PHYLOGENETIC AND GENETIC ANALYSIS OF TROPICAL BED BUG *Cimex hemipterus* (F.) POPULATION IN PENINSULAR MALAYSIA

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by

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LIST OF ABBREVIATIONS

AMOVA	Analysis of Molecular Variance
bp	Base pair
bp	Base pairs
CI	Confident Interval
dn	Dinucleotides
DNA	Deoxyribonucleic acid
E	East
F_{IS}	Inbreeding coefficient at the population level
F_{IT}	Inbreeding coefficient at total populations
F-Null	Null Allele (F-Statistic)
F_{ST}	Proportion of differentiation among populations
H_E	Expected heterozygosities
H_O	Observed heterozygosities
HWD	Hardy-Weinberg Deviation
HWE	Hardy-Weinberg Equilibrium
IAM	Infinite Allele Model
K	Number of cluster
Lat.	Latitude
Long	Longitude
min	Minutes
ml	Millilitre
mm	Millimetre
N	No
N	North
NA	Number of allele
NCBI	National Centre for Biotechnology Information
NGS	Next-Generation Sequencing
nm	Nanometre
PCR	Polymerase chain reaction
PIC	Polymorphic Information Criteria

r	Coefficient of relatedness
rpm	Revolutions per minute
rpm	Revolutions per minute
SD	Standard deviation
sec	Seconds
SMM	Stepwise Mutation Model
ssDNA	Single Stranded Deoxyribonucleic Acid
TPM	Two-Phase Mutation Model
trn	Trinucleotides
ttn	Tetranucleotides
v	Version
WGS	Whole Genome Shotgun
Y	Yes

ANALISIS FILOGENETIK DAN GENETIK POPULASI PEPIJAT KATIL TROPIKA *Cimex hemipterus* (F.) DI SEMENANJUNG MALAYSIA

ABSTRAK

Pepijat katil tropika, *Cimex hemipterus* (Fabricus) merupakan serangga tanpa sayap, yang diiktiraf secara saintifik sebagai masalah kesihatan awam yang signifikan. Hubungkaitan populasi pepijat katil di antara isolat dari segi lokasi geografi serta pemahaman yang mendalam dan signifikan adalah penting untuk merangka strategi kawalan yang berkesan. Kajian ini dijalankan untuk memahami corak pembiakan secara genetik ke atas populasi *C. hemipterus* dari segi variasi allelik, pencirian kepelbagaian genetik, struktur populasi dan filogeografi dalam kalangan populasi pepijat katil tropika di Semenanjung Malaysia. Kajian filogenetik menggunakan gen *cytochrome oxidase subunit I* (COI) mitokondria dijalankan ke atas dua puluh dua populasi pepijat tropika dengan tiga individu pepijat setiapnya. Analisis data gen COI menghasilkan pohon filogenetik dengan satu klad monofiletik utama yang terdiri daripada dua kumpulan iaitu Ch01 dan Ch02. Pepijat kumpulan Ch02 terdiri daripada populasi Bandar Hilir, berbeza dengan populasi lain yang membentuk satu klad tunggal. Pepijat kumpulan Ch01 menunjukkan persamaan dengan beberapa jujukan COI *C. hemipterus* dari Thailand, mencadangkan kemungkinan besar kesamaan variasi populasi. Namun begitu, variasi gen COI yang dicerap adalah rendah. Seterusnya, dari data NGS, 50 penanda mikrosatelit telah dibangunkan, dengan tujuh penanda mikrosatelit dipilih untuk kajian polimorfisme penanda. Sejumlah 220 sampel pepijat katil digenotipkan dengan tujuh penanda mikrosatelit yang spesifik spesies dan polimorfik menunjukkan 6-14 alel per lokus, dengan kepelbagaian genetik yang tinggi diamati di kalangan populasi. Nilai perbandingan purata dan jangkaan lokus heterozigos bagi keseluruhan populasi adalah 0.280 dan 0.828. Manakala, nilai perbandingan purata dan jangkaan lokus heterozigos di antara populasi adalah 0.281 dan 0.657. Populasi secara keseluruhan, berada dalam keseimbangan Hardy-Weinberg dengan lokus Ch_09ttn, Ch_01dn, dan Ch_13dn menunjukkan kehadiran alel null. Analisis genetik berdasarkan struktur genetik populasi mendapati pepijat katil tropika di Semenanjung Malaysia mempunyai diversiti genetik yang tinggi dengan perbezaan dan aliran genetik yang sederhana. Genetik populasinya pula didapati tidak terstruktur secara teratur. Hasil kajian ini membuktikan populasi pepijat katil tropika di Semenanjung Malaysia membiak dengan pasangan yang mempunyai persamaan yang tinggi secara genetik. Ia juga mencadangkan bahawa populasi pepijat ini dapat memulakan populasi baru dan menginfestasi habitat baru hanya dengan satu pendedahan sahaja. Hasil ini menunjukkan kawalan penghindaran infestasi perlu dimulakan sebaik sahaja dikesan untuk pencegahan yang lebih baik.

