoparasites in welfare homes

4.1.1 Specimen collection

Specimen collection from welfare homes produced two blood-feeding species of medically-important ectoparasites; the human head lice, *P. h. capitis*(Pthiraptera: Pediculidae)and bed bugs, *C. hemipterus*(Hemiptera: Cimicidae). Overall, 900 head lice from 143 individuals were collected from fifteen welfare homes across Greater KL/KV. In addition, 26 bedbugs were collected from Rumah Titian Kaseh located in Kuala Lumpur. In the state of Kedah, a total of 832 head lice were collected from 117 individuals in ten welfare homes.

Figures 4.1 and 4.2 show the images of human head lice, *P. h. capitis* and *C. hemipterus* bedbug collected in this study, respectively.

4.1.2 *P. h. capitis* infestation

The prevalence rate of pediculosis ranged from 13% to 100% and 34.3% to 100% in welfare homes in Greater KL/KV and state of Kedah, respectively. The overall prevalence rate for pediculosis in Kedah was slightly higher, i.e. 64.9%, compared to Greater KL/KV which prevalence rate was 59.2%. However, no statistical difference was found between the two values (p=0.56).

Tables 4.1 and 4.2 summarize the prevalence of pediculosis among occupants in welfare homes across the Greater KL/KV and state of Kedah, respectively.
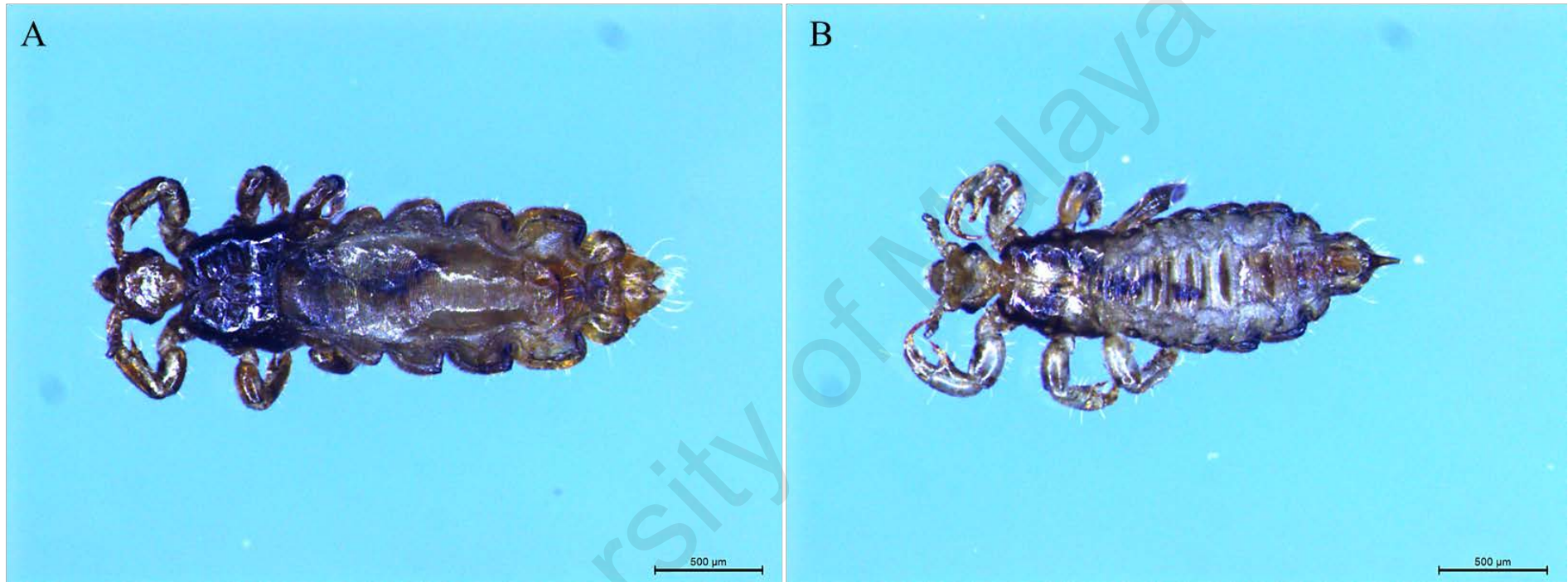
Figure 4.1:*P. h. capitis* collected in this study. (A) Dorsal view of ethanol-preserved female head louse collected from Rumah Anak Yatim Amal Solehah, Pendang, Kedah. The female is generally larger and relatively has broader abdomen. The abdomen terminates into two posterior lobes, giving a bi-lobed appearance. (B) Dorsal view of ethanol-preserved male head louse collected from Pusat Jagaan Nuri, Setapak, Kuala Lumpur. The male abdomen has dark transverse bands on the dorsum and its posterior end is rounded with dark pointed tip of the dilator.
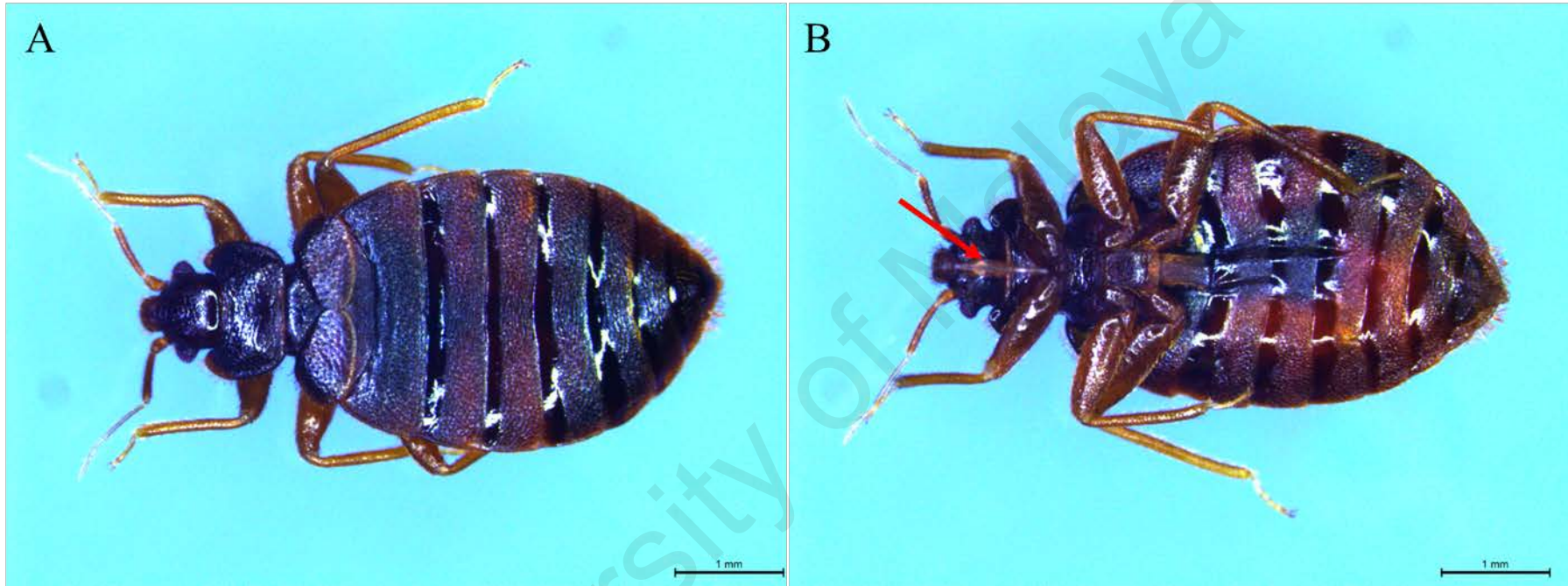
Figure 4.2: The tropical bedbug, *Cimex hemipterus*. Dorsal (A) and ventral view (B) of ethanol-preserved adult male bedbug collected from Rumah Titian Kaseh, Kuala Lumpur. All bedbugs collected in this study were dark-brown coloured. The bite unit (red arrow) is folded under the head.

Table 4.1: Prevalence of pediculosis in welfare homes across Greater KL/KV

	City	Welfare homes	No. of occupants examined	No. of occupants infested	No. of head lice	Prevalence (%)
1	Kuala Lumpur	Rumah Titian Kaseh	20 girls	20	155 head lice	100
2	Ampang	Rumah BAKTI Datuk Harun	16 girls	10	62 head lice	62.5
3	Rawang	Baitul Aini Selangor	23 boys	3	10 head lice	13.0
4	Rawang	Pusat Tahfiz Anak Yatim Nur Ikhlas	18 girls	8	42 head lice	44.4
5	Banting	Rumah Anak Yatim & Asnaf As-Sholihin	14 girls	11	58 head lice	78.6
6	Bandar Baru Bangi	Rumah Bakti Al-Kausar	11 girls	6	33 head lice	54.5
7	Kuala Lumpur	Pusat Jagaan Kasih Murni	25 girls	11	74 head lice	44.0
8	Sepang	Baitul Barokah Wal Mahabbah	29 girls	16	70 head lice	55.2
9	Petaling Jaya	Pusat Jagaan Rumah Kesayangan	16 girls	10	73 head lice	62.5
10	Kajang	Rumah Nur Hikmah	13 girls	8	101 head lice	61.5
11	Puchong	Rumah Amal Limpahan Kasih	6 girls	2	14 head lice	33.3
12	Shah Alam	Rumah Amal Al-Firdaus	22 girls	15	83 head lice	68.2
13	Setapak	Pusat Jagaan Nuri	9 girls, 5 boys	9	68 head lice	64.3
14	Cheras	Rumah Jalinan Kasih	13 girls	6	11 head lice	46.2
15	Klang	Pertubuhan Kebajikan Anak-Anak Yatim & Miskin Sungai Pinang	8 girls	8	46 head lice	100

Table 4.2: Prevalence of pediculosis in welfare homes in the state of Kedah

	Town/City	Welfare homes	No. of occupants examined	No. of occupants infested	No. of head lice	Prevalence (%)
1	Kuala Nerang	Pusat Jagaan Baitul Mahabbah Al-Hashimi	10 girls	5	11 head lice	50.0
2	Pendang	Rumah Anak Yatim Amal Solehah	14 girls	9	79 head lice	64.3
3	Alor Star	PERKIM Bahagian Negeri Kedah	35 girls	12	64 head lice	34.3
4	Baling	Persatuan Kebajikan Anak Yatim & Miskin Nur Hidayah	14 girls	14	102 head lice	100
5	Sik	Pertubuhan Asuhan dan Didikan Anak-Anak Yatim Islam Daerah Sik	48 girls	32	262 head lice	66.7
6	Gurun	Persatuan Kebajikan Anak Yatim dan Miskin Al-Munirah	13 girls	11	201 head lice	84.6
7	Yan	Rumah Budi Kedah	10 girls	4	13 head lice	40.0
8	Langkawi	Rumah Nur Kasih	11 girls	10	44 head lice	90.9
9	Kulim	Pertubuhan Pembangunan Anak-Anak Yatim Bekas Parajurit Malaysia Daerah Kulim	22 girls	7	20 head lice	31.8
10	Jitra	Pusat Jagaan IHMK	15 girls	13	42 head lice	86.7

4.1.3 *C. hemipterus* infestation

C. hemipterus were recovered from only one shelter, Rumah Titian Kaseh, out of fifteen welfare homes in the Greater KL/KV. All ten welfare homes in Kedah were free from bedbug infestations at the time of inspection.

4.2 DNA barcoding of ectoparasites collected from welfare homes

The *COI* partial sequences of approximately 599 bp (in head lice) and 650 bp (in bedbugs) were amplified, sequenced, trimmed and aligned with reference sequences available in BOLD.

4.2.1 *COI* analyses of *P.h. capitis*

DNA barcodes of 260 head lice were aligned with five Barcode Index Numbers (BINs) available in the BOLD: AAA1556, AAA1557, AAA1558, AAW5034 and ACR6059 representing clade A, B, D, C, D and E, respectively. After sequence correction, Neighbor-Joining (NJ) analysis of *COI* barcodes clearly identified the head lice as belonging to three clades of *P. h. capitis* corresponding with three BINs: BOLD: AAA1556 (clade A), BOLD: AAA1557 (clade B) and BOLD:AAW5034 (clade D) as shown in Figure 4.4.

Clade D head lice represents the majority of the overall population i.e., 57%, whereas clade A represents 41% of the head lice population. Clade B head lice poses the lowest population rate of 2%, and were detected only in two shelters located in Greater KL/KV.
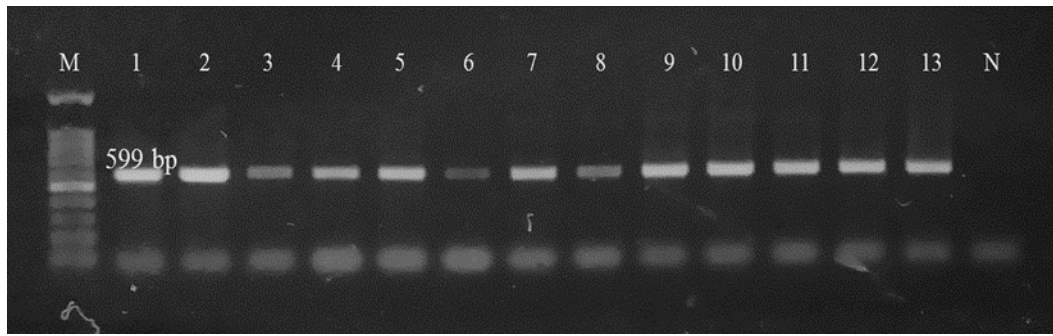
Figure 4.3: Amplification of 599 bp of *COI* partial gene by COIF/COIR primer pair. M: 100 bp DNA ladder; 1-13: head lice specimens; N: negative control (sterile distilled water).
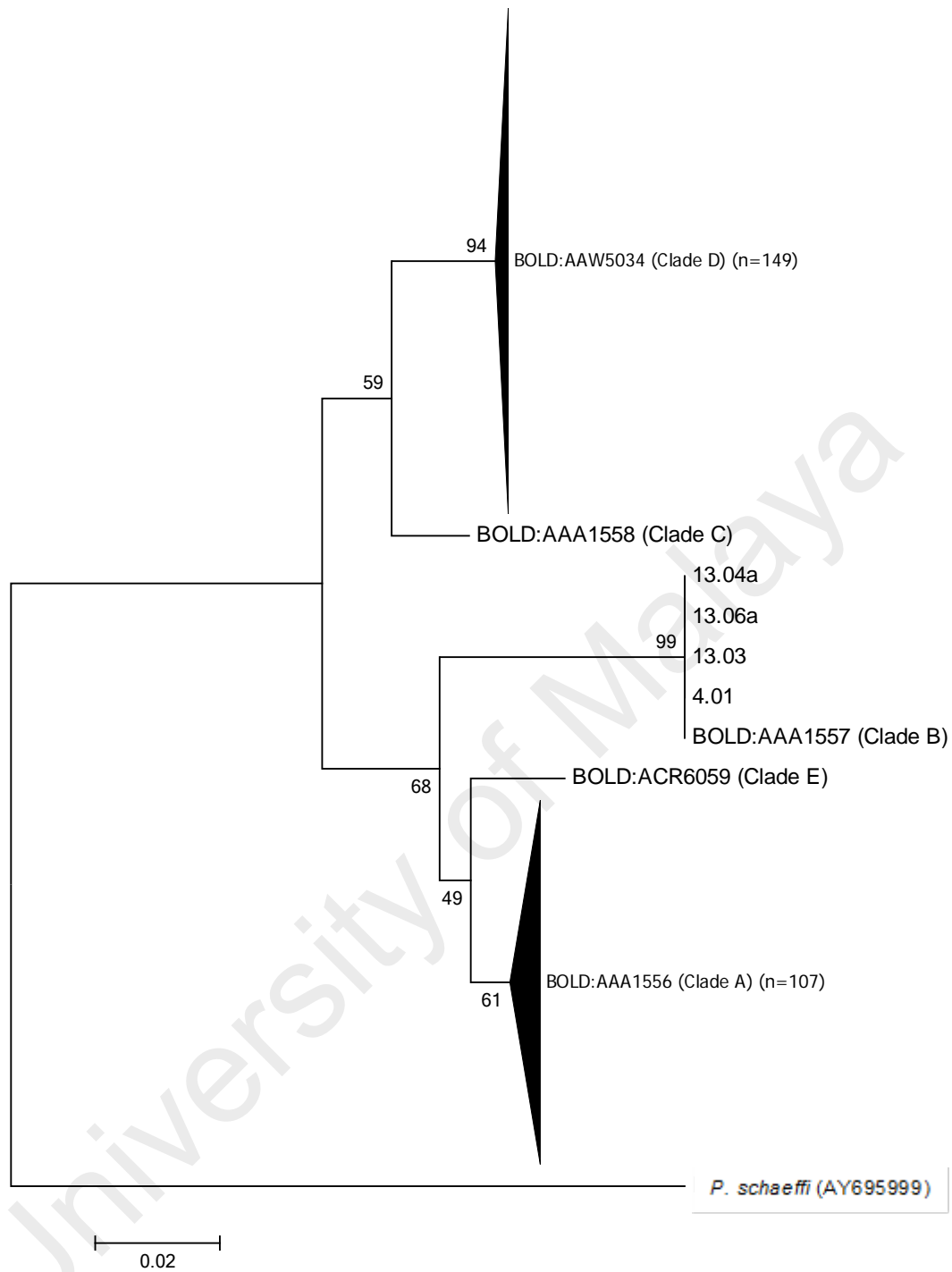
Figure 4.4: NJ cluster analysis based on partial *COI* sequences showing the phylogenetic placement of the 260 head lice into three BINs of Clade A, B and D. Bootstrap analysis was performed with 1000 replications. The scale bar shows K2P distances. *Pediculus schaeffi* (Accession Number: AY695999) was used as an outgroup.

