TROPICAL BED BUG
(HEMIPTERA: CIMICIDAE) INFESTATION, PUBLIC AWARENESS, MORPHOLOGICAL IDENTIFICATION AND POPULATION GENETIC STRUCTURE IN IRAQ

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LIST OF ABBREVIATIONS

AMOVA	Analysis of Molecular Variance
bp	Base pair
CI	Confident Interval
dn	Dinucleotides acid
trn	Trinucleotides
ttn	Tetranucleotides
DNA	Deoxyribonucleic acid
E	East
FIS	Inbreeding coefficient at the population level
FIT	Inbreeding coefficient at the total population level
FST	Proportion of differentiation among populations
F-Null Null	Allele (F-Statistic)
HE	Expected heterozygosities
HO	Observed heterozygosities
HWE	Hardy-Weinberg equilibrium
IAM	Infinite Allele Model
TPM	Two-Phase Mutation Model
SMM	Stepwise Mutation Model
K	Number of cluster
Lat.	Latitude
Long	Longitude
min	Minutes
ml	Millilitre
mm	Millimetre

N	No
NA	Number of allele
NCBI	National Centre for Biotechnology Information
PIC	Polymorphic Information Criteria
PCR	Polymerase chain reaction
r	Polymorphic Information Criteria
SD	Standard deviation
sec	Seconds
v	Version

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INFESTASI PEPIJAT TROPIKA (HEMIPTERA: CIMICIDAE), KESEDARAN AWAM, PENGECAMAN MORFOLOGI DAN STRUKTUR GENETIK POPULASI DI IRAQ

ABSTRAK

Cimex hemipterus (F.), atau pepijat tropika, adalah ektoparasit yang kebanyakannya memakan darah manusia. Pepijat tropika baru-baru ini menjadi isu penting dalam kesihatan awam dan mempunyai kepentingan sosial serta ekonomi. Kajian ini bertujuan untuk menentukan pembiakan pepijat, mengenal pasti spesies pepijat, dan menentukan tahap kesedaran dalam kalangan komuniti Iraq mengenai pembiakan pepijat di Iraq. Kajian ini juga meneliti struktur genetik dan kepelbagaian populasi *C. hemipterus* di Iraq. Dinamika pembiakan pepijat ditentukan melalui kajian 18 tapak pembiakan, termasuk rumah persendirian, hotel tempatan, dan asrama pekerja, di lapan wilayah Iraq antara tahun 2020 dan 2021. Sampel pepijat dikumpulkan dari setiap tapak pembiakan untuk kajian lanjut. Dari kajian, kebanyakan pembiakan pepijat di Iraq berpunca dari pekerja migran dan penggunaan semula perabot serta pakaian terpakai. Dari segi tempat persembunyian pilihan, perabot kayu adalah sumber yang paling biasa (91.6%). Walaupun sejumlah besar responden (43.8%) menyatakan "agak bimbang" tentang pembiakan pepijat di Iraq, terdapat tahap ketidaktahuan yang tinggi (53.3%) mengenai pembiakan pepijat. Nisbah lebar kepada panjang pronotum purata bagi sampel pepijat yang dikumpulkan adalah kurang daripada 2.5mm; sampel-sampel ini kemudian menjalani pengenalan molekul melalui urutan parsial COI mitokondria dan 16S rRNA. Berdasarkan ciri morfologi dan data urutan DNA mereka, dapat dipastikan bahawa semua pepijat yang dikumpul adalah *C. hemipterus*. Selain itu, hasilnya menunjukkan bahawa C. hemipterus adalah satu-

satunya spesies pepijat di Iraq. Lapan belas sampel dipilih secara rawak dari setiap tapak untuk analisis filogenetik menggunakan penanda DNA mitokondria gen Cytochrome Oxidase 1 (COI) dan 16S ribosomal RNA (16S rRNA). Untuk analisis filogenetik, kemungkinan maksimum (ML) dan pohon sambungan jiran (NJ) yang dibina menggunakan MEGA11 menunjukkan pengelompokan urutan yang diperoleh bersama dengan urutan rujukan C. hemipterus dari GenBank, dengan nilai bootstrap 100% untuk pohon COI dan 99% untuk pohon 16S. Analisis kepelbagaian genetik menggunakan urutan parsial COI dan 16S rRNA mendedahkan tiga haplotip dalam populasi pepijat di Iraq dengan kepelbagaian genetik yang rendah (0.0011 dan 0.0090, masing-masing). Seratus empat puluh sampel dari 14 tapak dipilih secara rawak dan digenotip menggunakan tujuh penanda spesies-spesifik polimorfik mikrosatelit. Kepelbagaian genetik tinggi dilihat antara populasi, dengan purata 2–9 alel per lokus. Bilangan alel merentasi tujuh lokus mikrosatelit antara 6 hingga 18. Walau bagaimanapun, semua lokus menunjukkan penyimpangan daripada Keseimbangan Hardy-Weinberg (HWE). Analisis varians molekul (AMOVA) mendedahkan bahawa 93% variabiliti genetik adalah dalam populasi, dan 7% adalah di antara mereka. Populasi keseluruhan menunjukkan heterozigositi purata diperhatikan sebanyak 0.175 dan heterozigositi purata dijangka sebanyak 0.730. Heterozigositi purata diperhatikan antara populasi adalah 0.173, manakala heterozigositi purata dijangka adalah 0.673. Analisis genetik berdasarkan struktur genetik populasi menunjukkan bahawa populasi pepijat di Iraq mempunyai pembezaan genetik yang rendah (F_{ST}=0.045) dan tahap pembiakbakaan yang tinggi (F_{IS}=0.761) dengan aliran gen sederhana antara populasi. Individu campuran telah didedahkan menggunakan struktur dan pohon filogenetik sambungan jiran, menunjukkan aliran gen sederhana antara populasi dan kekurangan struktur genetik dalam kumpulan wilayah.