PHYLOGENETIC AND GENETIC ANALYSIS OF TROPICAL BED BUG *Cimex hemipterus* (F.) POPULATION IN PENINSULAR MALAYSIA

ABSTRACT

The tropical bed bugs, *Cimex hemipterus* (Fabricus) wingless insects, is recognized scientifically as a significant public health problem. The association of bed bug populations between isolates in terms of geographical location with in-depth and significant understanding is important for developing effective control strategies. The study was conducted to understand the genetic reproduction pattern of the *C. hemipterus* population in terms of allelic variation, genetic diversity, population structure and phylogeography among tropical bed bug populations in Peninsular Malaysia. The phylogenetic study was carried out by using mitochondria cytochrome oxidase subunit I (COI) on twenty-two tropical bug populations with three pooled bed bugs respectively. The analysis of COI gene data produced a phylogenetic tree with a major monophyletic clade consisting of two groups of Ch01 and Ch02. The Ch02 group was consist of Bandar Hilir populations, in contrast to other populations that make up a single cluster. The Ch01 group shows similarities with some of the COI genes of the *C. hemipterus* from Thailand, suggesting the similarity of population variation. However, the variation of the observed COI gene is low. Next, from NGS analysis, 50 microsatellite markers were developed, with seven microsatellite markers selected for the polymorphisms study. A total of 220-bed bugs samples were genotyped with seven species-specific polymorphic microsatellite markers and showed 6-14 allele per locus, with high genetic diversity observed among populations. The mean observed and expected heterozygosity of the overall population were 0.280 and 0.828. Meanwhile, mean observed and expected heterozygosity between all population is 0.281 and 0.657. The population as a whole is in equilibrium with Hardy-Weinberg with locus Ch_09ttn, Ch_01dn, and Ch_13dn indicates the presence of null allele. Genetic analysis based on population genetic structure has observed that tropical bed bugs in Peninsular Malaysia have a high genetic diversity with moderate genetic differentiation and gene flow. Its population genetics are also weak and undefined. This study proved that the population of tropical bed bugs in Peninsular Malaysia were moderately inbreeding. It also suggests that this bed bug population can start new infestation with one introduction These results indicate that control of infestation should be initiated once detected for better prevention.

CHAPTER 1

INTRODUCTION

1.1 Background of Study

Bed bug is a reddish-brown and flat blood-feeding insect. Due to blood feeding behaviour, these two species have a significant contribution to medical health. They are *Cimex lectularius* Linnaeus which can be found in temperate zones or subtropical areas while *Cimex hemipterus* (F.) can be found in tropical regions. Both sexes of these insects feed on human and animal, with birds or bats are the primary hosts (Delaunay et al. 2011).

The attention gained towards this insect as an indication of the possibilities of the bed bug's capacity to transmit disease, management of eradication, and the economic impact of infestations. This is due to the lack of references since the current bed bug resurgence is most notable in the areas of chemical regulation and prevention towards resistance mechanism in bed bug. Infestations are now common in the urban environment, including single-family dwellings, apartments, rooming houses, hotels, healthcare facilities, and college dormitories (Hwang et al. 2005).

Current research towards bed bug was aimed to eradicate or control them to a minimal infestation level with minimum effects on health and economic. Mainly, investigations were done to explore their capability to express resistance towards pesticides such as deltamethrin and imidacloprid as insecticide resistance is now proven to be the primary source of the bed bug resurgence (Myamba et al. 2002; Doggett et al. 2004; Romero et al. 2007; Wang & Cooper 2011; Doggett et al. 2012).

Along with the investigation on their pesticide resistance, some studies also focus on their preferable habitation in spreading infestation through passive, active dispersal (Wang et al. 2010) and human-mediated transportation (Vail & Chandler 2017). Their dispersal has gained much attention to their morphology as they were proved to be able to escape from most common management method, the bed bug monitor trap (Kim et al. 2017).

However, with the modernisation of technology, the management of this pest species was also considering supplementary molecular knowledge or their genetic profile aspects. Few types of research were done to provide significant data on molecular-based management such as phylogenetic, population genetic structure, and knockdown resistance (kdr) mutation. These research findings can be a fundamental knowledge to further research on proposing a proper control method towards this pest as they will provide the genetic structure of the infested population.