K2P model calculation results using MEGA 6.06 showed that intra- and inter-specific genetic distance exists obvious difference. The intra-specific K2P divergence ranged from 0.000 % to 0.012 % with an average of 0.005 % (Table 4.3a). The inter-spesific K2P divergence between the three clades averaged 0.074 %, ranging from 0.070 % to 0.078 % (Table 4.3b). All inter-specific divergence values were greater than intra-specific values, more than 14 times.

The results of applying the ABGD algorithm to the *COI* dataset are presented in Figure 4.5. Distance values show a gap between the intra-specific and the inter-specific distances (Figure 4.5a). The data set was partitioned into three groups when the prior assumption of maximum intra-specific divergence was set as high as 1.29 % (Figure 4.5b).

Genetic diversity indices and the results of neutrality test for *COI* are shown in Table 4.4. The average number of pairwise nucleotide differences (k), nucleotide diversity (π) and haplotype diversity (Hd) varied among clades A and D. Four sequences of clade B show no diversity.

Table 4.3A: The intra -specific K2P divergence values (%) of *COI*

Clade	Intra-specific distance		
	Average	Minimum	Maximum
A	0.005	0.000	0.012
B	0.000	0.000	0.000
D	0.000	0.000	0.004
All	0.005	0.000	0.012

Table 4.3B: The inter-specific K2P divergence values (%) of *COI*

Comparison between clades	Distance
Clade A against Clade B	0.075
Clade A against Clade D	0.070
Clade B against Clade D	0.078
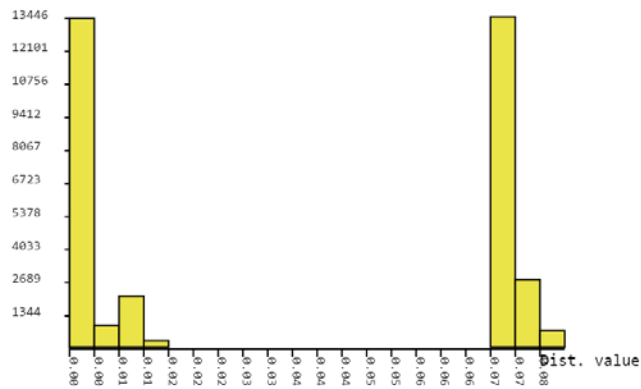
(A)
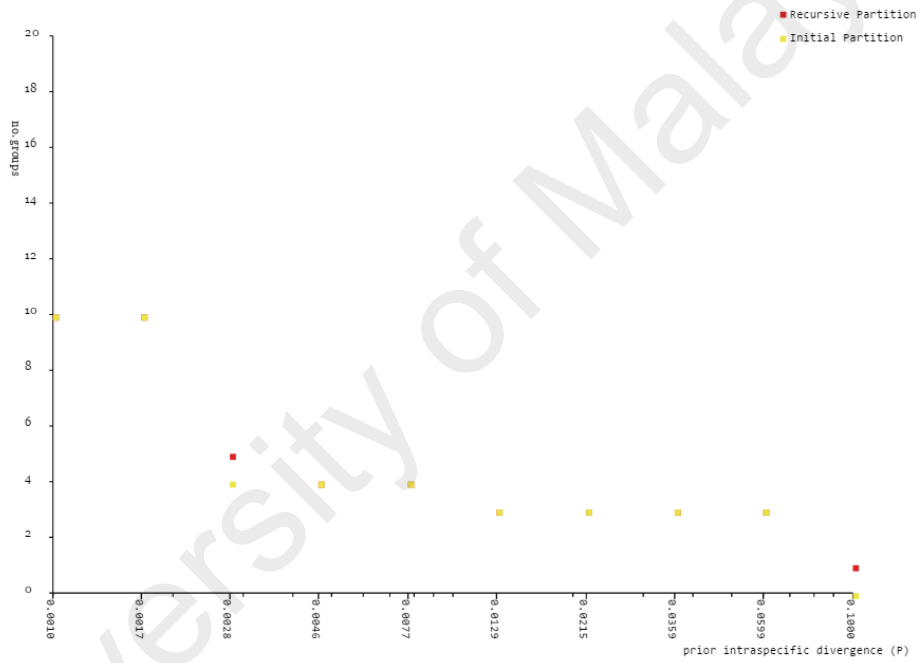
(B)

Figure 4.5: Histogram of pairwise (K2P) distances (A) and automatic partition results (B) among 260 *COI* sequences of head lice.

Table 4.4: Genetic diversity indices and neutrality test (Tajima's *D*) on the *COI* sequences of head lice

Clade	n	S	k	π ± SD	h	Hd ±SD	Tajima's *D*
A	107	9	2.581	0.00524 ± 0.00030	6	0.661 ± 0.031	1.24632
B	4	0	*	*	1	*	*
D	149	2	0.217	0.00044 ± 0.00009	3	0.215 ± 0.041	-0.56546

*Sequences show no diversity. n: number of sequences; S: number of polymorphic sites; k: average number of pairwise nucleotide differences; π: nucleotide diversity; h: number of haplotypes; Hd: haplotype diversity.

Tajima's *D*: A negative Tajima's *D* signifies an excess of low frequency polymorphisms relative to expectation. A positive Tajima's *D* signifies low levels of both low and high frequency polymorphisms. Statistical significance: Not significant, P > 0.10.

Interestingly, similar pattern of clade distribution was noted in both areas; where clade D lice was the highest population followed by lice of clade A (Figure 4.6).

Head lice collected in this study were not distinguishable by morphological characteristics. Head lice of different clade and within the same clade show colour variations; some were light-brown coloured, some were dark-brown coloured and some were black coloured as shown in Figures 4.7, 4.8 and 4.9.

Table 4.5 and Table 4.6 summarize the distribution of the three clades of head lice in the Greater KL/KV and state of Kedah, respectively.

Frequency

Greater KL/KV   Kedah

Figure 4.6: Distribution of clade A, B and D head lice in Greater KL/KV and the state of Kedah. Clade B lice was not detected in Kedah.
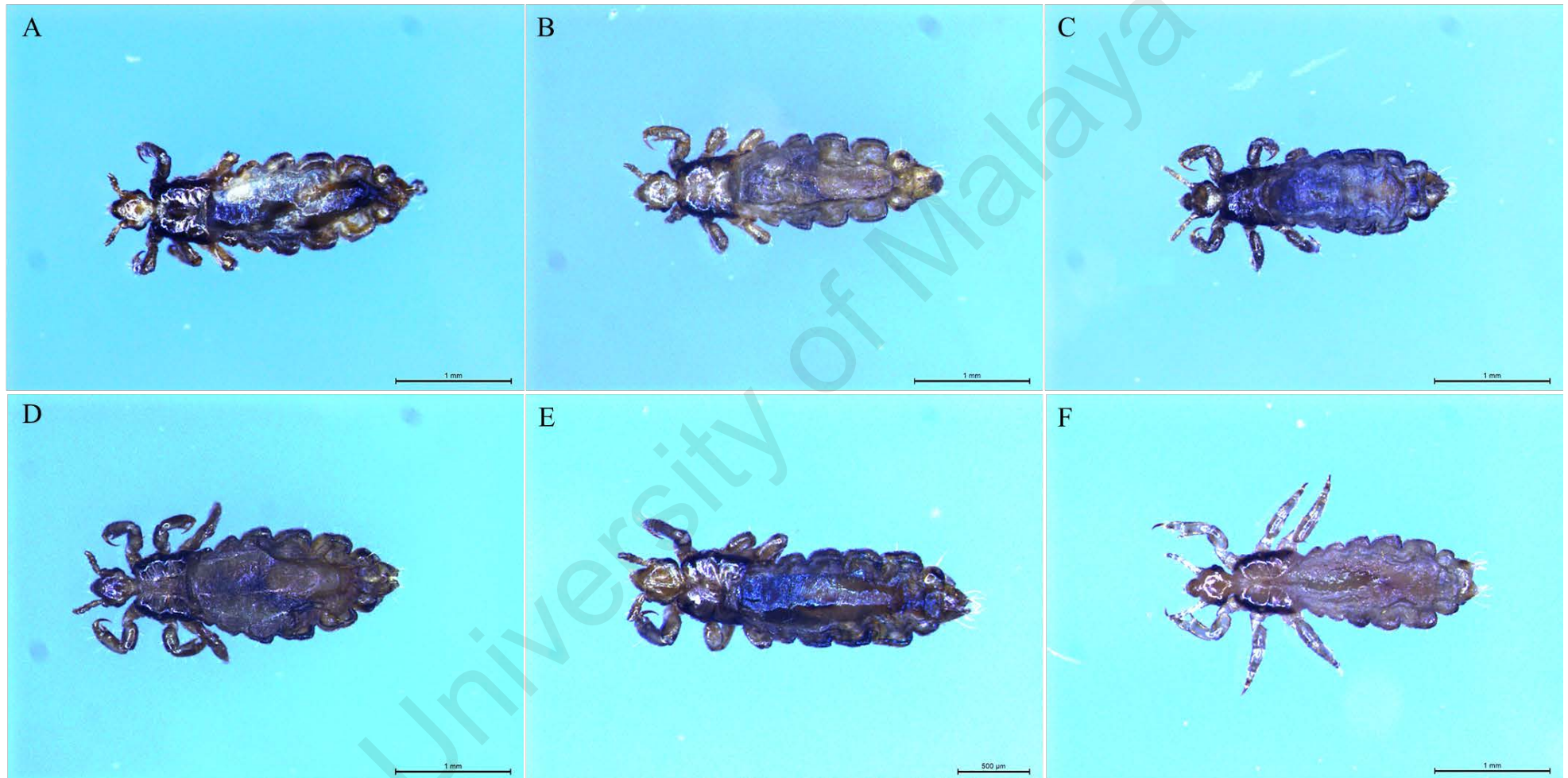
Figure 4.7: Head lice of clade A. These specimens were collected from Pusat Jagaan Kasih Murni (A), Baitul Barokah Wal Mahabbah (B) and Pusat Jagaan Rumah Kesayangan (C) located in Greater KL/KV; and Persatuan Kebajikan Anak Yatim Miskin Nur Hidayah (D), Pertubuhan Asuhan dan Didikan Anak-Anak Yatim Islam Daerah Sik (E) and Persatuan Kebajikan Anak Yatim dan Miskin Al-Munirah (F)located in the state of Kedah.
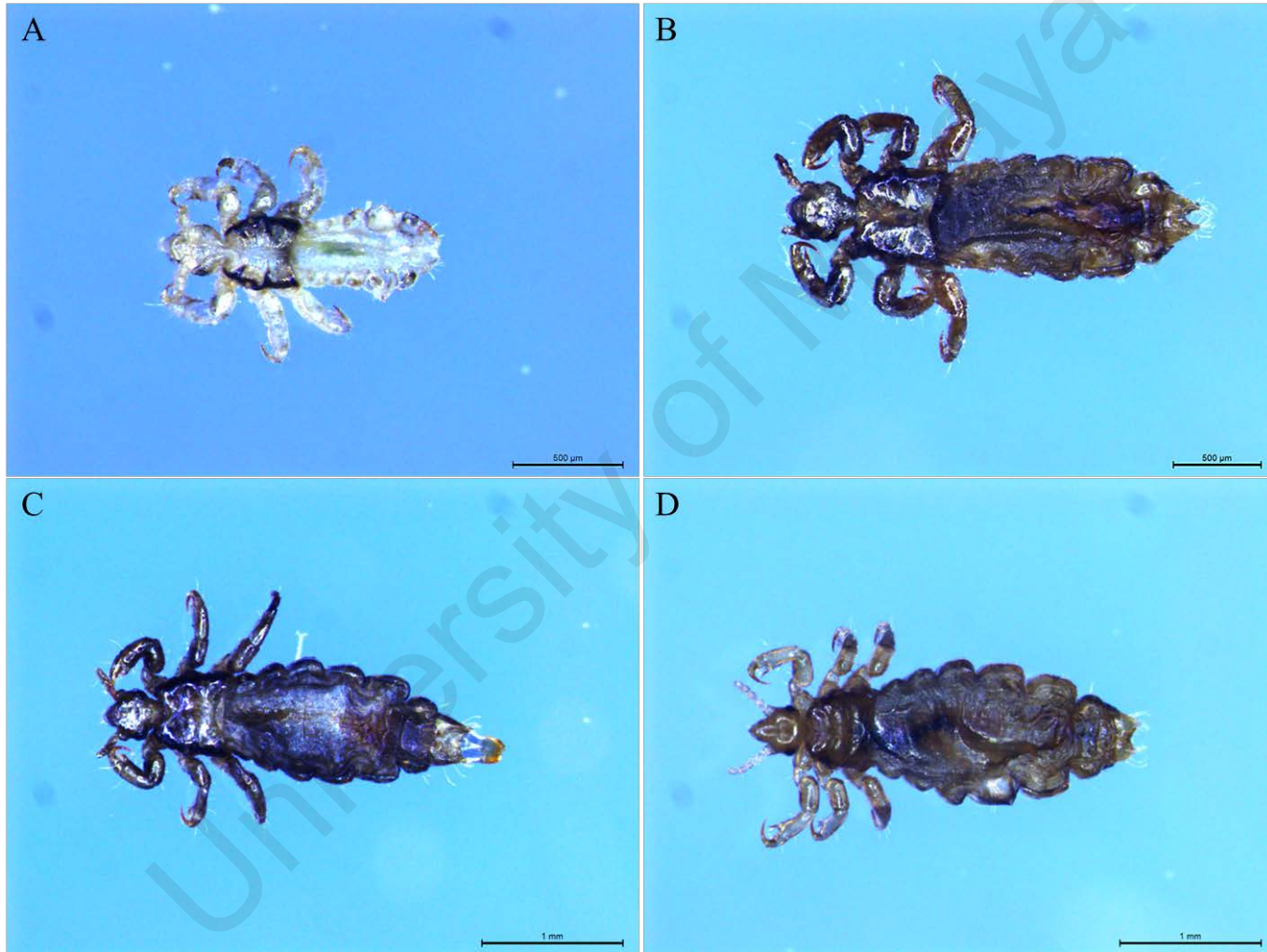
Figure 4.8: Head lice of clade B.These specimens were collected from Pusat Tahfiz Anak Yatim Nur Ikhlas (A) and Pusat Jagaan Nuri (B, C and D) located in Greater KL/KV.
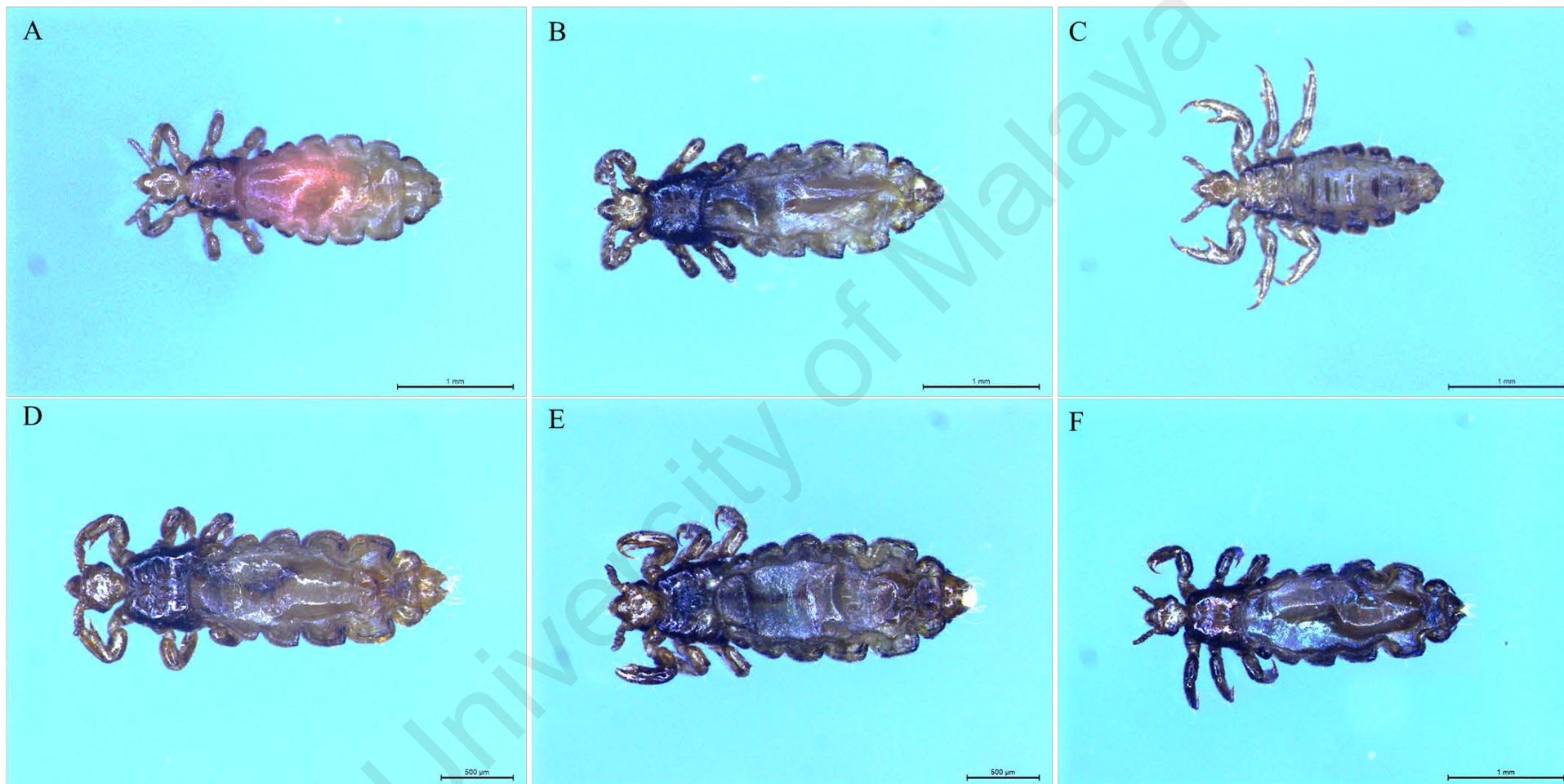
Figure 4.9: Head lice of clade D**.** These specimens were collected from Rumah Titian Kaseh (A), Rumah BAKTI Datuk Harun (B) and Rumah Nur Hikmah (C) located in Klang Valley; and Rumah Anak Yatim Amal Solehah (D), PERKIM Bahagian Negeri Kedah (E) and Rumah Nur Kasih (F) located in state of Kedah.