TROPICAL BED BUG (HEMIPTERA: CIMICIDAE) INFESTATION, PUBLIC AWARENESS, MORPHOLOGICAL IDENTIFICATION AND POPULATION GENETIC STRUCTURE IN IRAQ

ABSTRACT

Cimex hemipterus (F.), or tropical bed bugs, is an ectoparasite that primarily feeds on human blood. Tropical bed bugs have recently become in public health and are of social and economic importance. This study aimed to determine bed bug infestation, identify the bed bug species, and determine the level of awareness among the Iraqi community regarding bed bug infestations in Iraq. This study also examines the genetic structure and diversity of *C. hemitpterus* populations in Iraq. Bed bug infestation dynamics were determined by surveying 18 infested sites, including private homes, local hotels, and workers' dormitories, across eight Iraqi governorates between 2020 and 2021. Bed bug samples were collected from each infested site for further study. From the survey, most bed bug infestations in Iraq primarily stem from migrant workers and the reuse of second-hand furniture and clothing. In terms of preferred harborage, wooden furniture was the common source (91.6%). Although a large number of respondents (43.8%) expressed "somewhat concerned" about bed bug infestations in Iraq, there was a high level of unawareness (53.3%) regarding bed bug infestations. The mean pronotum width-to-length ratio of collected bed bug samples was less than 2.5mm these samples then underwent for molecular identification through partial sequences of mitochondrial COI and 16S rRNA. Based on their morphological characteristics and DNA sequence data, it can be affirmed that all collected bed bugs were *C. hemipterus*. Moreover, the results indicated that *C. hemipterus* is the sole bed bug species in Iraq. Eighteen samples were randomly

selected from each site for the phylogenetic analysis using mitochondrial DNA markers Cytochrome Oxidase 1 (COI) gene and 16S ribosomal RNA (16S rRNA). For phylogenetic analysis, maximum likelihood (ML) and neighbor-joining (NJ) tree constructed using MEGA11 showed clustering of the obtained sequences together with C. hemipterus reference sequences from GenBank, with bootstrap values of 100% for the COI tree and 99% for the 16S tree. Genetic diversity analysis using partial sequences of COI and 16S r RNA has revealed three haplotypes within bed bug population in Iraq with low genetic diversity (0.0011 and 0.0090, respectively). One hundred and forty samples from 14 sites were randomly selected and genotyped using seven species-specific polymorphic microsatellite markers. High genetic diversity was seen among populations, with an average of 2–9 alleles per locus. The number of alleles across seven microsatellite loci was between 6 to 18. However, all the loci have shown deviation from the Hardy-Weinberg Equilibrium (HWE). Analysis of molecular variance (AMOVA) revealed that 93% of the genetic variability was within the populations, and 7% was among them. The overall population exhibited an average observed heterozygosity of 0.175 and an average expected heterozygosity of 0.730. The average observed heterozygosity among the population was 0.173, while the average expected heterozygosity was 0.673. Genetic analysis based on population genetic structure demonstrates that the bed bug population in Iraq has a low genetic differentiation (F_{ST}=0.045) and a high degree of inbreeding (F_{IS}=0.761) with a moderate gene flow between populations. Admixed individuals were revealed using structure and neighbor-joining phylogenetic trees, demonstrating moderate gene flow between populations and a lack of genetic structure in the regional groups.

CHAPTER 1

INTRODUCTION

1.1 Background of study

The Bed bugs are small, flat, reddish-brown insects, a common urban nuisance that may be found worldwide (Krinsky, 2019). The *Cimex lectularius* species is more common temperate region, while the *Cimex hemipterus* species is more common in subtropical and tropical zones (Eddy & Jones, 2011; Doggett et al., 2018; Leong et al., 2020). Both sexes, throughout all life cycle stages (except for the egg stage), feed on human blood and other animals, such as birds and bats. Blood is necessary for their continued growth and development. As a result of this feeding, bed bugs can cause many health problems for humans (Delaunay et al., 2011; Doggett et al., 2012).

Between the 1950s and the late 1970s, bed bug infestations dropped substantially in many developed nations due to the extensive use of insecticides (Potter, 2011). However, the difficulty of controlling bed bugs has been rising in recent years, probably due to the development of their resistance against insecticides (Potter, 2011). Despite the possible resistance, many pest management companies still use insecticides in bed bug treatment despite the availability of many other control methods, including non-chemical approaches like vacuuming and extremes in temperature, which are routinely recommended for effective bed bug control (Zehnder et al., 2014; Potter et al., 2015; Doggett et al., 2018). Therefore, pest control specialists in the United States have reported that these insects are among the most difficult pests to deal with inside buildings (Lai et al., 2016).