1.2 Problem Statement

The bed bug is an insect with a medically significant and a nuisance to humans whereas its bites and blood feeding activities may cause skin reactions and capable of harbouring infectious agents (Kolb et al. 2009). Scratching wounds that develop after several bites may even trigger some secondary infections (Goddard & deShazo 2009). Repeated bites of this insect can also cause systemic hypersensitivity (Thomas et al. 2004). Delauney et al. (2011) reported 45 candidate pathogens potentially transmitted by a bed bug in the laboratory. Although there is no report of proved cause of human diseases cases, bed bugs severely reduce the quality of life by producing discomfort, anxiety and sleeplessness symptoms (Hwang et al. 2005).

In Australia, other than causing implication on human health, bed bugs proved as one of the significant reasons contributing to economic losses in the hospitality and leisure industry by their infestation in hotels and public accommodations (Doggett 2006). Malaysia's economic status and level are also determined by the tourism and hospitality industry, so bed bug resurgence worldwide might contribute to a negative impact on Malaysia economic.

In Malaysia, there are only a few publications related to bed bugs. There was a report on bed bugs of Kuala Lumpur strain observed as more resistants to DDT as compared to a lowland tea estate strain of bed bug in Malaysia (Reid 1960). How and Lee (2010) reported that *C. hemipterus* is the only species of bedbug found in Malaysia with the most common locations in accommodations were headboard and bedding. In 2016, with a diverse area of collection for *C. hemipterus* in Peninsular Malaysia, Zahran et al. (2016) have confirmed only *C. hemipterus* was found at all 185 infested sites.

As insecticide resistance and international travelling, may be the main factors in contributing to the resurgence (Tawatsin et al. 2011), the molecular approach may contribute genomic profile knowledge for effective bed bug infestation prevention. Remarkable improvement has been made in insect molecular genetics whereas molecular genetics used as tools to solve problems.

1.3 Significance of Study

This study was conducted to study *C. hemipterus* infestation in selected urban and suburban areas in Peninsular Malaysia. This study aimed to examine the phylogenetic relationship between all chosen bed bugs population to discover possible genetic connection leading to *C. hemipterus* infestation in Peninsular Malaysia. Their population genetic structure was analysed in determining and support the discovery of relationships between *C. hemipterus* population for their infestation record in Malaysia. Thus, this will allow a better understanding of the species genetic profile.

1.4 Limitations of Study

The study was only done on selected urban and suburban areas in Peninsular Malaysia as reported by Pest Control Operator, not including East Malaysia due to the difficulty to gain access to infested premises along with the process to obtain permission.

1.5 Objectives

This project aimed to provide initial molecular genetic data on *C. hemipterus* specifically on describing its phylogenetic and population genetic structure of the tropical bed bug population in Peninsular Malaysia. This data would be useful for the effective prevention of this pest with fundamental information to the body of knowledge. To accomplish this main goal, the research flows were set to meet a few objectives as stated below:

Objective 1 (Chapter 3): To determine the phylogenetic relationship of *C. hemipterus* in Malaysia, by using mitochondrial DNA, Cytochrome Oxidase Subunit I as genetic markers.

Objective 2 (Chapter 4): To design and analyse polymorphism of novel microsatellite markers for *C. hemipterus* using the next generation sequencing. These markers were eventually used as the molecular marker in this study.

Objective 3 and 4 (Chapter 5): To analyse population genetics and structures of *C. hemipterus* by using the selected polymorphic microsatellite markers by examining its breeding dispersal and genetic pattern.

CHAPTER 2

LITERATURE REVIEW

2.1 Bed Bugs: A Glance of Their Role in Life

In recent years there has been a nation and worldwide resurgence in bed bug infestation. As travel and tourism cost become more affordable with emerging more options on transportation, more new bed bug infestation will be dispersed. It is easy for travellers to accidentally carrying some or an even one-bed bug on their clothing or even in their luggage. A trend towards less toxic pesticides and less pesticide use, in general, may have contributed to the reoccurrence of these insects. Additionally, bed bugs have become more impervious to some current insecticides.

Recent increases in bed bug reported cases suggested the need for a nationwide action that would clarify the scope of this growing problem, after almost 50 years of little attention (Gangloff-Kaufmann et al. 2006). The international resurgence of bed bug infestations has been a significant issue in many countries, including the United States, the United Kingdom, Denmark, Europe, Canada, Italy, Australia, Korea, Malaysia and Singapore (How & Lee, 2010c).

For some fifty years before the current resurgence, a bed bug was not considered a severe public health nuisance as the infestations had become rare. The decline in this once common pest was attributed to the improvements in hygiene and the development of powerful residual insecticides such as Dichlorodiphenyltrichloroethane (DDT) (Paul & Bates 2000). However, during the 1970s, the organochloride insecticides, including DDT, were gradually phased out due to environmental reasons. The non-availability of such highly effective insecticides and the changing philosophy of pest management to a more targeted approach led inadvertently to a niche being opened, in which bed bug was soon to exploit. By the early 21st century, bed bug infestations were growing at an alarming exponential level, and it has been the accommodation industry that has been the most impacted (Davies et al. 2012).