Code	Town	Welfare homes	No. of head lice being processed	Clades (n)

KV1	Kuala Lumpur	Rumah Titian Kaseh	20	A (7), D (13)
KV2	Ampang	Rumah BAKTI Datuk Harun	10	D (10)
KV3	Rawang	Baitul Aini Selangor	3	D (3)
KV4	Rawang	Pusat Tahfiz Anak Yatim Nur Ikhlas	8	A (2), B (1), D (5)
KV5	Banting	Rumah Anak Yatim & Asnaf As-Sholihin	11	D (11)
KV6	Bandar Baru Bangi	Rumah Bakti Al-Kausar	6	D (6)
KV7	Kuala Lumpur	Pusat Jagaan Kasih Murni	11	A (8), D (3)
KV8	Sepang	Baitul Barokah Wal Mahabbah	16	A (11), D (5)
KV9	Petaling Jaya	Pusat Jagaan Rumah Kesayangan	10	A (3), D (7)
KV10	Kajang	Rumah Nur Hikmah	8	D (8)
KV11	Puchong	Rumah Amal Limpahan Kasih	2	A (2)
KV12	Shah Alam	Rumah Amal Al-Firdaus	15	A (12), D (3)
KV13	Setapak	Pusat Jagaan Nuri	9	A (2), B (3), D (4)
KV14	Cheras	Rumah Jalinan Kasih	6	A (2), D (4)
KV15	Klang	Pertubuhan Kebajikan Anak-Anak Yatim & Miskin Sungai Pinang	8	A (7), D (1)

Distribution
B and D
across
KL/KV

Table 4.5:
of clade A,
head lice
Greater

Table 4.6: Distribution of clade A and D head lice in the state of Kedah

Code	Town	Welfare homes	No. of head lice being processed	Clades (n)
K1	Kuala Nerang	Pusat Jagaan Baitul Mahabbah Al-Hashimi	5	A (3), D (2)
K2	Pendang	Rumah Anak Yatim Amal Solehah	9	A (1), D (8)
K3	Alor Star	PERKIM Bahagian Negeri Kedah	12	D (12)
K4	Baling	Persatuan Kebajikan Anak Yatim & Miskin Nur Hidayah	14	A (4), D (10)
K5	Sik	Pertubuhan Asuhan dan Didikan Anak-Anak Yatim Islam Daerah Sik	32	A (18), D (14)
K6	Gurun	Persatuan Kebajikan Anak Yatim dan Miskin Al-Munirah	11	A (10), D (1)
K7	Yan	Rumah Budi Kedah	4	D (4)
K8	Langkawi	Rumah Nur Kasih	10	A (1) ,D (9)
K9	Kulim	Pertubuhan Pembangunan Anak-Anak Yatim Bekas Parajurit Malaysia Daerah Kulim	7	A (1), D (6)
K10	Jitra	Pusat Jagaan IHMK	13	A (13)

4.2.1.1 Occurrence of multiple clades within same shelters

Two shelters in Greater KL/KV, Pusat Tahfiz Anak Yatim Nur Ikhlas in Rawang and Pusat Jagaan Nuri in Setapak presented with head lice belonging multiple clades (clades A, B, and D). Seventeen shelters (seven each in Greater KL/KV and Kedah) had occurrence of double clades (clades A and D).

4.2.1.2 Occurrence of multiple clades within same individual

NJ cluster analysis of four individuals fromPusat Jagaan Nuri located in Setapak, Kuala Lumpur revealed that one individual (individual 13.06) was having head lice of clade B (n=1) and D (n=5); whereas individual 13.01, 13.04 and 13.05 were having single head lice infestation of clade A, B and D, respectively (Figure 4.10).

4.2.2 *COI* analyses of *Cimex hemipterus*

Approximately 650 bp of *COI* partial sequences were amplified in bedbugs collected from Rumah Titian Kaseh (Figure 4.11). DNA barcoding confirmed the bedbugs as belonging to the species, *Cimex hemipterus*. The DNA barcodes of the 26 bedbugs shared 99.78% similarity to DNA barcode in the BOLD (assigned the BOLD BIN Number - BOLD: AAR9233 and BOLD ID: BBCH001-16) and nested within other DNA barcodes named *C. hemipterus* on a BOLD identification tree (Figure 4.12). Two specimens (1.02a and 1.02e) showed one nucleotide change (C instead of T) at position 511.

The resulting barcodes were uploaded to BOLD and are available in the public dataset

(http://www.boldsystems.org/index.php/Public_RecordView?processid=BBCH001-16).
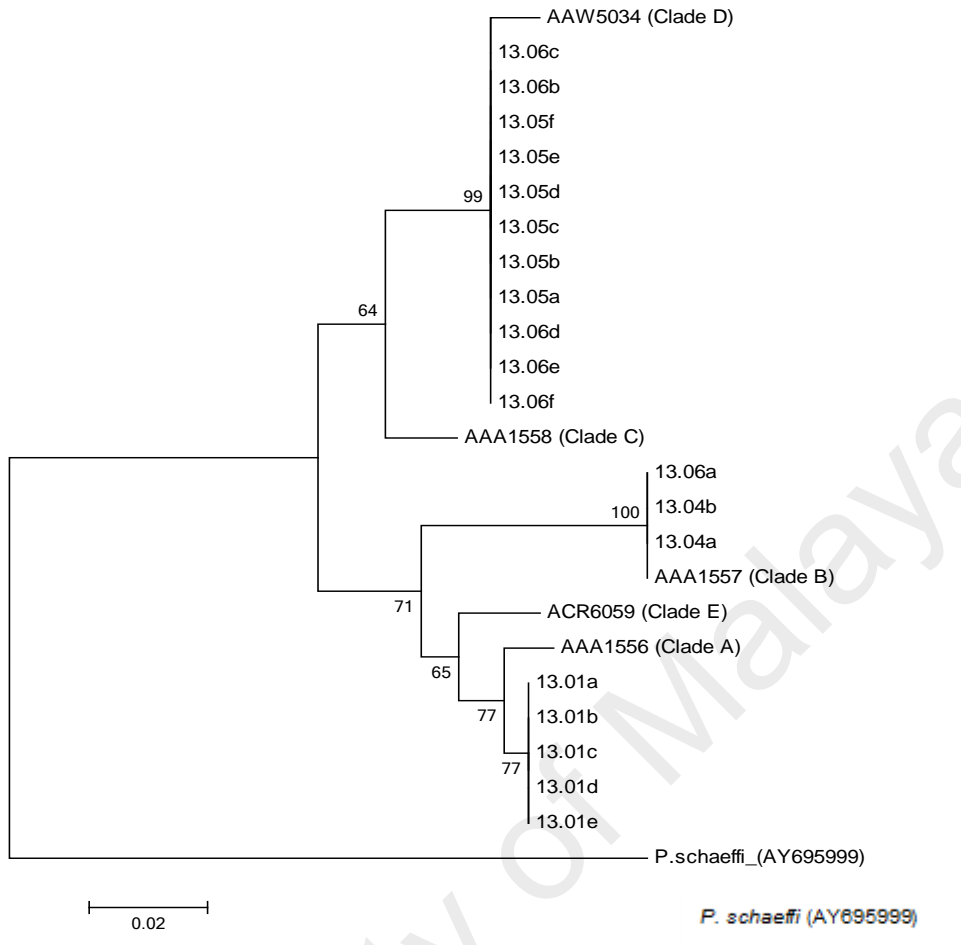
Figure 4.10:NJ cluster analysis of *COI* partial sequences of head lice collected from four individuals. Individual 13.06 was identified as having head lice infestation belonging to clade B (louse 13.06a) and D (lice 13.06c, 13.06b, 13.06d, 13.06e and 13.06f). Bootstrap analysis was performed with 1000 replications. The scale bar shows K2P distances. *P. schaeffi* (Accession Number: AY695999) was used as an outgroup.
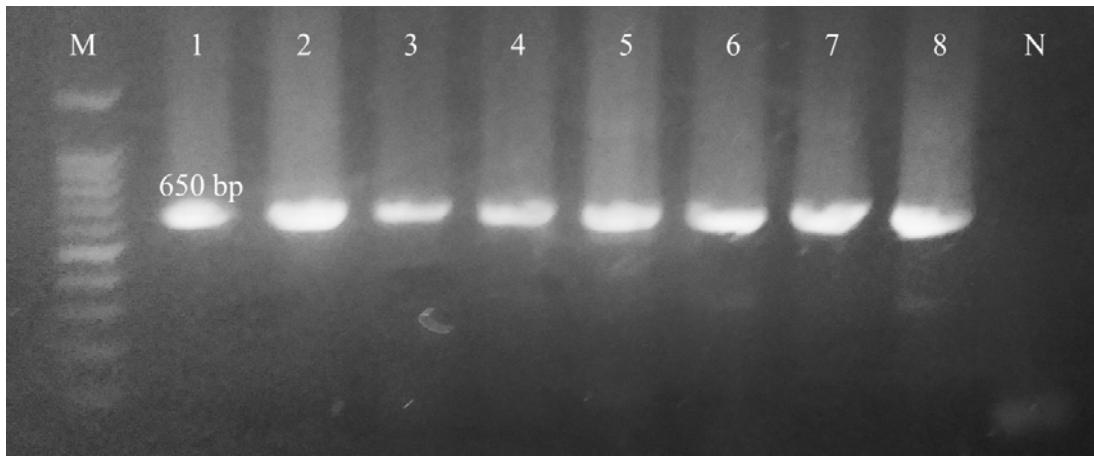
Figure 4.11: Amplification of 650 bp of *COI* partial gene in bedbugs collected from Rumah Titian Kaseh. M: 100 bp DNA ladder; 1-8: bedbug specimens; N: negative control (sterile distilled water).
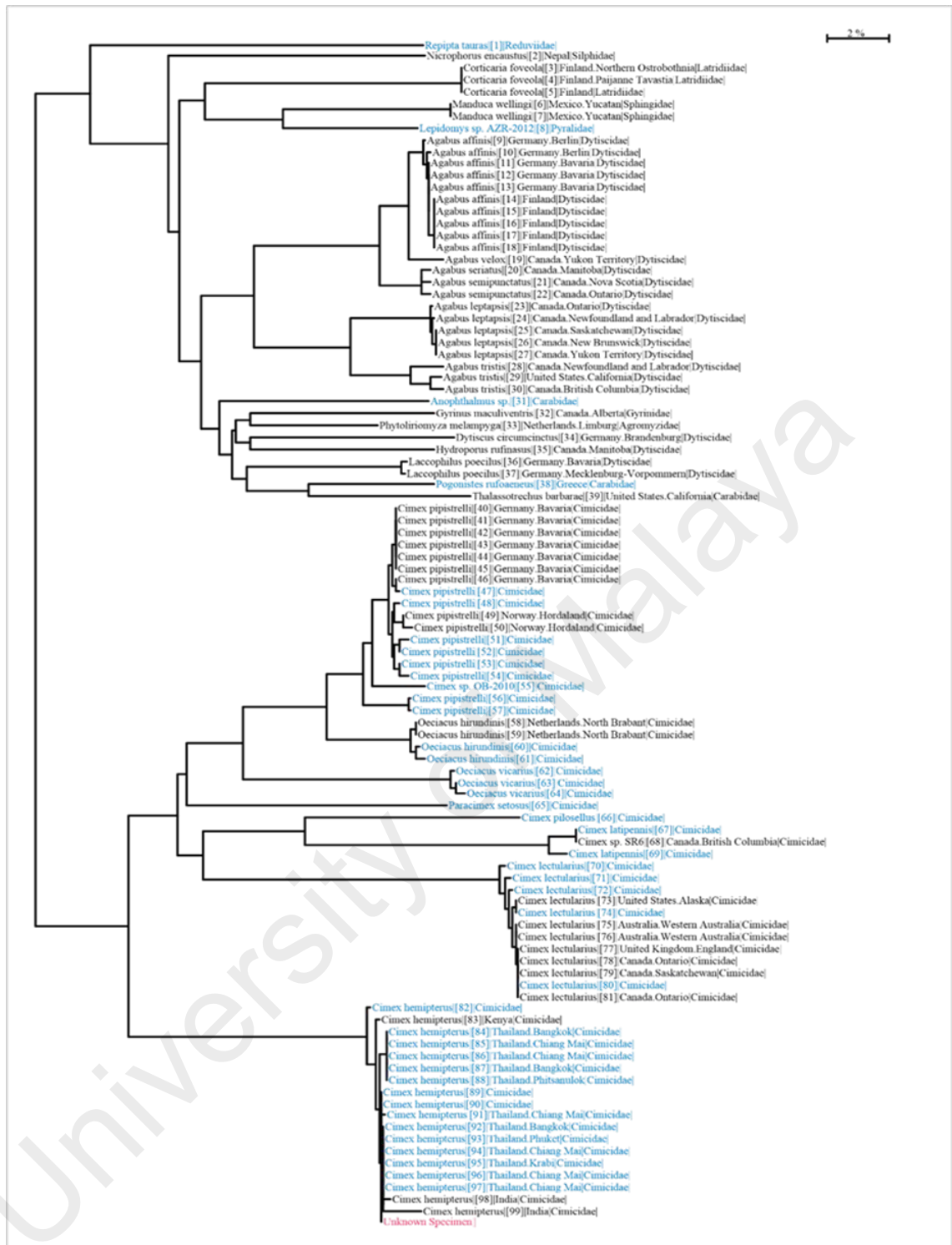
Figure 4.12:BOLD identification tree. This tree is produced by a full database sequence identification request in BOLD for the bedbug specimens. The *COI*sequence (designated as unknown specimen) groups closely with those from Thailand and Kenya; and the species is nested within a cluster of *C. hemipterus*.

4.3 Molecular detection of potential associated pathogens

A total of 260 head lice and 26 bedbugs were screened for the presence of their potential associated pathogens.

4.3.1 Molecular detection of bacteria in head lice

Head lice were subjected to PCRs of *Rickettsia* spp., *B. quintana*, *B. recurrentis*, *Acinetobacter* spp., *S. marcescens*, and *S. aureus*.

4.3.1.1 Molecular detection of *Rickettsia* spp., and *B. quintana*

In this study, the PCR investigations of *Rickettsia* spp., and *B. quintana*did not detect any positive specimens. Positive controls in PCRs of *Rickettsia* spp. and *B. quintana* amplified normally as shown in Figures 4.13A and 4.13B, respectively.

4.3.1.2 Molecular detection of *Acinetobacter* spp.

The DNA of *Acinetobacter* spp. were detected in 63 (24.2%) head lice belonging to clade A (n=18) and D (n=45). The sequences of *Acinetobacter* spp. were compared with those available in the GenBank database. The *rpoB* sequences obtained were 100% identical to previously reported sequence of *A. baumannii*(GenBank Accession No: LC102671) and *Acinetobacter junii* (GenBank Accession No: LC102684) based on the *rpoB*NJ cluster analysis (Figure 4.14). The *rpoB* of *Acinetobacter guillouiae*was 99.6% similar (with one nucleotide difference) to previously reported sequence of *A. guillouiae* (GenBank Accession No: LC102679), as shown in Figure 4.15.