Bed bugs' mechanisms to resist insecticides have been a popular research subject in order to find a way to control them. For example, many studies focused on spread of bed bugs via passive dispersal pathway through which bed bugs are transported unnoticed by the host to new location (Doggett et al., 2003; Reinhardt & Siva-Jothy 2007, Kilpinen et al., 2008; Dogget et al .2018). However, bed bugs are also capable of actively disperse over relatively shorter distance, such as moving from one room to another room within in a building (Reinhardt & Siva-Jothy, 2007; How & Lee, 2010; Wang et al., 2010). Both passive and active dispersal behaviors play a pivotal role in the proliferation of infestations (Wang et al., 2010; Vail & Chandler, 2017). The fact that they can evade the most prevalent form of control, the bed bug monitor trap, has sparked much interest in their morphology and its implications for their spread (Kim et al., 2017).

However, bed bug dispersal patterns can be better understood, and management strategies against these ectoparasites can be improved with more information on bed bugs' biology, epidemiology, and genetic structure. Research is limited on phylogenetics, population genetic structure, or any other information that would have been useful in providing substantial data on molecular-based management.

1.2 Problem Statement

Bed bugs feed exclusively on human blood and are a globally significant medical parasite. Their bites might exacerbate psychological disorders, disturb sleep, and cause additional health problems, including rashes, irritation, and allergies. Bed bugs are believed to transfer infectious pathogens to humans (Eddy & Jones, 2011). Delauney et al. (2011) state that bed bugs may harbor up to 45 pathogens or microorganisms. While *C. lectularius* has been demonstrated in laboratory settings to potentially serve as a biological vector for *Trypanosoma cruzi*, the pathogen causing Chagas disease, and *Bartonella quintana*, the agent responsible for trench fever

(Salazar et al., 2015; Leulmi et al., 2015; Lai et al., 2016), such evidence remains confined to experimental conditions and has not been definitively confirmed in natural settings, thus precluding its classification as a confirmed biological vector. Moreover, no field evidence links bed bugs to the spread of any human disease. This unproven theory suggests that bed bugs may have evolved specialized antipathogen defenses that hinder their ability to act as effective vectors (Ho et al.,2016; Potts et al., 2022).

In addition to causing health issues, bed bugs are accountable for a wide range of economic challenges that impact the cultural and tourism sectors (Doggett & Russell, 2008). Infestations of bed bugs are frequent in various settings, including homes, apartments, dormitories, hotels, motels, and even public transportation (Doggett et al., 2018). The tourism sector is considered one of the most critical sectors in the Iraqi economy, so the resurgence of bed bugs worldwide may have a negative impact on the Iraqi economy.

In Iraq, there is limited literature manuscripts on bed bugs. According to the results of an early study conducted by Patton (1919, 1920) it was hypothesized that *C. hemipterus* was the only species living among the Indian communities in Mesopotamian cities like Basra and Baghdad. After fifty-eight years, Abul-Hab (1978) reported that *C. hemipterus* was present in Iraq in the 1970s. Augul and Al-Saffar (2019) also confirmed the presence of *C. hemipterus*, collected from Baghdad dwelling houses in 2018. However, other studies indicate the presence of *C. lectularius* in Iraq as well. But these studies did not benefit from using molecular tools to identify bed bugs.

1.3 Significant of study

To our knowledge, this study is the first in Iraq to determine the infestation of bed bugs in urban and suburban areas in 8 among 18 Iraqi governorates. Also, this study was to determine the public awareness level of the Iraqi population about bed bugs; examining the phylogenetic relationship between all selected groups of bed bugs was one of the most important aims of this study to discover the possible genetic connection that leads to a bed bug infestation in Iraq. The genetic structure of their populations was studied to confirm and explain the connections between the several *C. hemipterus* populations responsible for the documented infestations in Iraq.

1.4 Limitation of study

This study relied on samples of bed bugs collected from the infested sites in southern and northern governorates, some areas of the capital Baghdad, and two governorates in central Iraq. Contacting residents and pest control companies served as the collection process's means. The sampling process did not include the governorates in Iraq's eastern and western regions because this process required obtaining permission. In addition, a lack of social cooperation negatively affects the sampling process.

1.5 Objectives

The aim of this study was to determine the population genetic structure and evolutionary relationships within tropical bed bug populations in Iraq, using partial genetic data for *C. hemipterus*, using partial genetic data for *C. hemipterus*. Integrating this data into the current knowledge base has the potential to enhance successful strategies for preventing this pest. Research methodologies are strategically designed to achieve multiple objectives, all of which contribute to the overall goal of understanding and preventing this pest. To accomplish the primary aim, the study was designed to achieve a number of objectives, enumerated as follow:

Objective 1 (Chapter Three): To determine bed bug infestation dynamics and public awareness level in Iraq

Objective 2: (Chapter Four): Morphological and molecular identification of bed bugs collected from Iraq using COI and 16S rRNA

Objective 3: (Chapter Five): To determine the genetic diversity and phylogenetic relationship of bed bug infestation and distribution in Iraq

Objective 4: (Chapter Six): To discover the population genetic structure and breeding pattern of bed bugs in Iraq.