Resistance to insecticides can be developed in insects through four modes. Firstly, penetration resistance, which the resistance is increased if the insect has a thicker cuticle for decreased the entry of insecticides. Next is behavioural resistance, in which the resistance is raised for the insect which capable to sense and avoid the toxic compounds. Target site resistance is characterised by the ability of knockdown resistance (kdr), altered acetylcholinesterase and resistance to dieldrin (RDL), while metabolic resistance is defined by higher ability to detoxify or metabolize the insecticide (Mamidala et al. 2011).

Among these four modes, target site resistance (kdr) has been well characterised in common bed bug, *Cimex lectularius* (Yoon et al. 2008) and tropical bed bug, *C. hemipterus* (Dang et al. 2015). More comprehensive and global understanding of *C. hemipterus* is necessary for improving existing control strategies or devising new ones. An emphasis must be placed on expanding our knowledge regarding the apparent and rapid spreading of this pest, especially when monitoring or control method does not currently exist.

2.2 Biology of *Cimex hemipterus* (F.), the Tropical Bed Bug

2.2.1 Taxonomic classification of *Cimex hemipterus* (F.)

The taxonomic classification of *C. hemipterus* according to the Integrated Taxonomy Information System (ITIS) is as follows:

Kingdom: Animalia

Phylum: Arthropoda

Class: Insecta

Order: Hemiptera

Family: Cimicidae

Genus: *Cimex*

Species: *Cimex hemipterus* (F.)

C. hemipterus (Plate 2.1) morphology as an adult can be best described as dorsoventrally flattened (similar to cockroach), wingless, have compound eyes and reddish-brown in colour. Their size may reach eight millimetres in length, bigger than the common bed bug which is around 5 to 7 millimetres (Williams & Willis 2012). They have four segments of antennae with the last two segments are functioning as sensory perception. The sensory also contributed by signal send by sensilla hair distributed all over their legs, thorax, and abdomen (Singh et al. 1996).

Their pronotum is twice bigger than their length. *C. hemipterus* can do rapid movement and with higher tenant hairs on their tibial pad, will allow them to climb in competition with *C. lectularius* (Kim et al. 2017). When bed bug faced treat from a predator, an oil scented gland located at the ventral side of the third thoracic will

(a)

(b)

Plate 2.1: The ventral (a) and dorsal (b) view of *C. hemipterus*

excrete the oily substances to repel them away. Sexual dimorphism of the bed bug is contributed by bigger females (Roberts & Janovy 2000) and have traumatic insemination as their mating method. The male‘s aedeagus will releases sperm into paragenital sinus, which is located at right side of the female's fifth abdominal segment (Usinger 1966). Before copulating, the males require a blood meal while the females require it for egg productions (Roberts & Janovy 2000).

2.2.2 Lifecycle and morphological characters of *Cimex hemipterus* (F.)

Two species of bed bugs are considered as human pests: the tropical bed bug, *Cimex hemipterus* (F.), which exists mainly in tropical countries including Malaysia, and the common bedbug, *Cimex lectularius* L., which appears in temperate areas of the world (Harlan et al. 2008). Their nuisance identity is contributed by their blood feeding activities as soon as they hatch until they are adults. Bed bug needs to feed on blood meals specifically from warm-blooded hosts, which are typically humans for *C. lectularius* and *C. hemipterus*, although other mammals and birds can be utilised in the absence of a human host. After they emerge from their eggs, they will undergo five nymphal stages with moulting to the next stage after a blood meal, with the final fifth stage, they will moult into an adult (Harlan 2006).

2.2.3 Habitat and distribution of *Cimex hemipterus* (F.)

The tropical bed bug, *C. hemipterus*, is an important economic and nuisance pest that infests the numerous places where people sleep, including houses, apartments, hotels and college dormitories. It can form infestations comprising up to thousands of individuals (Reinhardt et al. 2010; Wang et al. 2010). Outbreaks typically consist of aggregations of individuals located in a discrete location, for example, a crack in a bed frame.

It walks to the host and returns to it hidings when feeding is complete (Reinhardt & Siva-Jothy 2007). Being flightless, it can only move actively over limited distances, and much of their recent spread is attributed to long-distance passive dispersal facilitated by human movement (Doggett et al. 2004; Reinhardt & Siva-Jothy 2007; Potter et al. 2008; Szalanski et al. 2008).

These insects commonly live in blankets, mattresses, furniture, floors, and walls near sleeping areas. They survive by using specialised mouthparts to pierce through humans integumentary system to feed on their blood. With a size that could easily be seen by naked eyes, they ensure their survival by hiding in seams and crevices of any suitable structures. Besides homes and hotels, bed bugs emerge in schools, hospitals, theatres, offices, retail stores, libraries, day care centres, fire and police stations, ambulances, moving transportation, and memorial homes (Potter et al. 2010). Small cities and towns that had been secure for a while now come upon the same risk of infestations as larger urban areas (Potter, 2011).