Figure 4.13: Negative amplification of *Rickettsia* spp. and *Bartonella* spp.(A) Negative amplification of tyhus group (TG) and spotted fever group (SFG) rickettsiae by primers CS-78 and CS-323. M: 100 bp DNA ladder; 1: Positive control (*R. honei*); 2-12: head lice, N: negative control (sterile distilled water). (B) Negative amplification of *Bartonella* spp. by primers BhCS781p and BhCS1137n. M: 100 bp DNA ladder; 1: Positive control (*B. tribocorum*); 2-10: head lice, N: negative control (sterile distilled water).
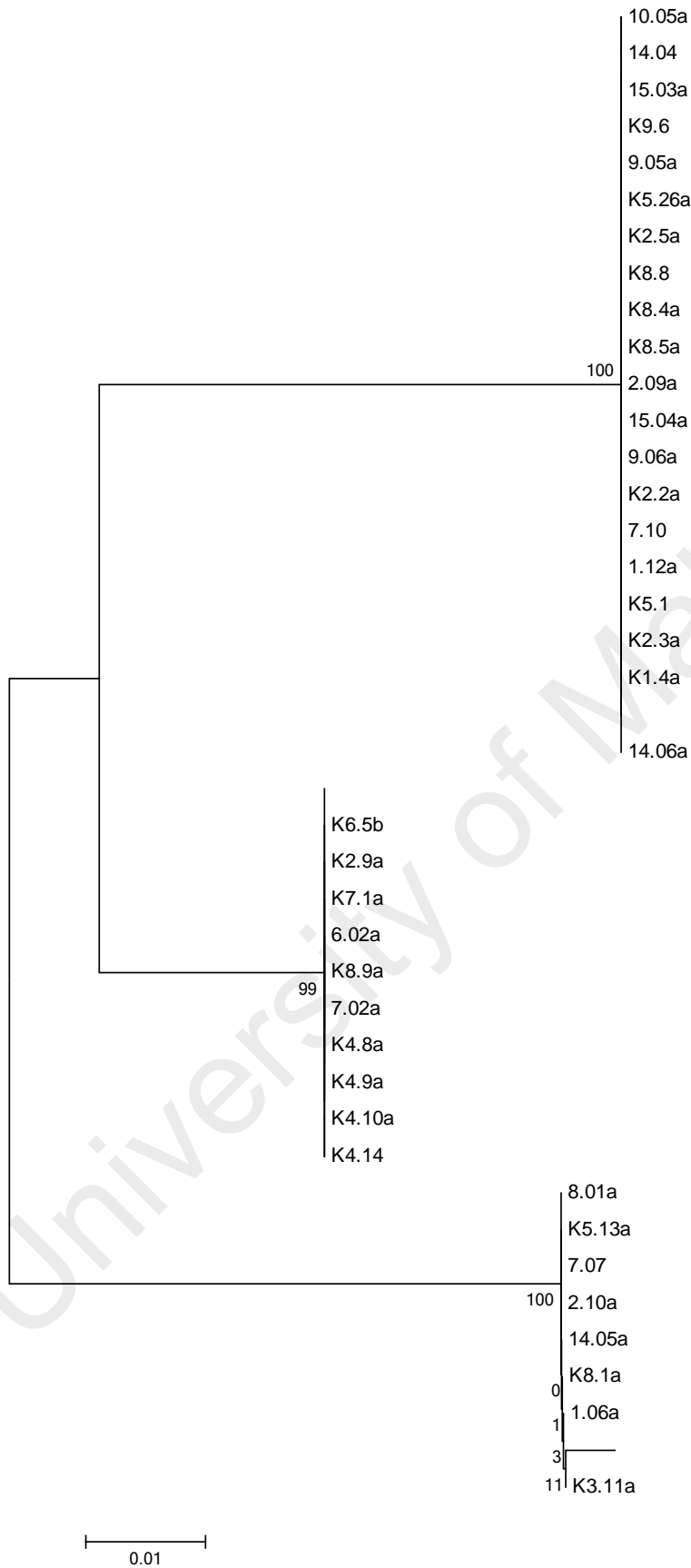
Figure 4.14: NJ cluster analysis of *rpoB* of three *Acinetobacter* spp. detected in head lice. The *rpoB* sequences were compared with reference strains (GenBank Accession Number: LC102684, LC102671 and LC102679) available in GenBank database. Bootstrap analysis was performed with 1000 replications. The scale bar shows K2P distances.
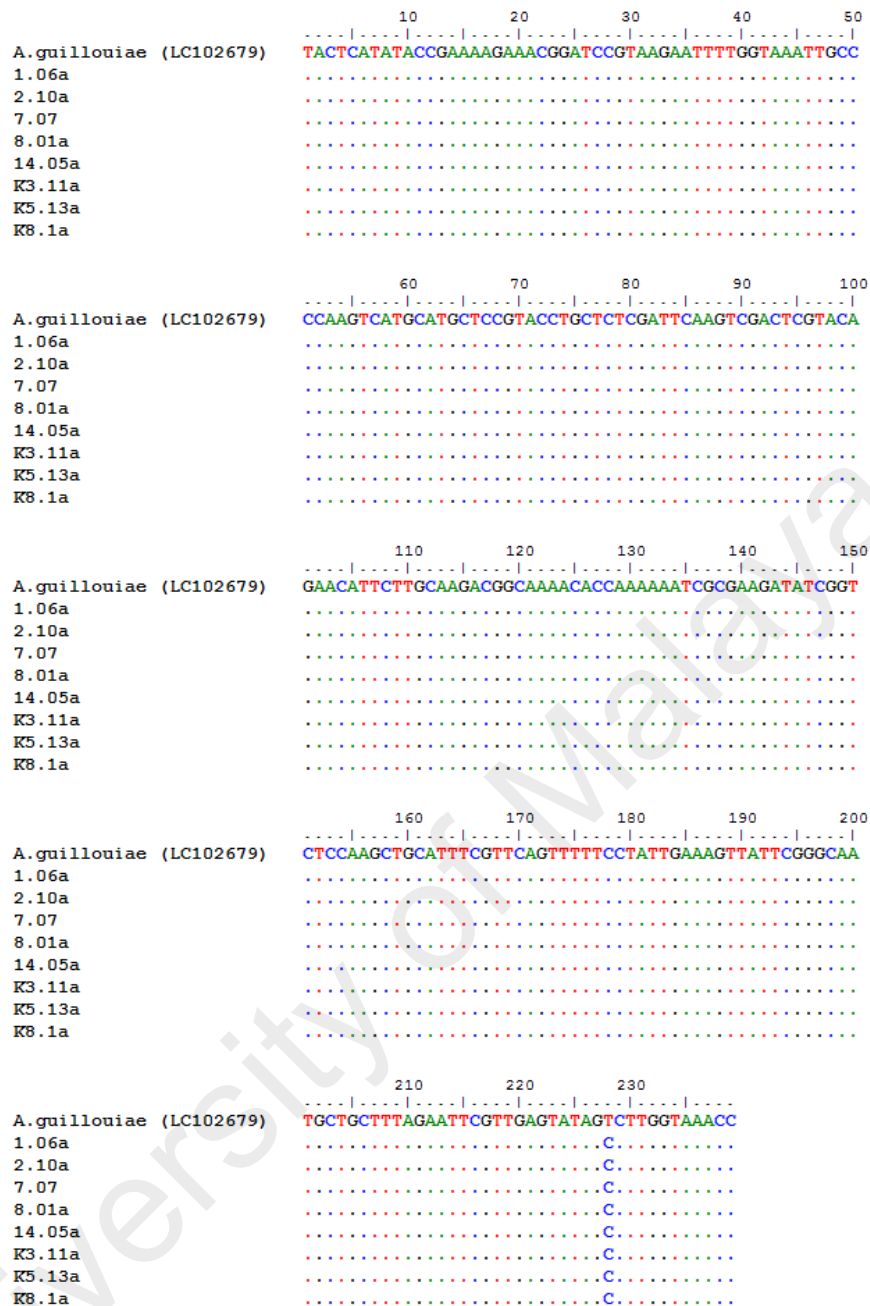
Figure 4.15: Sequence alignment of *A. guillouiae rpoB* sequences amplified from head lice with *A. guillouiae* strain KCTC23200 (GenBank Accession No:LC102679) as reference strain. The*rpoB* sequences amplified from eight head lice (1.06a, 2.10a, 7.07, 8.01a, 14.05a, K3.11a, K8.1a and K5.13a) showed one nucleotide change (C instead of T) at position 228.

The *recA* sequences were 100% identical to those sequences of *A. baumannii* (GenBank Accession No: LC014653), *A. nosocomialis* (GenBank Accession No: LC014643) and *A. guillouiae* (GenBank Accession No: LC014686) based on the *recA* NJ cluster analysis (Figure 4.16).

The infection rate of *A. baumannii*, *A. nosocomialis*, *A. junii*, and *A. guillouiae* was 5.8%, 1.5%, 7.7% and 9.2%, respectively. Detection of *Acinetobacter* spp. in KL/ KV and state of Kedah is summarized in Table 4.7 and Table 4.8, respectively.

No intra-specific genetic diversity was observed for all four *Acinetobacter* species detected in this study. The inter-specific genetic distances of *rpoB* and *recA* among the species are shown in Tables 4.9 and 4.10, respectively.
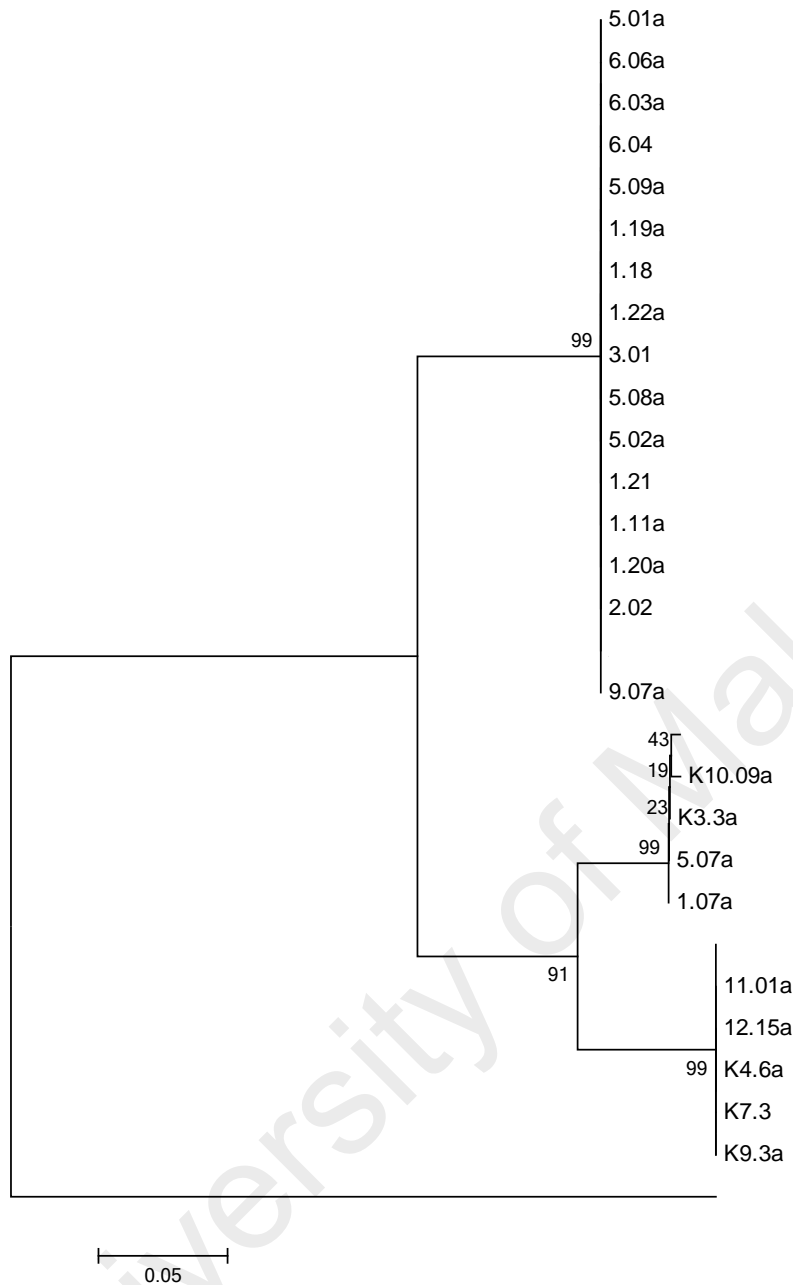
Figure 4.16:NJ analysis of *recA* of three *Acinetobacter* spp. detected in head lice. The *recA* sequences were compared with reference strains (Accession Number: LC014686, LC014643 and LC014653) available in GenBank database. Bootstrap analysis was performed with 1000 replications. The scale bar shows K2P distances. *P. aeruginosa* (GenBank Accession Number: GQ183951) was used as an outgroup.

Table 4.7: *Acinetobacter* spp. detection in head lice collected from Greater KL/KV

No.	Town	Orphanages / Shelters	No. of head lice tested	No. of head lice infected by *Acinetobacter* spp.	Species identification (n)
1	Kuala Lumpur	Rumah Titian Kaseh	20	9	*A. guillouiae* (7), *A. nosocomialis* (1), *A. junii* (1)
2	Ampang	Rumah BAKTI Datuk Harun	10	3	*A. guillouiae* (2), *A. junii* (1)
3	Rawang	Baitul Aini Selangor	3	1	*A. guillouiae* (1)
4	Rawang	Pusat Tahfiz Anak Yatim Nur Ikhlas	8	0	-
5	Banting	Rumah Anak Yatim & Asnaf As-Sholihin	11	5	*A. guillouiae* (4) *A. nosocomialis* (1)
6	Bandar Baru Bangi	Rumah Bakti Al-Kausar	6	4	*A. guillouiae* (3), *A. baumannii* (1)
7	Kuala Lumpur	Pusat Jagaan Kasih Murni	11	3	*A. guillouiae* (1), *A. junii* (1), *A. baumannii* (1)
8	Sepang	Baitul Barokah Wal Mahabbah	16	1	*A. guillouiae* (1)
9	Petaling Jaya	Pusat Jagaan Rumah Kesayangan	10	3	*A. guillouiae* (1), *A. junii* (2)
10	Kajang	Rumah Nur Hikmah	8	1	*A. junii* (1)
11	Puchong	Rumah Amal Limpahan Kasih	2	1	*A. baumannii* (1)
12	Shah Alam	Rumah Amal Al-Firdaus	15	1	*A. baumannii* (1)
13	Setapak	Pusat Jagaan Nuri	9	0	-
14	Cheras	Rumah Jalinan Kasih	6	3	*A. junii* (2), *A. guillouiae* (1)
15	Klang	Pertubuhan Kebajikan Anak-Anak Yatim & Miskin Sungai Pinang	8	2	*A. junii* (2)

Table 4.8: *Acinetobacter* spp. detection in head lice collected from the state of Kedah

No.	Town	Orphanages / Shelters	No. of head lice tested	No. of head lice infected by *Acinetobacter* spp.	Species identification (n)
1	Kuala Nerang	Pusat Jagaan Baitul Mahabbah Al-Hashimi	5	1	*A. junii* (1)
2	Pendang	Rumah Anak Yatim Amal Solehah	9	4	*A. junii* (3), *A. baumannii* (1)
3	Alor Star	PERKIM Bahagian Negeri Kedah	12	2	*A. nosocomialis* (1), *A. guillouiae* (1)
4	Baling	Persatuan Kebajikan Anak Yatim & Miskin Nur Hidayah	14	5	*A. baumannii* (5)
5	Sik	Pertubuhan Asuhan dan Didikan Anak-Anak Yatim Islam Daerah Sik	32	3	*A. guillouiae* (1), *A. junii* (2)
6	Gurun	Persatuan Kebajikan Anak Yatim dan Miskin Al-Munirah	11	1	*A. baumannii* (1)
7	Yan	Rumah Budi Kedah	4	2	*A. baumannii* (2)
8	Langkawi	Rumah Nur Kasih	10	5	*A. guillouiae* (1), *A. baumannii* (1), *A. junii* (3)
9	Kulim	Pertubuhan Pembangunan Anak-Anak Yatim Bekas Parajurit Malaysia Daerah Kulim	7	2	*A. baumannii* (1), *A. junii* (1)
10	Jitra	Pusat Jagaan IHMK	13	1	*A. nosocomialis* (1)

Table 4.9: The inter-specific K2P divergence values (%) of *rpoB*

Species 1	Species 2	Distance
A. baumannii	*A. guillouiae*	0.071
A. baumannii	*A. junii*	0.061
A. guillouiae	*A. junii*	0.094

Table 4.10: The inter-specific K2P divergence values (%) of *recA*

Species 1	Species 2	Distance
A. nosocomialis	*A. baumannii*	0.083
A. nosocomialis	*A. guillouiae*	0.159
A. baumannii	*A. guillouiae*	0.176

4.3.1.3 Molecular detection of *S. marcescens*

DNA of *S. marcescens* were detected in five head lice belonging to clades A (n=2) and D (n=3) collected from Rumah Titian Kaseh (n=2) and Pusat Jagaan Kasih Murni (n=1) located in Greater KL/KV; and PERKIM Bahagian Negeri Kedah located in state of Kedah (n=2). The 409 bp (Figure 4.17) of partial 16S *rRNA* sequences obtained were identical (384nt/384nt) to previously reported sequence of *S. marcescens* strainSmUNAM836(Genbank Accession No: CP012685), a multidrug-resistant strain isolated from a Mexican patient with obstructive pulmonary disease(Sandner-Miranda et al., 2016).