CHAPTER 2

LITERATURE REVIEW

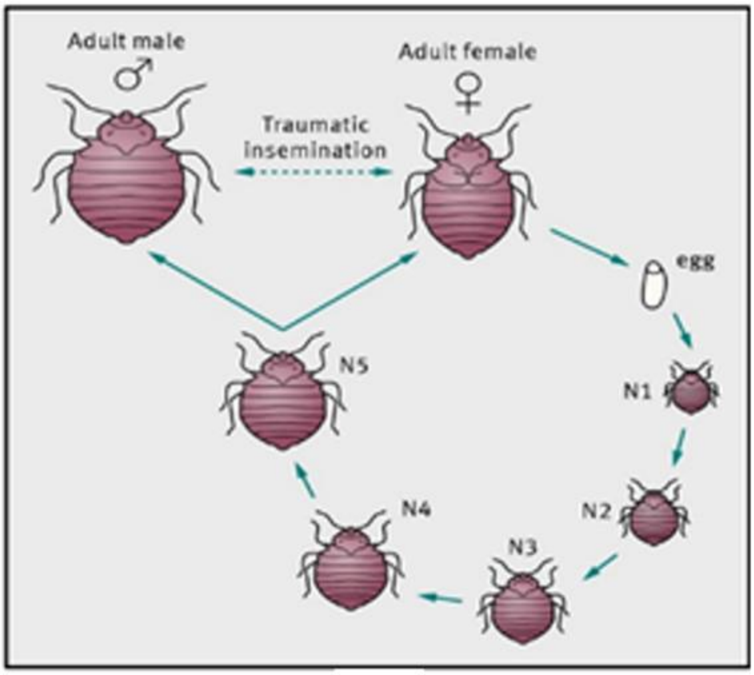
2.1 A glimpse at the role of tropical bed bugs

Over the past decade, there has been a worldwide resurgence of the common bed bug *Cimex lectularius* L. and the tropical bed bug *Cimex hemipter*us (F.). Homes, hotels, public lodgings, workplaces, libraries, healthcare institutions, transportation (bus, cruise ships, and aircraft), and even chicken farms have all been affected by this pest species in varying degrees (Doggett et al., 2018). It is believed that globalization, ineffective control strategies, and insecticide resistance are the primary factors behind the resurgence (Dang et al., 2017). In addition, the high prevalence of insecticide resistance suggests fewer chemical control options against bed bugs (Doggett et al., 2018). Moreover, it is shown that bed bugs are highly resistant to a wide range of insecticides (Romero et al., 2017). After World War II, reports of bed bug infestations dropped dramatically in several countries due to the broad use of dichloro-diphenyl trichloroethane (DDT). DDT was first invented in 1939 (Dang et al., 2017). However, after 50 years, bed bug complaints gained widespread media attention again, suggesting the comeback of the pest problem (Gangloff-Kaufmann & Pichler, 2008). The documented reports of a resurgence of bed bugs in 135 nations across five continents since the 1990s indicated a significant threat to human health and wellness (Zorrilla-Vaca et al., 2015). Doggett and Lee (2023) demonstrated, that by the early 21st century bed bugs had reached a widespread dispersal and the industry most significantly affected was the accommodation industry (Penn & Hu, 2020). Therefore, a global understanding of the patterns of spread of bed bugs and the establishment of invasive species in nonnative regions is critical, as the species is economically or medically damaging.

2.2 Biology and life cycle of bed bugs

Bed bugs are true bugs of the order Hemiptera (also written as Bed bugs) (Saenz et al., 2012). Only three species of the family Cimicidae, *Leptocimex boueti* (Brumpt), *C. hemipterus* (F.), and *C. lectularius* (L.) are often known as 'bed bugs' due to their close association with humans (Newberry, 1989). The bed bug *C. hemipterus* has a wingless, oval-shaped body, reddish brown, large compound eye, a pair of short antennae, and measures 4–7 mm in length (Williams & Willis, 2012). Their antennae have four segments, with the last two serving as sensory organs. Sensilla hairs are abundant on their legs, thorax, and abdomen and function as sensory perception (Khan & Rahman, 2012). The pronotum has a width-to-length ratio of less than 2.5mm, the lateral pronotal lobes are short, and the inner margins of the forewings or hemelytra are rounded (Usinger, 1966). *Cimex hemipterus* has more tenant hairs on its tibial pads than *C. lectularius*. These hairs were believed to give *C. hemipterus* a superior climbing ability by increasing the vertical friction force (Kim et al., 2017). The third thoracic somite has a pair of scent glands on its ventral side. These glands produce an oily secretion, which could act as a defense against predators. The bigger female bed bugs play a role in the species' sexual dimorphism (Robert & Janovy, 2000). Traumatic insemination transpires outside of the female genital tract: the male pierces the body of the female with hypodermic genitalia and deposits sperm inside her body cavity. On the right side of the fifth abdominal segment of females is a notch called the paragenital sinus, where the paramere is inserted (Usinger, 1966). To copulate, male *C. hemipterus* must consume a blood meal; females must consume a blood meal before ovipositing. In addition, males will repeatedly mate with females, and homosexual behavior in males is not unheard of (Usinger, 1966; Newberry, 1989; Roberts & Janovy, 2000).