2.3 Bed Bugs Resurgence

Several studies have shown the high ability of bed bugs as an infectious vector and have raised significant concerns regarding possible new, emerging, or severe health risks. There is also an advanced assessment within the medical community that suggests emerging cases involving this insect should be expected (Thomas et al. 2004, Goddard & deShazo 2009).

International travellers could mostly spread bed bugs or even using second-hand furniture. Inadequate knowledge of bed bug's biology, outdated pest management practices and resistance to pesticides has caused a resurgence of this pest. As their revival is no longer new to the medical and public health communities, an increase in awareness and research on bed bugs is taking place (Delaunay et al. 2011).

Bed bugs have resurged globally. United States of America (USA) and Canada, are mostly infested with *C. lectularius* due to its temperate regions. In USA, first cities infested are New York, Chicago and Cincinnati, and worsen with lack of bed bugs managing experience among the pest control operators (Potter 2011). As similar to USA, Canada also experienced the same inefficient bed bugs management that contributes to higher reported cases (Doggett et al. 2018).

In Latin America, horror towards bed bugs has increased with the fear of the community towards their in-laboratory ability to harbour Chagas disease's agent, *Trypanosoma cruzi* (Salazar et al. 2015). In the past, bed bug infestation was usually associated with low hygiene, an improper structure of housing, and highly populated density. However, along with their resurgence, they could also be found at better quality housing area and tourist attraction places. This could suggest their ability to do more passive and active dispersal.

In Asia, resurgence was detected the late 1990s with significant public awareness starting 2005 (Doggett et al. 2018). In China and Japan, both *C. lectularius* and *C. hemipterus* were found with the evidence of infestation discovered at tourist attraction area (Komatsu et al. 2016). Studies by How and Lee (2010c) discovered infestation by *C. lectularius* in Malaysia, Singapore and Thailand (Tawatsin et al. 2011). A recent investigation by Zahran et al. (2016) has discovered *C. hemipterus* species which infested all 185 sampled sites in Peninsular Malaysia.

Doggett and Russell (2008) suggested cheaper and backpacking accommodation may contribute to the resurgence contribution factor, as the cases in Australia. With increased affordable international transportation such as aeroplane and train, this has encouraged an increase of infestation worldwide (Tawatsin et al. 2011, Zahran et al. 2016). It may also be contributed by an elevated level of insecticide resistance in bed bugs when the insect was exposed to higher concentration and prolonged used of insecticide (Tawatsin et al. 2011; Dang et al. 2015).

The resurgence of the bed bugs has initiated several researchers to investigate the bed bugs' genetic diversity, phylogenetics, population dynamics, and dispersal using various molecular markers ranging from the microsatellite to the COI markers. Population infestation dynamics and dispersal of *C. lectularius* were reviewed by Reinhardt and Siva-Jothy (2007), as there are still many uncertainties surrounding the biological aspect of bed bugs.

2.4 *Cimex hemipterus* as Public Health Significance Pest

Bedbugs are nocturnal, bloodsucking insects that feed on humans and are mainly crucial as biting nuisance. People who are bitten by bedbugs may suffer from intense itching, inflammation, and secondary infection after scratching, and sleepless episodes (Tawatsin et al., 2011). Although bed bug has been found positively capable to transmit disease in the laboratory with blood-borne pathogens such as Hepatitis B and HIV viruses, they are not proven or reported as a competent vector of disease.

The primary medical importance is inflammation associated with their bites (due to allergic reactions to components in their saliva). Sometimes, bed bug bites are self-limiting and enquire a little more attention than any infection prevention at the bite site. Human risks associated with bed bugs include sleeplessness, itchy bites and skin welts, anaemia, tension, economic losses, and the higher chances to have an overexposure to pesticides that been used for bed bug management (Fountain et al., 2014).

Physical health effects related to bed bugs are included in hypertrophic scarring, and allergic responses (Goddard & deShazo 2009) which appear immediately or a week of post-exposure with some of them might be asymptomatic (Reinhardt et al. 2009). Some might experience systemic health effects such as anaemia, asthma. However, the effects are occasionally rare (Abou et al. 1991; Paulke-Korinek et al. 2012) Mental health effects such as depression after several disrupted sleep or sleep loss were also reported. The possibility for bed bugs to act as vectors for abundant human pathogens, such as human immunodeficiency virus (HIV), hepatitis C virus (HCV), and hepatitis B virus (HBV), has been investigated (Chen & Copes 2010; Delaunay et al. 2011; Goddard & deShazo 2009) with HBV as a potential candidate.