4.3.1.4 Molecular detection of *S. aureus*

Eleven head lice belonging to clades A (n=7) and D (n=4) collected from four welfare homes in Greater KL/KV: Rumah BAKTI Datuk Harun (n=1), Rumah Bakti Al-Kausar (n=1), Pusat Jagaan Kasih Murni (n=2) andPertubuhan Kebajikan Anak-Anak Yatim Miskin Sungai Pinang (n=2); and three welfare homes in state of Kedah: Pertubuhan Asuhan dan Didikan Anak-Anak Yatim Islam Daerah Sik (n=1), Persatuan Kebajikan Anak Yatim dan Miskin Al-Munirah (n=1), and Pusat Jagaan IHMK (n=3) were screened positive by *nucA* PCR amplification. The amplified *nucA* (Figure 4.18)sequences from seven head lice were identical (180nt/180nt) to previously reported strain of *S. aureus*(GenBank Accession No: DQ507382) whereas four head lice showed one nucleotide change (Figure 4.19). However, *mecA* PCR of the eleven specimens resulted in negative amplification.
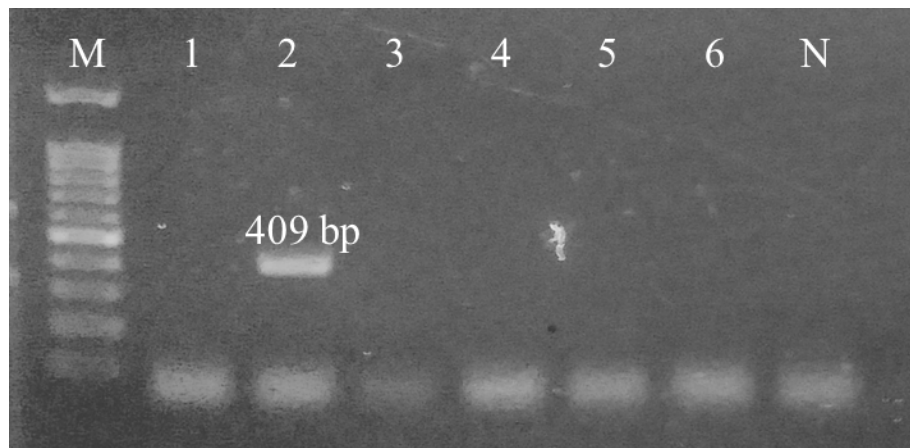
Figure 4.17: Amplification of 409 bp of *S. marcescens* partial 16S *rRNA* sequences inhead lice. M: 100 bp DNA ladder; 2: positive amplification of 16S *rRNA*; N: negative control (sterile distilled water).
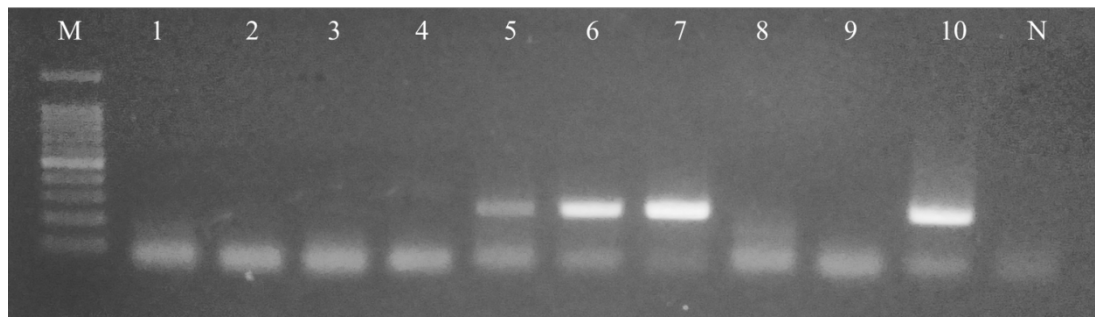
Figure 4.18: Amplification of 279 bp of *nucA* which is unambigious to *S. aureus*. M: 100 bp DNA ladder; 5-7 and 10: Positive amplification of *nucA*; N: negative control (sterile distilled water).

```
                    10        20        30        40        50
                ----|----|----|----|----|----|----|----|----|----|
S. aureus strain R18  GGTGTAGAGAAATATGGTCCTGAAGCAAGTGCATTTACGAAAAAAATGGT
2.08                  ..................................................
6.04                  ..................................................
7.02a                 ..................................................
7.09a                 ..................................................
15.03a                ..................................................
15.02a                ..................................................
K6.5a                 ..................................................
K5.32a                ................C.................................
K10.09a               ................C.................................
K10.10a               ................C.................................
K10.11a               ................C.................................

                    60        70        80        90        100
                ----|----|----|----|----|----|----|----|----|----|
S. aureus strain R18  AGAAAATGCAAAGAAAATTGAAGTCGAGTTTGACAAAGGTCAAAGAACTG
2.08                  ..................................................
6.04                  ..................................................
7.02a                 ..................................................
7.09a                 ..................................................
15.03a                ..................................................
15.02a                ..................................................
K6.5a                 ..................................................
K5.32a                ..................................................
K10.09a               ..................................................
K10.10a               ..................................................
K10.11a               ..................................................

                    110       120       130       140       150
                ----|----|----|----|----|----|----|----|----|----|
S. aureus strain R18  ATAAATATGGACGTGGCTTAGCGTATATTTATGCTGATGGAAAAATGGTA
2.08                  ..................................................
6.04                  ..................................................
7.02a                 ..................................................
7.09a                 ..................................................
15.03a                ..................................................
15.02a                ..................................................
K6.5a                 ..................................................
K5.32a                ..................................................
K10.09a               ..................................................
K10.10a               ..................................................
K10.11a               ..................................................

                    160       170       180
                ----|----|----|----|----|----|
S. aureus strain R18  AACGAAGCTTTAGTTCGTCAAGGCTTGGCT
2.08                  ..............................
6.04                  ..............................
7.02a                 ..............................
7.09a                 ..............................
15.03a                ..............................
15.02a                ..............................
K6.5a                 ..............................
K5.32a                ..............................
K10.09a               ..............................
K10.10a               ..............................
K10.11a               ..............................
```

Figure 4.19: Sequence alignment of *S. aureus nucA* sequences amplified from eleven head lice with *S. aureus* strain R18 (GenBank Accession No:DQ507382) as reference strain. The *nucA* sequences amplified from four head lice (K5.32a, K10.09a, K10.10a, and K10.11a) showed one nucleotide change (C instead of T) at position 18.

4.3.2 Molecular detection of pathogens in bedbugs

In this study, the PCR investigations of *S. aureusnucA* by primer pair nucAF/nucAR, fungal *rRNA* by primer pair ITS1/ITS4, rickettsial *gltA* by primer pair CS-78/CS-323, and *B. quintana gltA* and *ribC* by primer pairs BhCS781p/BhCS1137n and PBH-L1/PBQ-R1, respectively, did not detect any of the above pathogens.

4.4 DNA barcoding of arthropods in medical case reports

Arthropod specimens of three medical case reports submitted to the Department of Parasitology were identified using DNA barcoding.

4.4.1 Case report 1: Recurrent tick infestation of humans in Pekan, Malaysia

4.4.1.1 Clinical findings

A 4-year-old male child was brought to the Pekan clinic in state of Pahang, Malaysia in December 2011 complaining of itchiness of hisleft eye and lacrimation. The patient did not suffer from any symptoms such as runny nose, fever, rash, joint pain, or headache and had no history of trauma to the eyes. Overall, the patient was healthy and not agitated. His eyelids were not swollen nor were there any redness of the conjunctiva. Physical examination of the left eye revealed a small tick in the lower lid margin among the eyelashes (Figure 4.20A). The patient's parents were oblivious of the tick's presence. The tick was removed from under the eyelid with care using a pair of crocodile forceps (Figure 4.20B). The involved eye healed without complications.The patient is one of five children. His older sister had attended the sameclinic two months earlier complaining of pain in her left ear. Upon investigation, a tick was discovered and removed from the affected ear using a pair of crocodile forceps. The young girl too had recovered without complications upon removal of the tick.
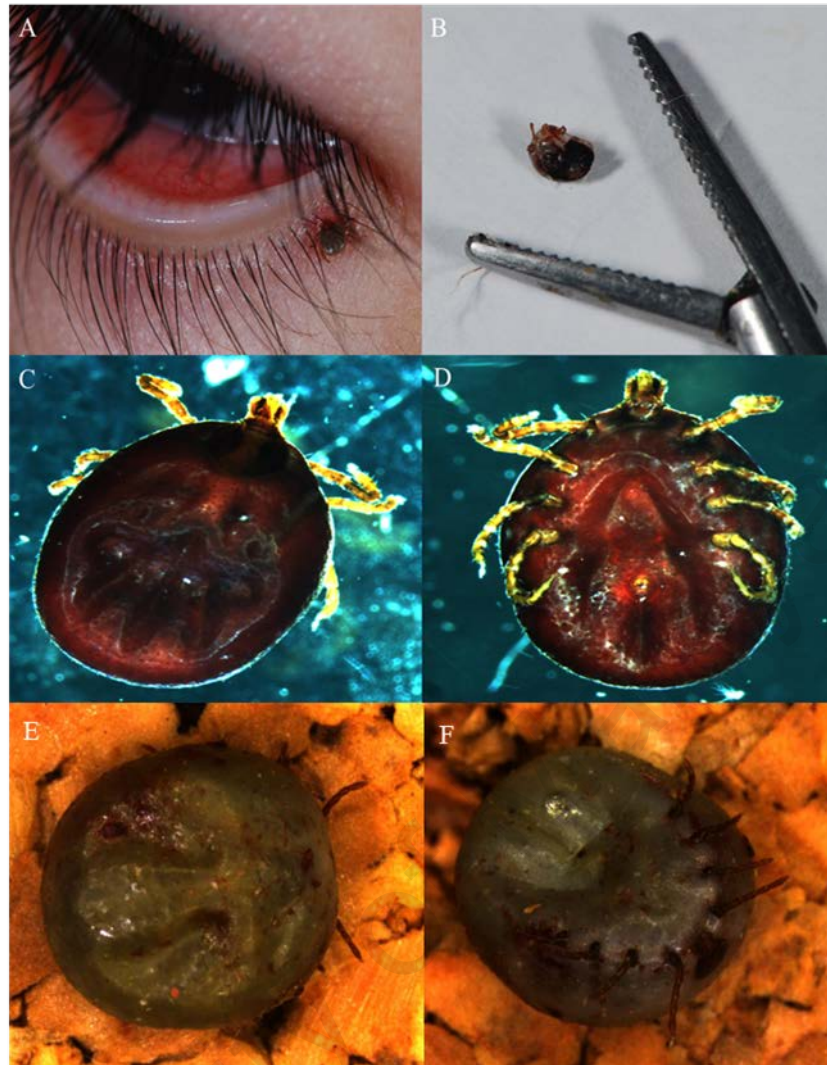
Figure 4.20: Patients with ocular and intra-aural tick infestation.(A) Tick at margin of lower eyelid causing inflammation to the eye. (B) Offending tick was removed from the lower eyelid margin using a crocodile forceps. Dorsal (C) and ventral (D) aspects of tick in boy patient. Dorsal (E) and ventral (F) aspects of tick in intra-aural infestation.

The patient's father works for the Department of Survey and Mapping Malaysia and had just returned from a course at the National Elephant Conservation Center, Kuala Gandah, Pahang. The course was located within the Krau Wildlife Reserve. Amini zoo housing deer and other animals is also in close proximity to the elephant sanctuary. However the father denied to have been in contact with any of these animals. The patient's father was also not aware of any ticks on his attire and has neverbeen bitten by one before. It was also noted that the family does not keep nor live in proximity todomestic animals.

A 49-year-old woman was seen at the same clinic in Pekan, Pahang, Malaysia reporting pain in her right ear lasting three days and associated with vomiting and dizziness.On physical examination, the patient was observed to have right facial nerve palsy. She was unable to close her right eye for the past three days and there was deviation of the mouth to the left side. No numbness or weakness of the body was detected. There was no fever and her blood pressure was 130/80 mmHg. Her left ear was normal. Examination of the right ear showedpresence of a tick, which was removed using crocodile forceps. There was no perforation of the right eardrum.

The patient is a widow living alone and works as a cleaner in a German factory in Pekan, Pahang. Sheneither kept cats nor other domestic animals and she has no contact with cattle. Occasionally she noticed the presence of wild boars in the forest fringes near her home. She has no history of entering the jungle. The patient was prescribed dimenhydrinate (oral tablet) for three days. The patient returned to the clinic one week later for a follow-up appointment. The right facial nerve palsy had not resolved but the vomiting had stopped, although she still reported slight dizziness. She was referred to the district hospital to rule out other causes of facial nerve palsy. No other causes of facial nerve palsy were detected. After physiotherapy she recovered fully in ten days without any residual effects and no further complications.

Isolated facial palsy is a rare complication of intra-aural tick infestation. Patients normally complain of severe ear pain and have lower motor neuron type of facial nerve paralysis. This patient had grade IV House Brackmann's grading facial nerve palsy. The palsy is self-limiting after removal of the tick.

4.4.1.2 Analysis of *COI*

The resulting DNA barcodes were uploaded to the BOLD and are available in the public dataset (http://www.boldsystems.org/index.php/Public_RecordView?processid=DERMA001-14).

The barcodes from all three tick specimens were identical. They are the same species (demonstrated by >99% sequence similarity) to the three ticks collected from Dong Nai, Vietnam and submitted to BOLD under the taxonomy of Ixodidae, but without genus or species names. This species (assigned the BOLD BIN Number - BOLD: AAH6690) nested within the genus *Dermacentor* on a BOLD identification tree (Figure 4.21), therefore, using a strict tree-based assignment model (Wilson et al., 2011), it is concluded that these specimens are ticks of the genus, *Dermacentor*.

Figure 4.21:BOLD identification tree.This tree is produced by a full database sequence identification request in BOLD for the tick specimens (designated as unknown specimen). The sequences groups closely with those from Dong Nai, Vietnam and the species is nested within a cluster of *Dermacentor* species.

4.4.2 Case report 2: Intestinal myiasis in a Malaysian patient

4.4.2.1 Clinical findings

A 41-year old female from an urban area was first seen at the general practitioner's clinic on 18th February 2015 with a complaint of passing out 'worms'. The 'worms' were noticed on the toilet floor after she cleaned up, post-defecation. She was prescribed albendazole (oral tablets) for three consecutive days as a treatment for helminthiasis. Physical and systemic examinations were unremarkable and she was well following the treatment. However, the patient travels frequently. The following relevant information was volunteered: the case occurred following a field excursion during which the patient consumed a meal in an area infested by flies. Following this, she experienced a brief, severe, abdominal colic associated with cold sweats on 4th February. The symptoms were transient and resolved spontaneously. On 26th March 2015, the patient presented again at the clinic with a similar complaint - 'worm' was found on the toilet floor after cleaning herself up. This time, she brought the 'worm' to the clinic. However, a stool specimen was not provided for examination. She was treated again with albendazole. The patient recovered without further complications.

The larva specimen was examined under a stereomicroscope (Figure 4.22) and identified as a psychodid larva.
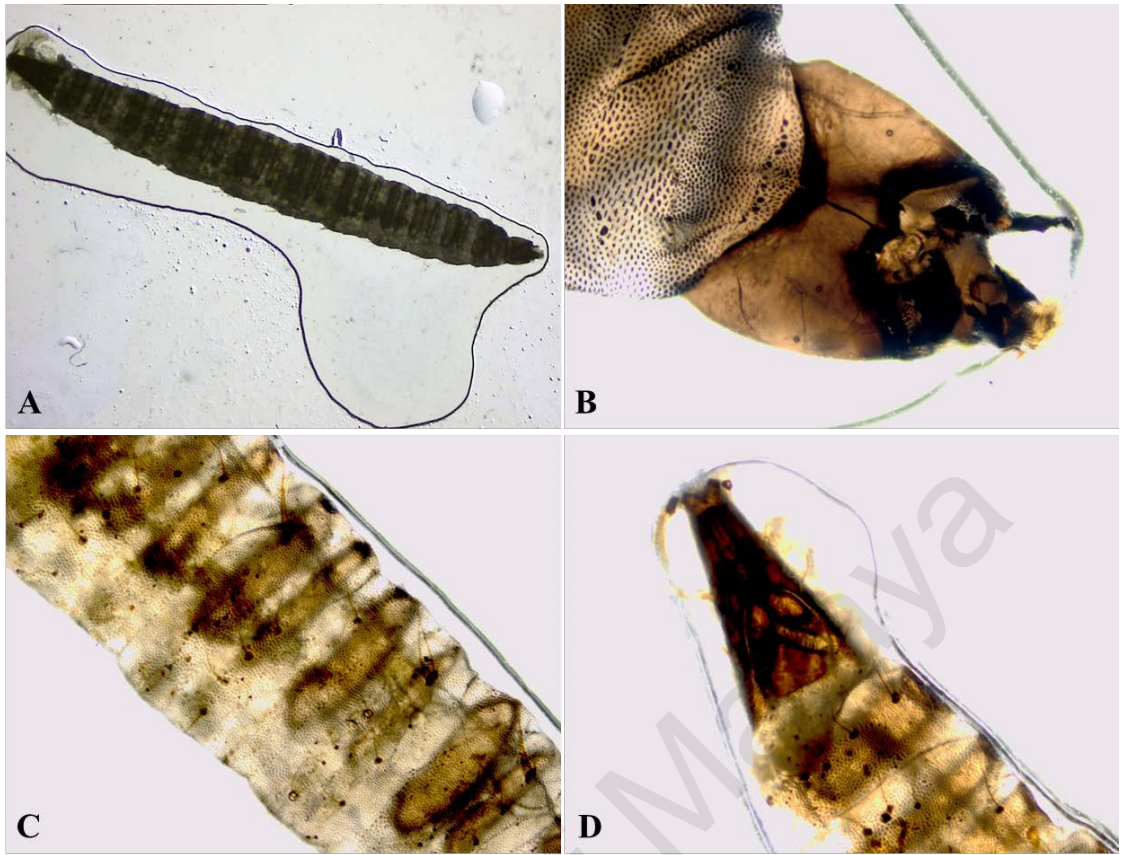
Figure 4.22: Larva of *C. albipunctatus* from the patient's faeces. (A) Dorsal view of the brown and slender larva. (B) Magnification of dorsal view of the head. (C) Magnification of lateral view of segments. (D) Magnification of the tail showing sclerotized breathing tube.

4.4.2.2 Analysis of *COI*

As identification of flies at larval stages is considered inconclusive using morphological characteristics, DNA barcoding was employed to establish the species identity of the specimen. The resulting DNA barcode was uploaded to the BOLD and is available in the public dataset(http://www.boldsystems.org/index.php/Public_RecordView?processid=IMTA001-15).