Cimex hemipterus female can produce up to 500 eggs throughout her lifetime (about one year), after mating with a male (Usinger, 1966) (Figure 2.1). Eggs are pearly white 1mm in length, and are curved and oval (Pinto et al., 2007). At the egg's tip is a sealed cap called an operculum (Usinger, 1966). Most bed bug species begin producing eggs 2-5 days following a blood meal, and most eggs are produced during the first four cycles of feeding and oviposition (How & Lee, 2010; Polanco et al., 2011). *Cimex hemipterus* prefers wood and paper structures over stone, plaster, or fabric structures. Bedbug "brood centers" grow large populations on a chosen surface. However, the fact that it is dry, rough, and dark is the most important requirement for a brood centre (Usinger, 1966; Newberry, 1989; Roberts & Janovy, 2000).



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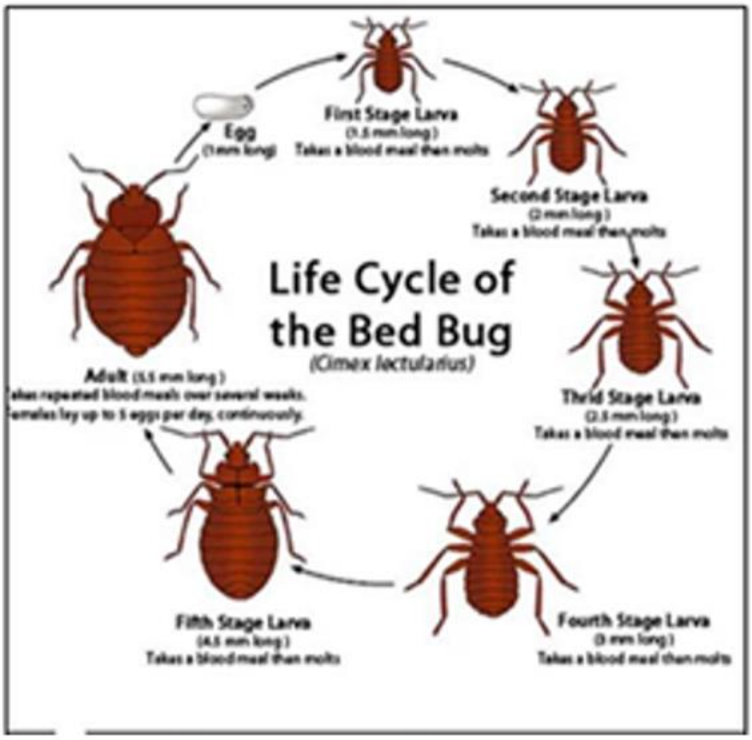

B

Figure 2.1 (A) Life cycle of *Cimex hemipterus*, (B) Life cycle of *Cimex lectularius* (Delaunay et al., 2011).

2.3 Taxonomy of Cimex lectularius and *Cimex hemipterus*

The main external differences between *C. hemipterus* and *C. lectularius* include the proportional width of their pronotum. In *C. hemipterus* the pronotum width is less than 2.5 times its length measured along the midline, while in *C. lectularius* it is more than 2.5 times its length measured along the midline (Usinger, 1966) (Figure 2.2). Another morphological characteristic that distinguishes between *C. hemipterus* and *C. lectularius* is the nature of their anterior margin. In *C. hemipterus,* the anterior margin is moderately furrowed, while in *C. lectularius,* it exhibits a deeply furrowed anterior margin (Ghauri, 1973) (Figure 2.3)