Nowadays, *C. hemipterus* is observed as resurfacing to be a significant economic and public health pest, and their population size is triggered by an unexpected global resurgence (Boase 2001; Doggett et al. 2004; Kilpinen et al. 2008; Potter et al. 2008; Richards et al. 2009). The origins of this sudden population size enlargement have continued as a mystery in which the management and control resolution have been hindered by a lack of research on the bed bug’s essential population and dispersal biology (Reinhardt & Siva-Jothy 2007).

2.5 Bed Bug Management and Control

Regardless of many control resources and approaches being offered for bed bug management, insecticide treatments endure being the most prevalent (Moore & Miller 2006; Potter 2008; Potter et al. 2013). Various new insecticide products for bed bug control have developed in recent years. Pyrethroid insecticides and its combinations are regularly used by pest control professionals. However, with pyrethroids as their active ingredients, the bed bug management seems to be not useful in exterminating bed bugs due to insecticide resistance (Potter 2005; Romero et al. 2007; Zhu et al. 2010; Zhu et al. 2013).

Evaluations of resurgence bed bug populations from across the world indicating to pyrethroid insecticides resistance is already prevalent. With the lacking of new development tactics for efficient bed bug management, further growth of this public health problem should be predictable (Romero et al. 2007). The incapability to manage bed bugs infestation with pyrethroids may require the development of products with new modes of action, relabeling of existing useful products, and greater dependence on alternate strategies such as heat treatment, vacuuming or mattress replacement. Moreover, there is increasing health concerns of human-insecticide exposure, as these pesticides are usually applied directly and extensively to the furniture and resting areas (Singh et al. 2015).

However, there are physical and financial boundaries as challenges appear in dealing with bed bug infestations. Existing treatment options such as the application of heat or pesticides can be useful if they are appropriately applied. Moreover, there are concerns about their pesticide resistance level. Here, an Integrated Pest Management (IPM) approach is suggested and, ongoing monitoring and prevention are also recommended as they are proven to be essential for positive outcomes (Comack & Lyons 2011). Academicians, researcher, regulatory officials, building management, and pest management professionals are encouraged to work together using a systematic method to address bed bug infestations by using IPM (Ratnapradipa et al. 2011; Rossi and Jennings 2010). IPM is a method that combines these approaches with updated and essential information on infestation severity, pest biology and genetic information, with any sustainable interventions for defining active management selections.

For example, identifying bed bug infestations as early as possible could increase the chances of successful treatment. Here, biology and genetic intervention are fundamental to provide crucial information as live or dead bed bugs, their moulted skins, faeces, or any blood stains can indicate active infestation. An infested unit should be inspected systematically to identify harbourage sites which can aid in determining the size of bed bug infested populations (Wang et al. 2009; Weeks et al. 2011) to assess their infestation dynamic.

2.6 Bed Bug Infestation Dynamic

In recent years, the application of high-resolution molecular markers has provided significant new perception into the population genetic structure and infestation dynamics of many insect pest species of public health concern (Conn & Mirabello 2007; Fitzpatrick et al. 2008; Paupy et al. 2008; Endersby et al. 2009; Crissman et al. 2010; Booth et al. 2011). New molecular tools could precisely recognise the number of populations actively invading a building (Pizarro et al. 2008, Crissman et al. 2010) and explain the infestation dynamics to understanding infestation patterns (Paupy et al. 2008, Piccinali et al. 2009). Significant information on genetic identification of species will be suitable to support the choice of proper and efficient bed bug management strategies. However, with the current powerful genetic tools to reveal information on the biology and management of bed bug, the infestation dynamics within human lodgings remains mostly unaddressed.

Tropical bed bug infestation dynamic has been investigated using their dispersal behaviour (How & Lee 2010a) that reported of control effort delays will increase the possibility of greater and wider infestation. For common bed bug, Booth et al. (2012) reveal of a small founding propagule introduction which could establish infestation and thus, is capable of rapidly spreading the outbreak to various apartments throughout the building. By knowing such information, it may educate the pest control operators on the choices of treatment method that are most likely to be efficient under such circumstances. This information may also provide the dispersal pattern as well as determine the possibility of reinfestations of treated premises.

2.7 Dispersal Pattern of Bed Bug

By using bed bug infestation dynamic information, the ability of bed bug to disperse and their pattern could attribute to provide the complete bed bug population structure. Ronce (2007) describes dispersal as any individuals or propagules movement that could lead to gene flow as it is vital for it fundamental information to the population structure and dynamics (Bowler & Benton 2004).

Therefore, understanding the possible cause of dispersal is essential to study the population genetic structure. For the natural population, a variance degree of spatial heterogeneity which defined as an uneven distribution of various concentrations of the species within an area may cause the individual in the population to face a variety of dispersal reasons (North et al. 2011). As for an unstable population, the possibility of dispersing may increase with spatial heterogeneity (Cadet et al. 2003; Parvinen et al. 2003).