The barcode demonstrated 98% similarity to a DNA barcode from Tamil Nadu, India, (named *Telmatoscopus* (=*Clogmia*) *albipunctatus* and was nested within a cluster of DNA barcodes named *C. albipunctatus* (assigned the BOLD BIN Number - BOLD: AAF9305) on a BOLD identification tree (Figure 4.23). By using a strict tree-based assignment model (Wilson et al., 2011), it is concluded that the specimen is the larval stage of *C. albipunctatus*.
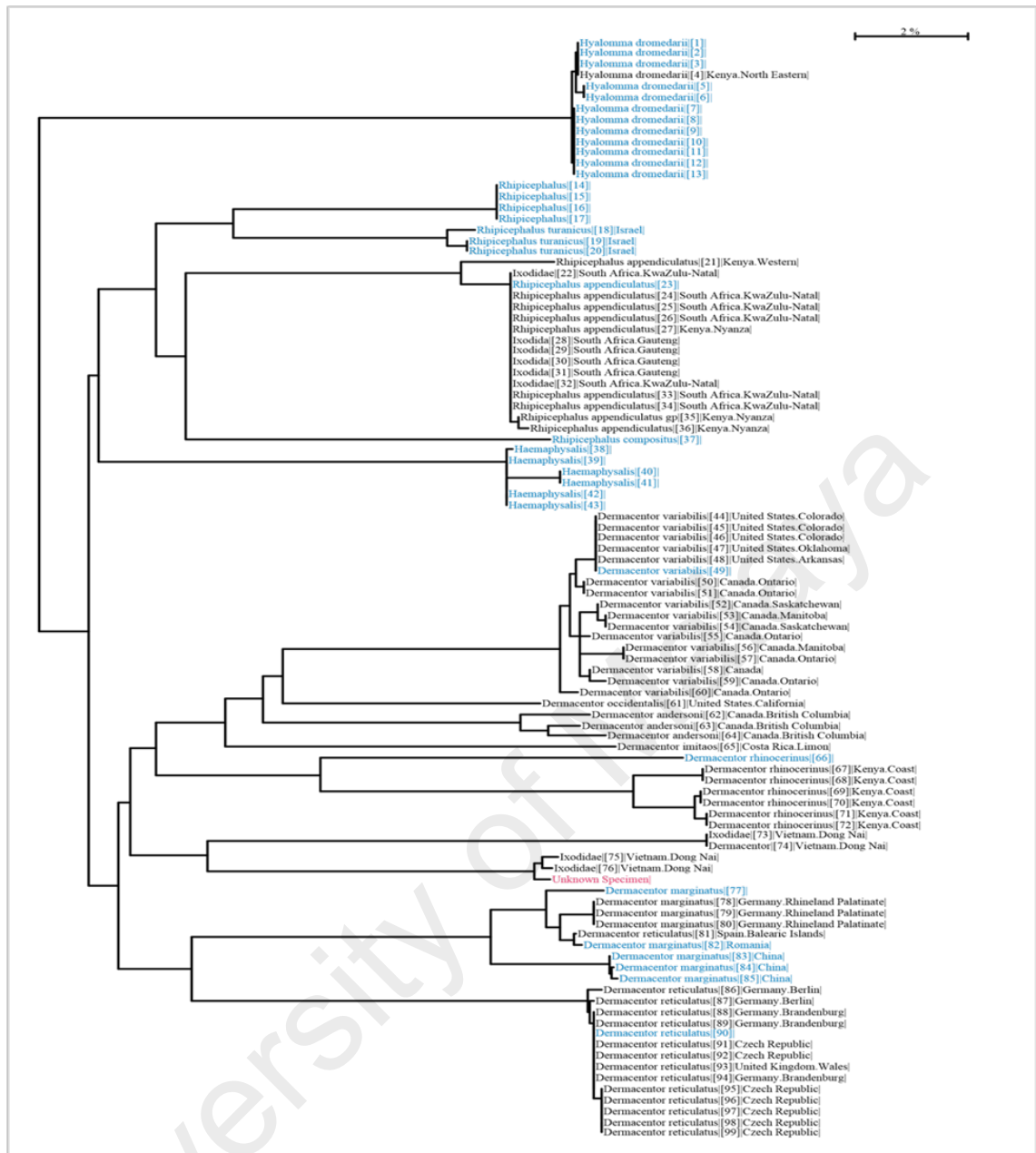
Figure 4.23: BOLD identification tree. This tree is produced by a full database sequence identification request in BOLD for the specimen coded W1. The sequence groups closely with those from Tamil Nadu, India, and the species is nested within a cluster of *C. albipunctatus* species.

4.4.3 Case report 3: Canthariasis in an infant

4.4.3.1 Clinical findings

A one-year-old baby girl was brought to thePaediatric Emergency Department in November 2015, with a 3-dayhistory of fever and one episode of passing multiple larvae in her stool (Figure 4.24). In the last 2 daysshe also had frequent bowel movements of 4 to 5 times a day. The stool consistency was normal and it was not associated with blood or mucous. Her oral intake was good and she had no vomiting. However, she was slightly irritable and the parents were unsure why the child was crying on and off, especially at night. There was no history of respiratory or urinary tract infections.

Upon examination, the child weighed 9.0 kg and was febrile with a body temperature of 39.6 °C. Blood pressure and heart rate were 86/60mmHg and 130/min, respectively. General physical and systemic examinations were unremarkable. Since larvae were found in the patient's stool, the child was given a single dose of syrup albendazole 200 mg as a treatment for parasitic infection,to be repeated in two weeks; and syrup paracetamol 125 mg four times a day to treat fever. At the 1-week follow up appointment, it was reported that the child continued to pass larvae until day-4 of illness. Her fever subsided by day-5 and she recovered without further complications.

Four larvalspecimens were examined under a stereomicroscope, revealing approximately 3.5 mm yellowish-white, hairy, full grown larvae, with visible legs and light brown coloured head (Figure 4.25).

Figure 4.24: Larvae in the patient's stool.Macroscopic examination of the stool sample showing multiple white coloured, scarab-like shaped larvae.
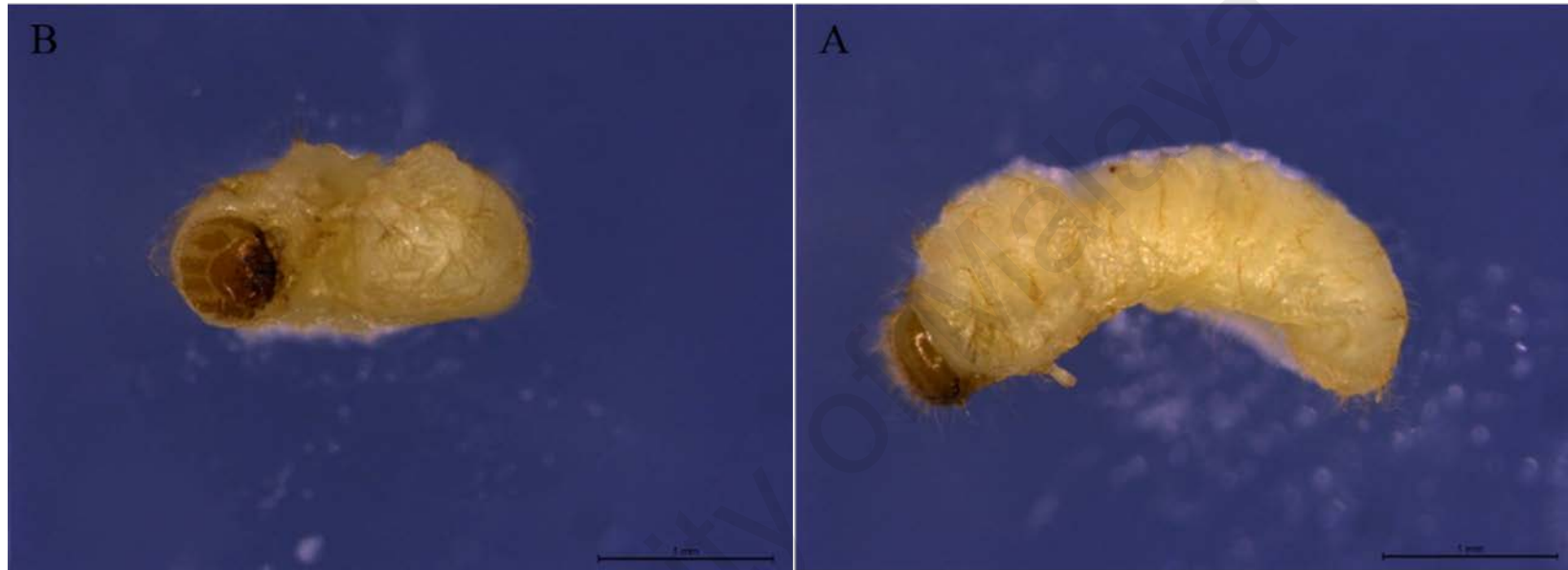
Figure 4.25: Stereomicroscopic images of cigarette beetle larva, *L. serricorne*. The lateral (A) and ventral (B) views revealed a C-shaped, yellowish-white larva, thinly covered with fine brown hairs with visible legs. The light brown coloured head is evenly rounded dorsally.

4.4.3.2 Analysis of *COI*

The resulting DNA barcode was uploaded to the BOLD andassigned the BOLD BIN Number - BOLD: ACG7582. Itis available in the public dataset (http://www.boldsystems.org/index.php/Public_BarcodeCluster?clusteruri=BOLD:ACG 7582).

The DNA barcode from the specimen shared 99.85% similarity with the closest-matching DNA barcode in the BOLD (BOLD ID: CICRP097-15) and nested within other DNA barcodes named *L. serricorne* on a BOLD identification tree (Figure 4.26). Therefore, using a strict tree-based assignment model (Wilson et al., 2011), we concluded that this specimen is the larval stage of *L. serricorne*.
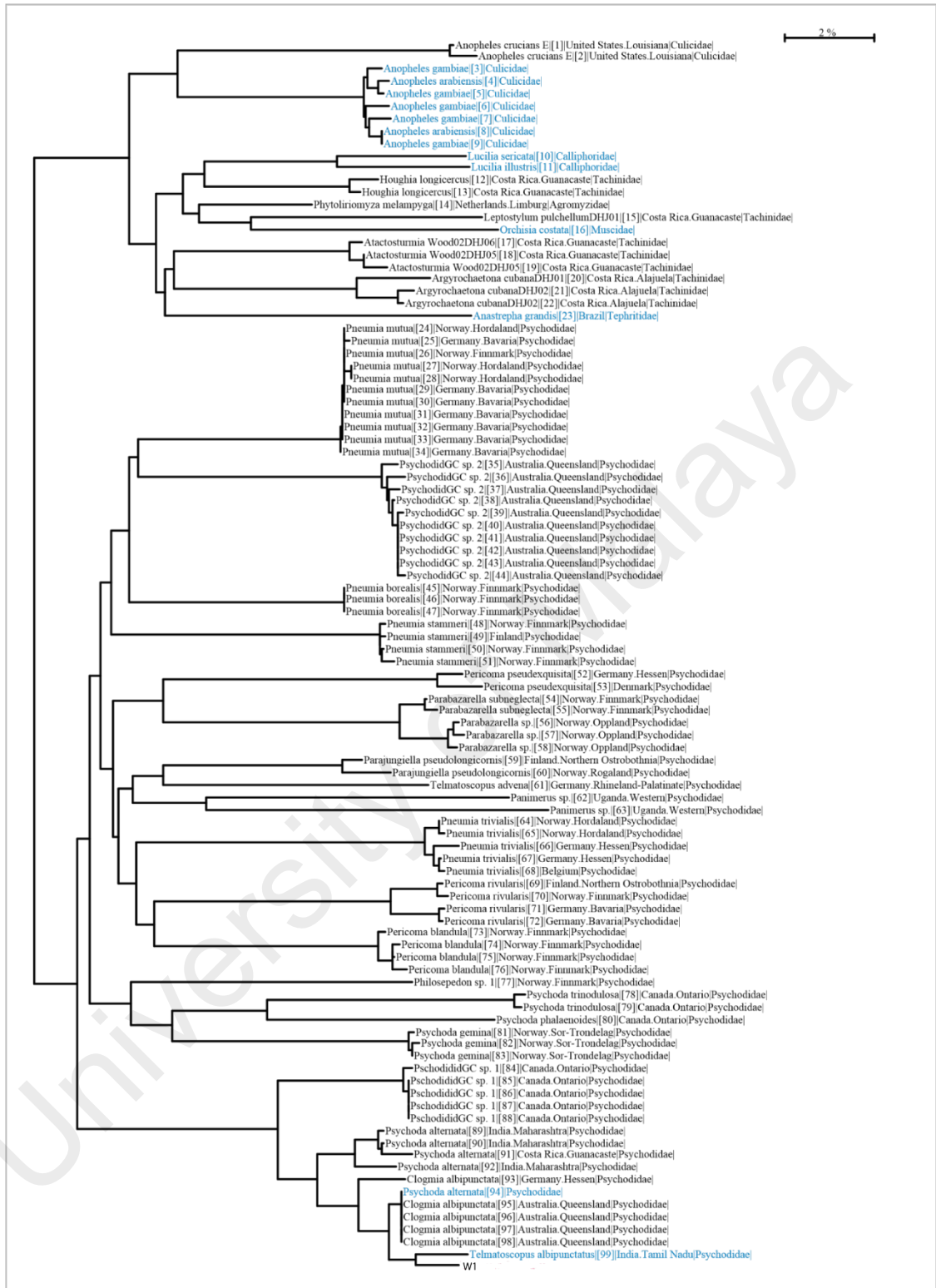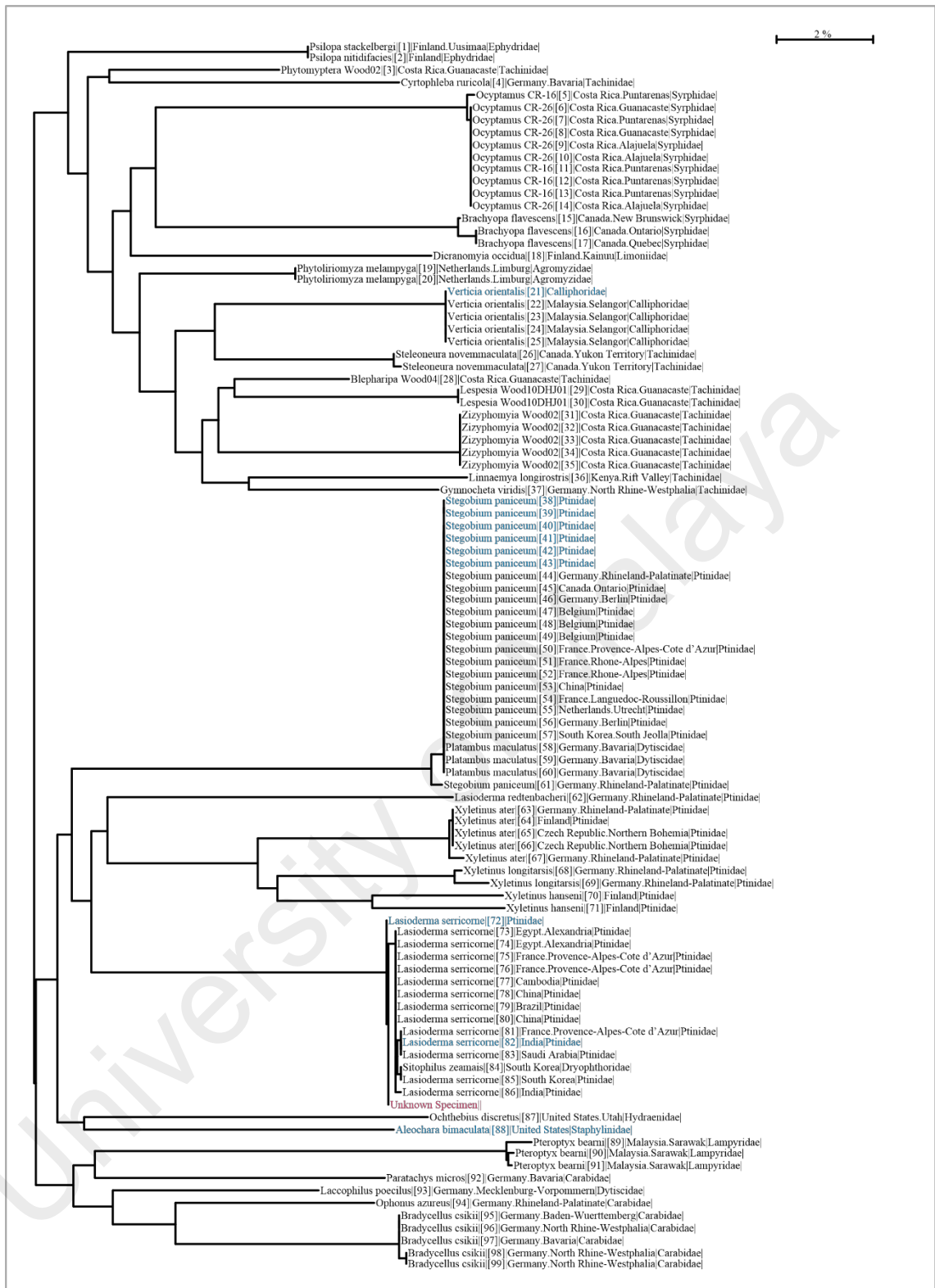
Figure 4.26: BOLD identification tree. This tree is produced by a full database sequence identification request in BOLD for the larval specimen. The sequence (designated as unknown specimen) groups closely with those from France, Egypt, India, Finland and Saudi Arabia; and the species is nested within a cluster of *L. serricorne*.

CHAPTER 5: DISCUSSION

5.1 Medically-important ectoparasites in welfare homes

The occurence of two ectoparasite species in the welfare homes; human head lice *P. h. capitis* and tropical bedbugs *C. hemipterus*, are discussed below.

5.1.1 Prevalence of pediculosis capitis and its contributing factors

Pediculosis capitis is not uncommon in this country, particularly in overcrowded institutions including welfare homes. Numerous reports on the infestation of head lice reflects that pediculosis capitis remains a health-care problem for children in Malaysia. The prevalence of 59.2% (in Greater KL/KV) and 64.9% (in the state of Kedah) obtained in this study were higher than the prevalence last reported in year 2010 by Yap et al. (24.7% in the state of Sarawak) and Muhammad-Zayyid et al. (49% in the state of Penang).

The factors that may have resulted in this higher rate is because the majority of study subjects were girls which numbered 412 in comparison to boys numbering 28. In this study, boys were under-represented because most welfare homes had their boys shave their heads bald; therefore, they were less likely to be at risk of infection. Nevertheless, this is in accordance with other studies that showed clear preponderance among girls (Gulgun et al., 2013; Muhammad Zayyid et al., 2010; Rassami & Soonwera, 2012; Yap et al., 2010). Head lice prefer long and thick hair, which is also difficult to groom and keep clean (Govere et al., 2003). It was noted that the female subjects in this study wore their hair long, therefore this might explain the high infestation rates. Gender-related behavioural differences such as pairs among girls and tendency for covering hair in girls (Davarpanah et al., 2013; Govere et al., 2003) increases the predominance of head lice infestation in girls. Most of the female subjects in this study covered their hair (only in the public) due to religious practice (The Holy

Quran, n. d.). Covering the head theoretically decreases head lice transmission, however, it facilitates the infestation by creating an ideal scalp humidity for the lice to thrive and multiply (Bachok et al., 2006). In addition, the study subjects were in close contact in their dormitories, thus promoting direct transmission of head lice. They also tended to share their headgears and accessories including scarves and combs, and this may serve as a source of indirect transmission of head lice (Gulgun et al., 2013).