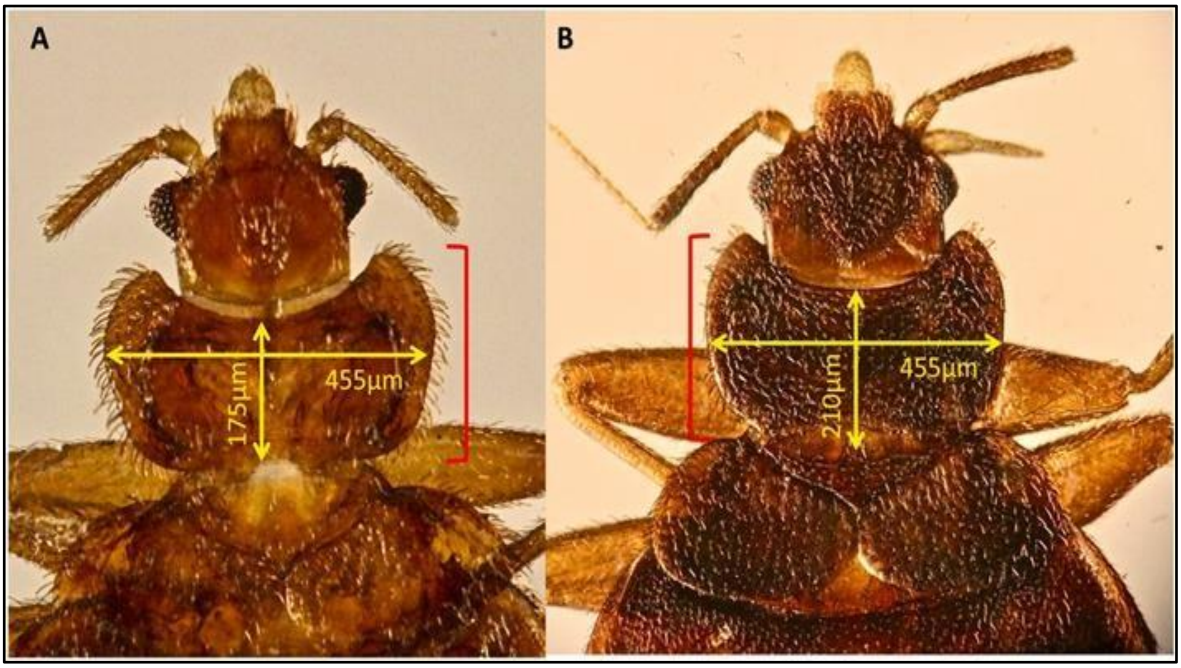

Figure 2.2 Morphological differences (A) *Cimex lectularius,* the pronotum measures more 2.5mm. (B) *Cimex hemipterus,* the pronotum measures less than 2.5mm (Benkacimi et al., 2020)

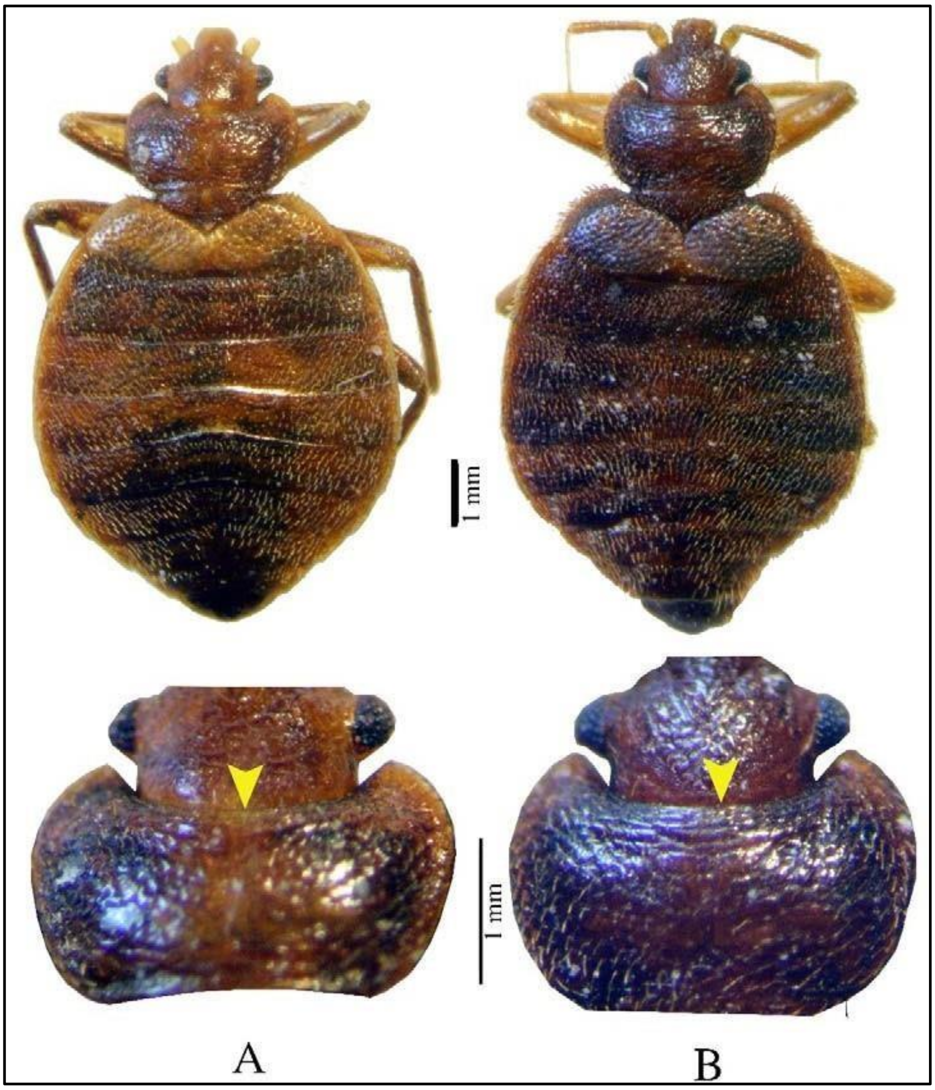

Figure 2.3 The differentiation between *Cimex hemipterus* and *Cimex lectularius* by examining the dorsal view of adult bed bugs. (A) *Cimex hemipterus*, the anterior margin is moderately furrowed. (B) *Cimex lectularius*, the anterior margin deeply furrowed (Hosseini-Chegeni et al., 2019)

According to the Integrated Taxonomy Information System (ITIS), taxonomic classification of *C. hemipterus* and *C. lectularius* is as follows:

Kingdom: Animalia

Phylum: Arthropoda

Class: Insecta

Order: Hemiptera

Family: Cimicidae

Genus: *Cimex*

Species: *Cimex hemipterus* (F.)

Species: *Cimex lectularius* L.