A study by Booth et al. (2012) revealed that the reinfestation of the residence could occur if an incomplete treatment did not eliminate all individuals with high possibility of interbreeding between the remaining past and present bed bug population. Active dispersal of unrelated bed bugs from outside of the residence was also readily infested earlier by the outside introductionary population. Additionally, Wang et al. (2010) have discovered some active dispersal in multistory buildings. Therefore it cannot be assumed as the cause of the extraordinary high genetic diversity of the bed bug infestation.

2.8 Molecular Genetic Marker

The application of molecular genetic markers could provide a powerful way to determine the breeding pattern and genetic structure (Husseneder et al. 2003). Molecular markers such as microsatellite, are especially useful for the investigation of population genetic differentiation (Vargo 2003). In addition to providing a powerful technique to determine the breeding pattern and population genetic structure in insect, molecular genetic markers are also useful for effectiveness evaluation of management practices.

2.8.1 Next-Generation Sequencing

Eukaryote genomes contain tandem repeated DNA motifs with a range of one to six base pairs. With an increased high throughput next-generation sequencing (NGS) application, microsatellite could be quickly developed and widely used as a molecular marker. Microsatellites can produce high variability (Guichoux et al. 2011) in population genetic structure (Rosenberg 2003), linkage disequilibrium studies (Ohashi & Tokunaga 2003) and sibling reconstruction (Wang & Santure 2009). The next-generation sequencing, such as the Roche 454 GS-FLX Titanium shotgun sequencing platform, offers better potential for the isolation of this repetitive DNA markers from the genome of species with no prior reference genome available (Margulies et al. 2005; Malausa et al. 2011).

This technology is a choice for any cost-constraint experiment compared to time-consuming Sanger technology. Moreover, this 454-shotgun sequencing offers a high throughput process and quicker time than manual sequencing (Rothberg & Leamon 2008). Therefore, this technology is still an option for many researchers to isolate microsatellite markers from insect genomic DNA (Rasmussen & Noor 2009; Santana et al. 2009; Smith et al. 2011). NGS technique has been used to isolate microsatellite markers from *C. lectularius* (Lowe & Eddy 1997; Rosenfeld et al. 2016) but not *C.hemipterus*.

2.8.2 Application of genetic markers for studying insects

The resurgence of bed bug outbreaks has stimulated interest in the development of novel control strategies for this pest. Current chemical methods especially the excessive usage of pyrethroid insecticide have rapidly developed resistance in natural bed bug populations. Nowadays, molecular control methods have been a promising alternative pest management technique to reduce or eliminate the pest populations.

Excessive and inadequate indiscriminating use of insecticide has prompted scientific communities to double their efforts to develop an alternative method such as genetic studies. Some molecular research has been done on bed bug to help the scientists to have a better understanding of this reemergence pest to provide new tools for bed bug management. Sakamoto and Rasgon (2006) have done broad experiment scale on endosymbiotic bacteria effects on bed bugs by using an idea of manipulating or eliminate obligate symbiotic bacteria required by bed bug for blood meal digestion.

2.8.3 Mitochondrial Cytochrome Oxidase I (COI) and its applications in studying insect phylogenetic relationship

COI is a subunit of the cytochrome oxidase complex involves in the electron transport chain. Hillis et al. (1996) mentioned that the sequences of COI are highly conserved across the species. Thus, it is possible to design a useful universal primer. This molecular marker has been used to examine the phylogenetic relationship in relatively lower levels of animal classification such as in families, genera, species or populations (Hwang & Kim 1999).

Hebert et al. (2003) argued in favour of a 50 fragment of the mitochondrial gene for cytochrome oxidase subunit I (COI) as a universal marker among animals because of its two critical advantages. First, the universal primers for this gene are very robust, enabling recovery of it's 5’-end (Folmer et al. 1994; Zhang & Hewitt 1997).

Second, COI appears to possess a greater range of phylogenetic signal than any other mitochondrial gene. Their third-position nucleotides show a high frequency of base switches that lead to a molecular evolution greater than 12S or 16S rDNA (Knowlton & Weigt 1998). COI was the first PCR-based marker and became the most widely used molecular marker for population genetic analysis. Sequences of COI are proven to be useful in identifying various animals at a species-level. However, empirical, theoretical evidence argued that COI sequences may not track the species evolution and diversification (Narain et al. 2015; Beltrà et al. 2012).

Nevertheless, COI sequences are continuously used to represent a standardised and universal DNA barcoding identification system for animals, of which the sequences provide a framework for species identification as well as for large-scale biodiversity assessments (Hebert et al. 2003). Numerous researchers have used COI sequences to identify the unknown, cryptic or invasive species in entomology, botany, ornithology and ichthyology (Hebert et al. 2003; Lahaye et al. 2008; Narain et al. 2015; Takeuchi et al. 2017).