In developing countries, pediculosis capitis is considered a poverty-associated disease; together with scabies, cutaneous larva migrans, and tungiasis (Feldmeier & Heukelbach, 2009). Based on the information gathered from the administrators, the welfare homes sustained their needs mainly from public donations. It is expected that welfare homes in Greater KL/KV received more donations compared to those in Kedah due to higher income of the population. With regard to this study, differences in socioeconomic status that might also reflect in standards of living and practices in welfare homes (such as better sanitary security and the availability of medical care) were not a contributing factor as welfare homes in both areas showed high prevalence. Several studies have proven that there is no significant association between head lice infestation with low socioeconomic status (Borges & Mendes, 2002; Doroodgar et al., 2014; Kokturk et al., 2003). However, association of head lice infestation with the economically disadvantaged has been demonstrated in recent years (AlBashtawy & Hasna, 2012; Lesshafft et al., 2013; Omidi et al., 2013).

In the current study, poor personal hygiene behaviour and the tendency of sharing headgears among the occupants (observed during sample collection) contributed to the high rate of pediculosis capitis in welfare homes, irrespective of the location and economic strata of the population. These factors, especially the personal hygiene behaviour may be of key importance in determining the spread of pediculosis capitis in the welfare homes. The negligence of the welfare homes administrators to provide

appropriate medical care including awareness of the disease has also worsened the infestation. A comprehensive study conducted in European Union by Bartosik et al. (2015) reported that the biggest number of infestations was observed from schools attended by children from orphanages and single-parent homes. This suggests that the transmission of pediculosis capitis in the present study might continue to occur in schools; hence, justifies the administrators' duty to prevent futher spread.

In Malaysia, data on the head lice resistance to pediculicides is lacking and outdated. To date, only three studies by Sinniah and colleagues evaluated the efficacy of chemical agents in treating pediculosis capitis. Sinniah and Sinniah (1982) documented low efficacy of dichlorodiphenyltrichloroethane (DDT), diluted in coconut oil and applied on the scalps of schoolchildren in ten states of Peninsular Malaysia, suggesting that head lice have developed resistance to this insecticide. Sinniah and Sinniah (1983) then performed a comparative study of DDT, carbaryl and actellic in the treatment of pediculosis capitis among schoolchildren of low socioeconomic status in Kuala Lumpur; their findings showed that DDT was ineffective but carbaryl and actellic proved to be very effective against head lice. In the following year, Sinniah et al. (1984) tested the head lice susceptibility against malathion, permethrin, carbaryl and kerosene; all four pediculicides showed high success rates in eliminating head lice collected from schoolchildren in Selangor.

Most of the above said pediculicides are no longer available in the market or are falling out of favour. DDT,the primary agent used in eliminating body lice infestations during World War II is now off the market due to its toxic environmental effects(Ko & Elston, 2004). Carbaryl has fallen out of favour due to reports of having carcinogenic and mutagenic potentials (Nutanson et al., 2008). Despite its high efficacy, the high alcohol content in malathion makes it highly flammable, its content containing terpenoids may cause stinging, and there is a risk of severe respiratory depression in the

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case of accidental ingestion(Downs, 2004; Nash, 2003; Nutanson et al., 2008). Evidence suggested permethrin resistance has been increasing in various countries including United States, United Kingdom, Spain, Italy, Australia, Canada (Burgess et al., 2013; Downs, 2004; Ko & Elston, 2004; Yoon et al., 2014b), and this could well follow in Malaysia.

As most of the affected occupants never received appropriate treatment for pediculosis capitis, a safe approach was taken to provide pesticide-free dimethicone shampoo to eliminate head lice. Dimethicone is a silicone-based polymer that works mechanically to lubricate hair to aid the removal of nits and lice, while physically occluding the respiratory system of the louse(Ihde et al., 2015). This obstruction inhibits water excretion causing physiological stress that leads to the louse death, thus potentially eliminating pesticide-resistance issue (Burgess, 2009; Ihde et al., 2015). Dimethicone is proven safe for children (Ihde et al., 2015) and several studies have demonstrated its ovicidal and pediculicidal efficacy (Burgess, 2009; Burgess & Burgess, 2011; Gallardo et al., 2012; Heukelbach et al., 2011; Ihde et al., 2015; Strycharz et al., 2012). It is anticipated that dimethicone lotion may serve as an alternative to pesticide-containing product or even as a first line treatment in treating pediculosis capitis.

Standard care and practices were explained to the occupants to prevent re-infestation in the welfare homes. They were advised to wash and dry all affected clothing, bedding, towels, and headgears at high temperature (preferably above 55 °C) to kill viable eggs and lice. In addition, the administrators were strongly advised to do routine inspection and they were also encouraged to screen the newcomers upon admission so that treatment can be provided promptly if pediculosis capitis was detected.

5.1.2 Genetic diversity of *P. h. capitis*

Sequence diversity of *COI* in *P. h. capitis*in Southeast Asian regions received little attention, in fact it has never been investigated in Malaysia. To date, only one such study was conducted in Thailand, in which the researchers reported the occurrence of clades A and C head lice (Sunantaraporn et al., 2015). This study is the first to successfully demonstrate the genetic variations in head lice collected from two regions of Peninsular Malaysia (Greater KL/KV and the state of Kedah) using *COI* DNA barcoding. The NJ cluster analysis based on partial *COI* sequences clearly showed the phylogenetic placement of the 260 head lice into three Barcode Index Number (BINs) of Clade A, B and D.

The first BIN of clade A, BOLD: AAA1556,comprised of *COI* sequences deposited in BOLD (http://www.boldsystems.org) from 22 countries: Egypt, Thailand, United States, Pakistan, Philippines, Papua New Guinea, Australia, Canada, United Kingdom, Ecuador, Honduras, Norway, Colombia, Burundi, Russia, France, Mongolia, Zimbabwe, Argentina, French Polynesia, Cook Islands, and Yemen. The detection of clade A lice from Greater KL/KV and state of Kedah in this study confirmed its global distribution.

The detection of clade B (restricted to Greater KL/KV), assigned the BOLD BIN Number - BOLD: AAA1557, is unique; occurred in low rate (2%) compared to clades A (41%) and D (57%). Clade B consisted only head lice (Light et al., 2008) and originated in America (Boutellis et al., 2013b). Its detection in the present study was the first in Asia thus supported the likelihood of further expansion as suggested by Light et al. (2008). Previous studies showed that clade B has limited distribution, restricted to only

Australia, Europe (United Kingdom, Germany, Portugal), North and Central America (United States, Honduras, Panama),South Africa and Algeria (Ashfaq et al., 2015; Bonilla et al., 2013; Boutellis et al., 2015; Light et al., 2008).

The new clade D, consisted of both head and body lice, was first found in Democratic Republic of the Congo(Drali et al., 2015). Ashfaq et al. (2015) recorded the occurrence of clade D in Pakistan, Nepal and Ethiopia and they were assigned to BINof BOLD: AAW5034. Herein, clade D represented as the most prevalent clade with a prevalence rate of 57%.

Clade C was not encountered in this study, so it remains restricted to Africa(Ashfaq et al., 2015; Bonilla et al., 2013; Light et al., 2008). However, Sunantaraporn et al. (2015) reported the occurrence of clade C in Thailand, in sympatry with clade A lice. The new Clade E, only known from Ethiopia (Ashfaq et al., 2015) was also not detected in this study.

Data in this study show that head lice of clade A, B and D can live in sympatry, for the first time, as evidenced from two shelters in Greater KL/KV that recorded multiple occurrence of all three clades. In addition, seventeen shelters (in Greater KL/KV and Kedah) had occurrence of double clades (clades A and D). Previous reports have shown that at least two clades can live in sympatry, as evidenced by concurrent occurrence of clade A and B in Chile (Boutellis et al., 2013b) and Algeria (Boutellis et al., 2015); clade A cohabitated with clade C in Ethiopia (Angelakis et al., 2011b; Veracx et al., 2013), Thailand (Sunantaraporn et al., 2015),and in Senegal (Boutellis et al., 2012); and clade A and D coexisted inCongo(Drali et al., 2015). Interestingly, one occupant from a welfare home located in Kuala Lumpur had lice of clade B and D living in sympatry, which was described for the first time here.

The suggested standard divergence threshold value in *COI* barcoding is ten times (10x) the mean intra-specific variation (Hebert et al., 2004). The 14 times higher divergence value among clades than within clades detected in this study has proven *COI* as a favourable DNA barcode in identification of head lice; the *COI* provides a robust DNA barcode in identifying clades A, B and D with non-overlapping genetic distance between intra- and inter-specific clades, as shown in Figure 4.5A.

Based on the contribution of clade A and D (in regard to prevalence) to the head lice diversity in Malaysia, it is suggested that recombination events could have occurred between these two clades as evidenced by the high-divergence of *COI* phylogenetic placement. This hypothesis is supported by Drali et al. (2015); clade D constituted 71% of the lice population and co-infestation of clades A and D were observed in three patients in Congo. Veracx et al. (2013) also suggested recombinant events have occurred between Clade A and Clade C lice in Africa, based on the greater diversity of the two clades. Indeed, the recombinant between clade A and D is plausible due to two possible reasons; the fact that head lice lack of spermathecal thus allowing them to mate more frequently, and their sympatric lifestyle as observed by gene exchanges increases the possibility of their mating (Drali et al., 2015; Veracx et al., 2013).

Both clades A and D comprised head and body lice (Drali et al., 2015; Light et al., 2008) that have been shown to transmit and carry pathogens, therefore exchange of pathogens can occur between these two clades (Drali et al., 2015). This perhaps explaining the detection of pathogens in clade A and D in the current study. To the best of my knowledge, clade B head lice have not been reported to carry pathogens. A similar finding was obtained in this study; no pathogens were detected in clade B lice. The molecular detection of potential associated pathogens is discussed further in section 5.2.

5.1.3 Prevalence of *C. hemipterus*

Low prevalence of *C. hemipterus* infestations was encountered in this study. This is an agreement with the report by Ab-Majid and Zahran (2015); in Kuala Lumpur and state of Selangor, dormitories were the least affected compared to other type of premises, including residential houses, apartments, hotels and motels.

In this study, *C. hemipterus* was recovered from mattresses, pillows and comforters in the dormitory. These sleeping gears were second hand items donated from the public and were cluttered, not properly cleaned and maintained. In Rumah Titian Kaseh where the *C. hemipterus* were recovered, several girls were noted having cutaneous reactions on their extremities, but were not further investigated. A pest control operator was called up by the research team to eradicate the infestation, however, re-infestation had reported to occur. Sleeping gears that have been pre-infested with bedbugs, received by the welfare home may be the source of re-infestation. The underprivileged children of Rumah Titian Kaseh could also have returned to their parents' houses (that potentially had infestations) during weekend and later re-introduced the bedbugs to the welfare home. The resurgence of bedbugs could also be due to the lack of insecticide efficacy used by the pest control company, however, it was not further investigated.

Bedbugs' resistance to organochlorines (DDT and dieldrin) has long been reported in Malaysia (Cheong, 1964; Reid, 1960). After the cessation of organochlorines usage, pyrethroids application was preferred in pest control spraying, however resistance due knockdown resistance (*kdr*)-type resistance mechanism

identified in *C. hemipterus* has been reported (Dang et al., 2015). The *kdr* resistance due to V419L and L925I point mutations on the voltage-gated sodium channel gene have been recognised to decrease the target site sensitivity of DDT and pyrethroids, hence resulting in high tolerance of bedbugs to both agents (Dang et al., 2015; Rinkevich et al., 2013).

Despite the low prevalence encountered in this study, the physical and mental health of the affected occupants that might be impacted by bedbug infestations should be taken into consideration. The medical nuisance following bites experienced by the affected persons (How & Lee, 2010) and delusional parasitosis due to cryptic behaviour of bedbugs (Hinkle, 2000) could further complicate the infestation scenario, especially in people receiving social care but with limited access to health services. Severe cases of anaemia attributed to bedbug bites have been reported (Paulke-Korinek et al., 2012; Pritchard & Hwang, 2009), indicating appropriate measures should be taken immediately in response to bedbug infestations. Based on the degree of infestations, thorough inspection of the likely infested items (including mattresses, bedframes and bedside furniture), disposal of highly infested furniture, removal or encasement of infested items, and application of insecticides by pest control operator should be performed to eliminate the infestations (Goddard & deShazo, 2009).

In addition, bedbug infestations also caused economic losses in the tourism industry and gained worldwide attention (Doggett, 2013), and this could well follow in Malaysia since bedbug infestations has resurged especially in hotels and low-cost motels in Kuala Lumpur and state of Selangor (Ab-Majid & Zahran, 2015; How & Lee, 2010). Nevertheless, bedbug infestations in welfare homes are still under control, at least in Greater KL/KV and state of Kedah as evidenced in this study.

5.1.4 *COI* barcoding of *C. hemipterus*

The *COI* barcodes generated from 26 bedbugs collected from Rumah Titian Kaseh were assigned the BOLD BIN Number - BOLD: AAR9233, which included *C. hemipterus* collected in Thailand and Kenya. The locality information gained from BOLD confirmed the tropical distribution of *C. hemipterus*, in concert with its occurrence in tropical regions of Brazil (Bernardes et al., 2015) and India (Bandyopadhyay et al., 2015). *COI* barcoding has also confirmed that *C. hemipterus* was the only species found in Malaysia, in agreement with studies conducted by How and Lee (2010) and Zahran et al. (2016).

No genetic diversity was observed within the *C. hemipterus*collected in this study except one point mutation (C substituted T) in two specimens; this transition could be due to sequencing errors.

5.2 Molecular detection of potential associated pathogens

Addressing the potential of head lice to transmit pathogens is imperative because of the high prevalence of pediculosis capitis in Malaysia setting. Study focusing on molecular detection of the pathogens is fundamental to provide insight into bigger perspective, including their transmission dynamics.

This study is the first to provide molecular evidence on the type of pathogens carried by head lice in Malaysia. *Rickettsia prowazekii* was not detected in this study, therefore the body louse, *P. h. humanus*remains the principal vector of *R. prowazekii*. *B. quintana*, primarily detected in body lice, has been reported in head lice (Bonilla et al., 2009; Sasaki et al., 2006). However, this pathogen yet was not detected in the current study.

COI DNA barcoding employed in this study revealed the head lice genetic diversity as well as served as an internal control.*COI* partial sequences were

successfully amplified in 260 head lice tested, demonstrating the lack of PCR inhibitors in head lice that might affect the detection of pathogens.

As head lice live on the scalp, their external surfaces are expected to be contaminated by various microorganisms, including the bacteria detected in this study. Evenso, as the specimens were kept in vial containing 70% alcohol after collection (then rinsed in distilled water and dried on a sterile filter paper prior DNA extraction), this method should be sufficient to decontaminate those microorganisms. It is therefore, suggested that *Acinetobacter* spp., *S. marcescens* and *S. aureus* were detected within thehead lice; possibly within the lice intestinal tract and not due to surface contaminations. As head lice ingest only blood, the inoculation of intestinal tract would be from the blood of children in welfare homes having bacteraemia. This hypothesis however needs to be further clarified.

5.2.1 *Acinetobacter* spp. in *P. h. capitis*

Four *Acinetobacter* spp. namely *A. baumannii*, *A. nosocomialis*, *A. junii*, and *A. guillouiae* were detected in this study. Of these, *A. baumannii* and its close relative*A. nosocomialis*are important opportunistic, nosocomial pathogens responsible for epidemic outbreaks of infection that occurred often in but rarely outside the clinical settings (Visca et al., 2011). Their abilities to cause substantial morbidity and mortality particularly in critically ill patients and the pandrug-resistant characteristic exhibited by these species make them a significant threat to human health(Carruthers et al., 2013; Visca et al., 2011). The endemicity of multidrug-resistance *Acinetobacter calcoaceticus-baumannii* Complex (comprising four species: *A. baumannii, A. nosocomialis, A. pittii and A. calcoaceticus*) has been reported in ICUs in Malaysia(Dhanoa et al., 2015; Janahiraman et al., 2015).

Human infections by *A. junii* even though rarely reported, have caused septicaemia outbreaks in neonates(Bernards et al., 1997) and paediatric oncology patients(Kappstein et al., 2000), catheter-related bacteraemia (Linde et al., 2002; Traglia et al., 2014), community-acquired meningitis in adults (Chang et al., 2000), refractory peritonitis (Borràs et al., 2007), and ocular infections (Prashanth et al., 2000).

A. guillouiae is an environmental species and is not known to cause human infections (Yoon et al., 2014a). Nonetheless, *A. guillouiae*had been isolated from human faeces (Visca et al., 2011) and metallo-β-lactamases (MBL)-positive *A. guillouiae* was isolated from the drain of a child hospitalized in a paediatric ICU (Bošnjak et al., 2014).

In the current study, the infection rate of *Acinetobacter* spp. in head lice was 24.2% and *A. baumannii* was detected in 5.8% of the head lice population. This was found to be much higher than that in Malaysia's neighbouring country Thailand, which recorded *A. baumannii* infection rate of 1.5% (Sunantaraporn et al., 2015). Moreover, higher rates of *A. baumannii* infection have been reported in European countries and Africa: Portugal (10%), the Netherlands (18%), France (32%), Ethiopia (52%), and Rwanda (58%) (Bouvresse et al., 2011; Kempf et al., 2012). *A. baumannii*was frequently isolated in body lice compared to head lice (Bonilla et al., 2013) and this perhaps might explain the lower infection rate encountered in current study.

The detection of *Acinetobacter* spp. in this study, especially *A. baumannii*, *A. nosocomialis,*and *A. junii* suggesting head lice as potential environmental reservoir that could pose a health threat to the infested children. Head lice were shown experimentally to excrete faeces with viable *A. baumannii*(Houhamdi & Raoult, 2006)and this bacterium was able to survive for a long time on dry surfaces (Jawad et al., 1998), therefore its transmission to human is possible via contamination of the bite wound site when scratched into the skin(Kempf et al., 2012).