2.4 Distribution of tropical bed bug *Cimex hemipterus*

The distribution of *C. hemipterus* is widespread in the tropics and subtropics, namely the area bounded 30 degrees north and south of the equator (Usinger, 1966). The *C. lectularius* species is more common in temperate regions (Doggett et al., 2018; Wang et al., 2018). Nevertheless, both species can coexist peacefully in locations as diverse as Africa, Hawaii, Taiwan, Australia, and Florida (Dang et al., 2017; Doggett et al., 2018). Despite this, *C. hemipterus* has been found in temperate places, such as the Middle East (Linnavuori et al., 2011), Florida and Hawaii (Campbell et al., 2016; Lewis et al. 2020), Italy (Masini et al., 2020), North Australia (Doggett et al., 2003; Doggett& Russell, 2008), Russia (Gapon, 2016), Iran (Hosseini-Chegeni et al., 2019), and Paris (Chebbah et al., 2021) suggesting *C. hemipterus* to expand outside the tropical range.

In comparison, during 1995 and 1996 in Tanzania (Myamba et al., 2002), 2001 and 2003 in Sri Lanka (Karunaratne et al., 2007), 2005 in Brazil (Araujo et al., 2009), 2006 in Malaysia and Singapore (How & Lee, 2010), 2007 in Bangladesh (Khan & Rahman, 2012), 2011 in Rwanda (Angelakis et al., 2013), and 2011 in multiple provinces of Thailand (Tawatsin et al., 2013) *C. hemipterus* pest issue was documented.

2.5 Habitat and distribution of *Cimex hemipterus*

Tropical bed bugs, *C. hemipterus*, are nidicolous parasites that feed at night on the blood of their hosts. Tropical bed bugs avoid light and conceal themselves in tiny cracks and crevices during the day. *C. hemipterus* favors wooden or paper dwellings (Usinger, 1966; Roberts & Janovy, 2000). However, *C. hemipterus* is a significant economic pest, and infestations could occur practically anywhere provided the insects contact their hosts. In addition, human-mediated transmission has dramatically increased bed bug infestations during the past two decades (Zulaikha & Hassan, 2016; Chebbah et al., 2021). The *C. hemipterus* congregate in hiding places close to their human hosts when not feeding on human blood (Weeks et al., 2020). They can feed on human blood as frequently as once every three days and can go months without feeding (Reinhardt et al., 2010).

Tropical bed bugs can get around by walking through active displacement. A bed bug travels on the bodies of humans, sleeping or relaxing, to feed on their blood. The three critical attractive elements are: releasing body heat, carbon dioxide, and maybe human kairomones during nighttime. The bed bugs return to the resting area to digest their blood meal, molt or lay eggs after each blood meal (Harraca et al., 2012). Besides, tropical bed bugs can passively disperse, implying that the insect may be accidentally transported to a new location by the host (Hentley et al., 2017).

2.6 Bed Ecology

Bed bugs are primarily indoor pests that inhabit human dwellings, including homes, hotels, dormitories, and hospitals. They are widespread worldwide and can be found in urban and rural areas. Additionally, bed bugs exhibit photophobia, avoid light and therefore prefer dark places, especially near sleeping areas, including mattresses, bed frames, furniture, and cracks in walls (Doggett et al., 2018). Additionally, bed bugs exhibit a behavior known as thigmotaxi, which indicates their preference for constant contact with hard surfaces. Due to their flat body shape, they typically seek refuge in narrow cracks and crevices, making

Bed bugs are cryptic creatures that have evolved to be stealthy and elusive. They have specialized mouthparts adapted for piercing skin and extracting blood, and they secrete anticoagulant-containing saliva while feeding. They can quickly locate a suitable feeding site in response to the warmth emanating from the host's body and tend to avoid prolonged contact with humans. Their peak feeding activity occurs between 1 and 5 am, when people are in their deepest sleep, after which they return to their hiding places to digest blood (Doggett et al., 2012; Krinsky, 2019).

Bed bugs exhibit specific behaviors to improve their survival and reproduction. These include aggregational behaviors, where bed bugs gather in groups to increase mating opportunities and protect themselves from predators and environmental stressors (Reinhardt & Siva-Jothy, 2007; Cooper, 2016b). Bed bugs stay in close to each other, and extensive infestations often have a recognizable, sickly-sweet odor. This odor, which some liken to a "sickly sweet" smell, is similar to the stink bugs frequently found on citrus trees (Mbuta et al., 2022).

They also exhibit dispersal behavior, allowing them to colonize new locations and infestations. Passive dispersal is the primary method wingless cimicids use to search for new hosts by tagging themselves along during human activities such as travelling, transporting luggage, and moving furniture (Naylor, 2012). Active dispersal in bed bugs refers to their ability to move independently from one location to another

Bed bugs can travel short distances independently, searching for ideal conditions to feed and reproduction (Akhoundi et al., 2015).

2.7 Resurgence of bed bugs

Over the past 18 years, bed bug resurgence has dramatically increased and is still on the rise. Other studies have demonstrated bed bugs' ability as an infectious vector, raising major worries about potential new, emerging health risks (Goddard & DeShazo, 2009, Ashbrook et al., 2017, Romero et al., 2017).