2.8.4 Microsatellite and its applications in studying insect population genetic structure and breeding pattern

Bed bug resurgence has created various concern on their level of population differentiation and breeding pattern due to the deficiency of genetic-based studies to design efficient management toward this pest, including their population genetic structure. Population genetic structure is the direct consequence of biogeography which provides invaluable statistics on patterns of species dynamics and isolation. By analysing population genetics study outcome, the result can be associated with phylogenetic study outcome to obtain information on the source of population and their breeding pattern (Perdereau et al. 2011; Perdereau et al. 2013). Understanding this information is crucial in developing effective management method and strategies against these pest species. Population genetic studies are among the most likely research explored by a molecular entomologist to investigate the species that disperse primarily by human transport as it can provide information on the role of human activity in the spread of the pest. It may also allow the identification of source populations and routes of transport which can be targeted to reduce the possibility of future introductions (Suarez & Tsutui 2008).

This population genetic studies can also provide ideas on the genetic structure of populations. Genetic markers have long been recognised as beneficial tools for the study of population genetic structure for the inference of past demographic events, including dispersal and gene flow (Avise 2004). Once the molecular genetic technique reveals the molecular data on bed bug specific strain and species, these can be further exploited for management and control of bed bug.

Efficient bed bug management is hampered by the lack of ecology, dispersal, genetic structure, and biological knowledge of its population. These types of information would be aided by the development of species-specific genetic tools such as microsatellite markers. Microsatellite markers are widely used genetic markers with a proven application on diversity, gene flow, dispersal, population genetic structure, and phylogeny studies (Sunnucks 2000; Lee 2002; Zhang & Hewitt 2003; Avise 2004). Thus, much information can be gained by using microsatellite markers for assessment of the spatial distribution, knowledge of the geographic origin, breeding system, and the relationship among any given population (Dunley & Croft 1992; Jeger 1999; Ross 2001; Vargo et al. 2003).

In population genetic research, Saenz et al. (2012) suggest that nearly all the studied infestations in the Eastern United States were started by a small propagule and all infestations were strongly and genetically differentiated from each other. However, there is no substantial evidence of a geographic pattern of genetic structure, indicating the infestations are in close proximity to each other and also are genetically differentiated as those located hundreds of kilometres away. This gives result in understanding the patterns of dispersal as well as the implications for managing the spread of bed bugs via vector control.

The critical usage of molecular genetic markers to the populations of bed bug could provide a powerful way to discriminate among the population as well as to determine population breeding structure (Husseneder et al. 2003). There is a growing number of genetic research using molecular markers in bed bug populations to elucidate their breeding structure (Vargo et al. 2006).

With increased travel and connectivity, there are now many opportunities for the human-facilitated dispersal of organisms, with disease vectors and pests of economic importance presenting a particular concern (Estoup et al. 2004; Grapputo et al. 2005; Tatem et al. 2006; Niggemann et al. 2009; Lawson Handley et al. 2011). The population significantly affect how genetic diversity is distributed, both within and between local populations (Hastings & Harrison 1994).

In a bed bug population, human dwellings form habitat patches, and small numbers of individuals bed bug find new infestations. Bed bugs have been observed to actively dispersing throughout buildings (Doggett & Russell 2008; Wang et al. 2010) and become the mechanism by which individuals move into portable items, leading to passive dispersal (Fountain et al. 2014).

Development of suitable effective management strategies to be able to mitigate infestation and illness in urban areas is by the determination of their dispersal patterns and gene flow (Hampton et al. 2004). Between populations, gene flow measured could be strongly influenced by their dispersal ability and geographical structure of the possible infested area (Colautti et al. 2005; Therriault et al. 2005; Booth et al. 2009). These factors, are more likely to significantly impede or encourage a population's evolutionary and adaptive capacity (Garant et al. 2007).

In the absence of contiguous suitable habitat through which active dispersal can occur, population differentiation may be strongly driven by passive movement in the form of human-mediated dispersal. However, few studies document the impact of this dispersal mechanism on population genetic structure in species for which it is necessarily the only means of interpopulation movement.

Bed bug represents an ideal species to study genetic diversity and connectivity among geographically-separated populations that are linked solely by human-mediated dispersal. As this species was recognised globally as a prominent household pest of public health and economic significance, they exhibit a relative behaviour of strict human commensalism. The effect of this behaviour on their genetic diversity and population differentiation observed in the urban setting has been addressed in some investigation using various molecular genetic markers (Booth et al., 2011).

Studies using microsatellite markers by Narain et al. (2015) indicated a high genetic diversity of *C. lectularius* in the Midwest United States. This population dynamics research also helps to find new alleles for the bed bugs population. A study on the genetic variation of *C. lectularius* in the United States showed a moderate