In addition, this study is the first showing the presence of *A. nosocomialis, A. junii* and *A. guillouiae* in head lice. Other studies reported the presence of non-baumannii isolates of *Acinetobacter radioresistens*, *Acinetobacter schindleri*, and *Acinetobacter rhizosphaerae* (Kempf et al., 2012; Sunantaraporn et al., 2015).

5.2.2 *S. marcescens* in *P. h. capitis*

S. marcescens is an opportunistic, Gram-negative bacillus belonging to Enterobacteriaceae family and is becoming increasingly associated with nosocomial infections including endocarditis, septicemia, and infections of the upper respiratory tract and urinary system (Hawkey & Choy, 2015). *S. marcescens* commonly affects immunocompromised patients including those receiving broad spectrum antibiotic therapy or having indwelling urinary or intravenous catheters(Mahlen, 2011). Increasing numbers in outbreaks of multidrug-resistant*S. marcescens* have been reported in hospital settings which includes Taiwan (Jang et al., 2001), Korea, (Suh et al., 2010; Yoon et al., 2005), China (Cai et al., 2008), Italy (Bagattini et al., 2004; Casolari et al., 2005), UK (David et al., 2006), Argentina (Merkier et al., 2013), Hungary (Ivady et al., 2014), Germany (Dawczynski et al., 2016), and Spain (Morillo et al., 2016) to name a few; mostly involved critically ill neonates and patients in ICUs.

This is the first report of *S. marcescens* detection in head lice; La Scola et al. (2001) previously isolated *S. marcescens*from body lice. The pathogenicity of the strain detected in this study is unknown, however, the partial 16S *rRNA* sequences were identical to a multidrug-resistant strain isolated from a Mexican patient with obstructive pulmonary disease (Sandner-Miranda et al., 2016). Therefore, there is now a need for a

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detailed investigation to determine the virulence of *S. marcescens*strain detected in this study.

5.2.3 *S. aureus* in *P. h. capitis*

S. aureus is both ahuman pathogen and commensal Gram-positive bacteriumbelonging to the phylum of *Firmicutes* that capable of causing a wide range of clinical infections (Dayan et al., 2016). Infection with *S. aureus* is the leading cause of bacteraemia and infective endocarditis, as well as osteoarticular, skin and soft tissue, pleuropulmonary, and devicerelated infections(Tong et al., 2015). Its pathogenic strain, the MRSA, has been known to cause nosocomial infections since 1960s and the epidemiology of MRSA infections have dramatically spread into community-acquired MRSA during the late 1990s, hitherto (Gaymard et al., 2016).

In this study, eleven head lice were positive for *S. aureus* infection, however negative amplification of *mecA* indicated they were not MRSA. Nonetheless, the detection of methicillin-susceptible *S. aureus* (either within head lice or from their surface) may initiate skin and soft tissue infections in the infested children; for instance, scratching due to pruritus may lead to breaches of skin or mucosal barriers thus, allowing *S. aureus* access to adjoining tissues or the bloodstream (Lowy, 1998). In addition, folliculitis, a superficial inflammation of the hair follicles due to *S. aureus* infection commonly seen in children (Sladden & Johnston, 2004) may also develop.

5.2.4 Bedbugs-borne pathogens

Detection of MRSA and VRE in *C. lectularius* collected from three patients of impoverished community in Vancouver, Canada (Lowe & Romney, 2011) has sparked a renewed interest in bedbugs' vector potential. Barbarin et al. (2014) on the other hand, suggested that *C. lectularius*transmission of MRSA is highly unlikely based on experimental evidence that showed MRSA did not amplify and survive longer than nine

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days within the midgut of the bedbugs. Nonetheless, Angelakis et al. (2013) identified *B. quintana* in *C. hemipterus* collected from a prison in Rwanda, where the bacterium has previously been identified in body lice from the same prison (Fournier et al., 2002). Leulmi et al. (2015) later demonstrated the vector competency of bedbugs to transmit *B. quintana*; *C. lectularius* can acquire, maintain for more than two weeks and release viable *B. quintana* following a stercorarial shedding. Bedbugs were artificially infected with *Rickettsia parkeri*, a pathogen of humans that belongs to the SFG rickettsiae; the bacterium survived in bedbugs' salivary gland for two weeks (Goddard et al., 2012). In addition, *Aspergillus flavus*, *Penicillium*spp., and *Scopulariopsis*sp. are among fungal species that have been identified in *C. lectularius*(Reinhardt et al., 2005).

Despite these reportedly found pathogens, DNA of fungi, *S. aureus*, *B. quintana*, and *Rickettsia* spp. was not amplified from any *C. hemipterus* collected in this study. The successful amplification of *COI* partial sequences in *C. hemipterus* (in DNA barcoding) confirmed that the negative PCR results of tested pathogens are due to the absence of pathogens' DNA, not due to PCR inhibitors. This finding may suggest that *C. hemipterus* found in this country is unlikely to transmit pathogens to humans, especially those in the welfare homes. However, sampling in larger geographic regions that would potentially result in increased sample size might show different results.

5.3 DNA barcoding of arthropods in medical case reports

Arthropod infestations in humans that have been identified through *COI* DNA barcoding are discussed in detail below.

5.3.1 Case report 1: Recurrent tick infestation of humans in Pekan, Malaysia

Tick infestation of human eyelids is rare or under-reported in Malaysia. In Malaysia, reports of tick infestations generally concern intra-aural infestations(Abdul-Rahim et al., 2013; Lazim et al., 2012; Shibghatullah et al., 2012; Srinovianti & Raja-

Ahmad, 2003; Zamzil-Amin et al., 2007), such as seen in the49-year-old female patient in case 3 (described in Section 4.4.1.1). However, the ticks were not identified to species (or the identification was not reported). Intra-aural cases are noteworthy due to the clinical manifestations observed, including otalgia(Dogan et al., 2012; Prakairungthong et al., 2012; Shibghatullah et al., 2012), facial paralysis (Miller, 2002; Patil et al., 2012) and labyrinthitis (Shibghatullah et al., 2012).

In this present case of eyelid infestation, the patient had a full recovery. Bites confined to the eyelid may present conditions ranging from transient pruritus to severe blepharitis (Dagdelen et al., 2013), although these are mild compared to tick infestations involving the eye globe (Rai et al., 2016). Further complications such as abscesses and granulomas can develop especially when a segment of the tick is left *in situ* due to incomplete removal of the tick (Rai et al., 2016). A case has been reported from Singapore (Singh et al., 2006) where the patient experienced erythema of the eyelid following infestation by a tick of the genus *Ixodes*. Eyelid infestations by *Dermacentor* tickshave been reported from India (*Dermacentor auratus*) (Kirwan, 1935), and the United States (*Dermacentor variabilis*) (Price & Woodward, 2009; Terry & Williams, 1980).

Six species of *Dermacentor* are known from Southeast Asia (*Dermacentor atrosignatus*, *D. auratus*, *Dermacentor compactus*, *Dermacentor limbooliati*, *Dermacentor steini* and *Dermacentor taiwanensi*) (Apanaskevich & Apanaskevich, 2015; Petney et al., 2007). No human pathogens were reported from these species, except for *D. auratus* which has been reported to carry *Rickettsia* and *Anaplasma* in Thailand, near the Thai-Myanmar border (Parola et al., 2003), and Kyasanur Forest disease virus in India (Service & Ashford, 2001). Lanjan virus, which is morphologically similar to bunyavirus,but with unknown pathogenicity in humans

(Brian, 2009), has been isolated from *D. auratus* infesting wild rodents in Malaysia (Tan et al., 1967).

The main host of *Dermacentor* species in Malaysia is the wild pig, *Sus* spp. (Petney & Keirans, 1996) with Sambar deer, *Cervus unicolor*, various species of bats and other small mammals being other common hosts (Mariana et al., 2008b). We postulate the *Dermacentor* ticks were accidentally acquired from vegetation in the Kuala Gandah forest by the father, then transferred to the children. A similar case was reported from the Thai-Myanmar border, whereby two engorged *Dermacentor* ticks were removed from the ears of two children, believed to have been acquired from their fathers who had recently returned home from the jungle (Parola et al., 2003). Parola et al. (2003) further reported that *Dermacentor* ticks, particularly *D. auratus*, are the major ticks infesting humans during their research work in Thailand. In the present cases, the pathogenicity of the ticks remains unknown as the involved eye and ear healed with no further clinical manifestations.

This is the first documented case of atick infesting the eyelid in Malaysia. The ability of these ticks to harbour pathogens is not known, but given previous reports of pathogens carried by *Dermacentor*, investigations of tick-borne pathogens and the potential of transmission following tick bites are needed.

5.3.2 Case report 2: Intestinal myiasis in a patient from urban area

Most reported myiasis cases in Malaysia, were attributed to infestations of *Chrysomya bezziana* flies(Abu-Bakar et al., 1984; Johari & Khanijow, 1993; Lee et al., 2005; Ramalingam, 1982; Ramalingam et al., 1980; Reid, 1953; Rohela et al., 2006). In addition, *Chrysomya megacephala*(Abu-Bakar et al., 1984; Lee & Yong, 1991), *Sarcophaga* spp.(Ahmad et al., 2009; Baharuddin et al., 1995; Thomas et al., 1980), *T. albipunctatus* (=*C. albipunctatus*) (Smith & Thomas, 1979), *Eristalis* spp. (Lee, 1989),

Hermetia illucens(Lee et al., 1995), and *Lucilia cuprina*(Nazni et al., 2011) flies were among the causative species.

Reid (Reid, 1953) documented the first case of human myiasis (cutaneous myiasis) in Malaysia caused by the Old World screwworm fly, *C. bezziana*. In 1984, Oothuman and Jeffery (1984) reviewed all known cases of human myiasis and classified the infestations as urogenital, cutaneous and intestinal myiasis according to Zumpt's (1965) nomenclature. Since then, more cases of human myiasis have been reported, which include oral myiasis (Lee & Cheong, 1985; Roszalina & Rosalan, 2002), urogenital myiasis (Lee, 1989), aural myiasis (Ahmad et al., 2009; Johari & Khanijow, 1993; Lee & Yong, 1991; Rohela et al., 2006), intestinal myiasis(Baharuddin et al., 1995; Cheong et al., 1973; Lee et al., 1995), nasopharyngeal myiasis (Lee et al., 2005; Nazni et al., 2011), ophthalmomyiasis (Alhady et al., 2008) and cutaneous myiasis (Rahoma & Latif, 2010).

The life cycle of *C. albipunctatus* is completed in one to three weeks(Rocha et al., 2011). Larvae of *C. albipunctatus* can cause myiasis(Costa et al., 1998; Tu et al., 2007), or trigger bronchial asthma in people who accidentally inhale fragments of their disintegrated body parts (Robinson, 1996). The first documented case of human intestinal myiasis due to infestation of *T.* (=*Clogmia*) *albipunctatus* larvae was by Tokunaga (Tokunaga, 1953) in Japan. In Malaysia, the first case of intestinal myiasis caused by this fly was reported by Smith and Thomas (1979) with another report from several years later in Taiwan (Tu et al., 2007).

The present case is the fourth documented case of intestinal myiasis in Malaysia and the second caused by *C. albipunctatus*. Based on careful history taking by the patient, it is postulated that the patient developed intestinal myiasis due to ingestion of eggs oviposited by female flies contaminating her meal during fieldwork. The time interval between the day of ingestion (4[th]February) to the day of first larval discharge

(18th February) is fourteen days. It is speculated that the larvae passed out in the patient's faeces developed in the intestine after ingestion given that larval period of *C. albipunctatus* ranges from twelve to fifteen days (Williams, 1943). However, the patient may have had repeated exposure to the eggs or early-stage larvae from contaminated food that resulted in persistent infestation. It is also noteworthy that the patient is from an urban area, whereas myiasis is usually associated with patients from rural areas with poor hygiene and low economic status (Fernandes et al., 2009).

The present case may serve as a note to physicians to include intestinal myiasis as a possible cause of gastro-intestinal disorders. At present, there is no specific treatment for intestinal myiasis; however, purgatives, albendazole, mebendazole, and levamizole were reported to cure some patients(Francesconi & Lupi, 2012), as seen in this case. Preventive measures such as high levels of personal hygiene, careful storage of food and other vector control measures are needed to prevent possible re-exposure.

5.3.3 Case report 3: Canthariasis in an infant

Gastrointestinal infection in infants is usually self-limiting and treatable. However, severe complications can occasionally arise, particularly when the infection manifests as acute infectious enteritis which can be fatal in children (Thapar & Sanderson, 2004). In developing countries, cases of gastrointestinal infection remains high with an estimated 1.8 billion episodes of childhood diarrhoea and is an important clinical problem in children despite improvements in public health and economic status (Casburn-Jones & Farthing, 2004).

The life cycle of *L. serricorne* is completed in 45 to 70 days (Retief & Nicholas, 1988). Females oviposit as many as 100 eggs singly onto dried food materials and the eggs hatch in six to eight days. The larvae undergo four to six larval stages before they transform into inactive pupae and emerge into fully developed adult beetles in about

seven to eighteen days, depending on the environmental temperature and humidity (Reed & Vinzant, 1942; Retief & Nicholas, 1988). The adult is small, stout, oval and brownish red in colour and lives for two to four weeks. When fully grown, both adults and larvae are two to three millimetres long.

The present case is noteworthy as canthariasis has never been associated with the larvae of *L. serricorne*; it is not known to be medically important. Sun et al. (2016) concurrently reported a similar case in China in which an eight-month-old baby girlhad an infestation with the larvae of *L. serricorne* in the gastrointestine. The patient in present case was a full-term baby weighing three kilogram at birth. Her developmental milestones were appropriate and she received up-to-date immunisations. The family lives in a healthy and hygienic environment with a domestic helper taking care of the patient. The working parents are well-educated and they have another healthy and asymptomatic three-year-old daughter.

At the time of infection, the patient was no longer breast-feeding and had started eating solid food. The larvae passed out in the patient's faeces may have developed in the intestine after the baby ingested eggs from contaminated food prepared from ingredients that do not involve cooking, such as cereals and biscuits infested with cigarette beetles. The previous cases of infant canthariasis, which involved dermestid beetle larvae in the US, reported the presence of beetle larvae in the cereal being fed to the infants (Okumura, 1967). Alternatively, the patient may have become infected by drinking contaminated infant formula. Intestinal disorder of a newborn due to consumption of milk powder contaminated with eggs and larvae of *Musca domestica* has been reported from India (Shekhawat et al., 1993). Babies, by nature, have the habit of putting things into their mouth as was observed by the parents in the present case. It is therefore also plausible that the patient picked up contaminated materials (food or non-food substances infested with eggs or larvae) off the floor that led to the infection.

Two dogs also reside in the house, and the source of infection could be pet food. Pet food is commonly infested by *L. serricorne* in houses (Choe, 2013). As the source of the infection was not investigated further we can only speculate.

The risk of gastrointestinal infections in infancy can be reduced through good hygiene practices by parents and caregivers. Cigarette beetle infestations in food products can be prevented and controlled by locating and eliminating the source of infestation. Infested items can be cold-treated (sixteen days at 2 °C, seven days at -4 °C or four to seven days at 0°C) or heat-treated (88°C for an hour, 49 °C for sixteen to 24 hours) to eliminate infestation of all stages of *L. serricorne*(Cabrera, 2014). Healthcare professionals can raise awareness and provide advice on proper treatment for the underlying cause of gastrointestinal infections particularly when the symptoms signify serious complications.

5.4 Research limitations

Lack of cooperation from the management of several welfare homes has limited the number of welfare homes involved in this study; however, substantial efforts have been made to ensure every city and district were represented by at least one welfare home.

Due to budget constraints, only 260 out of 1732 head lice were subjected to *COI* DNA barcoding; one louse per individual was randomly chosen to accommodate strategic specimen processing. The occurrence of non-detectable clades in this study (clade C and E) might have been missed out due to this caveat.

CHAPTER 6: CONCLUSION

COI DNA barcoding has revealed the genetic diversity of head lice collected from welfare homes across two geographical regions of Peninsular Malaysia. The *COI* barcodes submitted to BOLD contribute to the reference head lice sequences available worldwide and also serve as a foundation for a larger library of head lice sequences from all around Malaysia. The sympatricity of clades A and D head lice may be crucial as both clades carried pathogens, as shown in this study. It is also interesting to note that clade B head lice occurs in Malaysia, despite its restricted geographical distribution.These findings suggest that there is tremendous scope for further enlightening works to be performed in the area of pediculosis in Malaysia in the future. Welfare homes that shelter other ethnic groups including the Indian and Chinese should be included to assess the head lice genetic variations among different ethnicities.In addition, *COI* DNA barcoding has enabled rapid and accurate identification of

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arthropod specimens of medical importance, thereby demonstrating the value of this robust technique in assisting the diagnosis of human diseases.

Molecular detection of *Acinetobacter* spp, *S. marcescens* and *S. aureus* in head lice suggests that pediculosis capitis, even though common, should be considered a public health priority. This suggests the need to conduct extensive studies to verify whether these agents are capable of causing illnesses in the occupants of the welfare homes. The high prevalence of pediculosis capitis and the presence of pathogens in the head lice recorded in this study highlight the need to monitor head lice infestations in welfare homes. Based on the significant findings made in this study, continuous research is needed to determine the role of head lice in the maintenance and transmission of pathogens to humans. Broaden studies of the vector competence of head lice and the susceptibility of human host to the potential pathogens carried by head lice will be required to determine their significance for public health. Further investigations into the virulence and pathogenicity of the bacterial strains detected in this study by isolating and inoculating the bacteria from head lice and clinical samples are also warranted to determine their pathogenic role to the affected persons. In addition, the insecticide resistance in the Malaysian head lice and bedbugs should be investigated to ensure the effectiveness of practised control measures.

It is also anticipated that these findingsprovide baseline data useful for the surveillance, prevention and control of pediculosis capitis in this country. Health education programmes emphasizing on personal hygiene and effective management strategies are crucial to prevent further spread therefore collaborative efforts from the relevant authorities are very much needed to financially assist these actions.

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Appendix A