The emergence of pesticide resistance in both common and tropical bed bug species is the primary factor contributing to the global spread (Doggett et al., 2018). Several studies indicated that knock-down resistance (kdr) to pyrethrin, pyrethroids, and some organochlorines has only been studied because of mutations in the sodium channel gene. The resurgence of the bed bug is widely blamed on amino acid alterations in the sodium channel gene, which have occurred in both *C. hemipterus* and *C. lectularius* (Booth et al., 2015; Palenchar et al., 2015; Dang et al., 2015; Balvín & Booth, 2018; Holleman et al., 2019). Target site insensitivity, enhanced metabolic detoxication, and penetration resistance are the three primary resistance mechanisms responsible for the development of pesticide resistance in bed bugs (Davies et al., 2012; Dang et al., 2017).

Increased international travel has played a role in the global resurgence of bed bugs by aiding the spread of resistant strains from one region to another (Doggett et al., 2018). Most people have to travel because of education, work, or leisure, and this problem is exacerbated when the intended destinations are infested with bed bugs (Khan et al., 2021). Additionally, increasing low-cost international travel is partly attributed to the worldwide resurgence of bed bugs (Hentley et al., 2017). According to United Nations World Tourism Organization (2015), the number of foreign travelers hit 1.1

billion in 2014 (53% of which were on vacation) and is estimated to reach 1.8 billion by 2030.

Several other factors have contributed to the resurgence of bed bugs, including the increased trade in pre-owned furnishings, the decline in the use of broad-spectrum insecticides in favor of more targeted approaches to pest management, and a general lack of knowledge about the problem among pest control operators and medical professionals (Doggett & Lee, 2023). Furthermore, pesticides like dichlorodiphenyltrichloroethane (DDT) have been banned due to environmental concerns. For example, The Centres for Disease Control and Prevention also reports 111 cases of poisoning from improper pesticide application by people in an effort to get rid of bed bugs (Workowski & Berman, 2011).

It was previously believed that bed bugs originated in the Middle East and regions near the Mediterranean Sea (Usinger, 1966). However, according to the results of an early study conducted by Patton (1919, 1920). It was theorized that in Indian neighborhoods of Mesopotamian cities like Basra and Baghdad, *C. hemipterus* was the only species present. After more than sixty years, Kandil (1986) reported that flies, bed bugs, and fleas were common in the homes of rural villagers in an Iraqi village. According to Abul-Hab et al. (1989), *C. lectularius* was collected from 44 locations across Baghdad, Iraq. Abul-Hab and Shihab (1990) and Abul-Hab (1997) were the first to recognize *C. lectularius* as an ectoparasite of bats in Iraq. Due to an increase in documented infestations, a study was conducted by Barzangi et al. (1988) to investigate the susceptibility of *C. lectularius* to deltamethrin, malathion, and dieldrin across a large number of hotels, offices, and residences in Baghdad. This study provided evidence of the prevalence of *C. lectularius* during that period. Moreover, according to the U.S. Armed Forces Pest Medical Board, *C. lectularius* has established itself in hotels, homes, and office buildings in Baghdad, Iraq (Armed Forces Pest Management Board, 1999). A recent survey by Ameen (2019) indicated that *C. lectularius* was the only species in Kurdistan region of Iraq. While Abul-Hab (1978) stated that there was a population of *C. hemipterus* in Iraq during the 1970s. In addition, Ibrahim et al. (2017) also noted that *C. hemipterus* was present in Iraq. More recently, the presence of tropical bed bugs was reported by Augul and Al-Saffar (2019) in the Al-Hurriyah district of Baghdad in 2018. In Israel, *C. lectularius* and *C. hemipterus* caused significant health problems (Rosen et al., 1987). In Iran, *C. lectularius* was prevalent in human settlements in Bahnamir, Iran (Haghi et al., 2014). For Gulf countries, El-Azazy et al. (2013) reported the resurgence of *C. lectularius*. In Dubai, U.A.E. Balfour (2003) reported that *C. hemipterus* was a nuisance, especially prevalent in densely populated staff accommodations such as labor camps and older urban homes with many living spaces. According to Ramachandran (2012), the tropical bed bug, *C. hemipterus* is the most abundant species in the United Arab Emirates, corroborating the previous findings. Doggett et al. (2018) suggested that the region's quick industrialization, population growth, and relocation of residents can be traced back to the finding of oil in the region. This circumstance has likely aided in the spread of bed bugs in the region, and their presence has grown recently. However, the increasing bed bug population has not been met with a concerted effort. Similar to America, European and Australian cities have the same struggle with bed bug infestation due to the economic boom (Doggett et al., 2008; Potter, 2011).

2.8 Public health importance of *Cimex hemipterus*

Bed bugs are wingless, bloodsucking insects that preferentially feed on humans; their nontoxic bites often go unreported. However, bites can lead to weals, purpura, petechiae, vesicles, pustules, papular urticaria, local infection, and, in rare cases, an anaphylaxis reaction is possible (Shipman et al., 2023). In addition, secondary infections can develop in bite lesions, such as cellulitis or impetigo (Amoateng et al., 2022). Bed bugs can be a significant nuisance, but the magnitude of the problem caused by bed bugs varies widely depending on the extent of the infestation (Newberry, 1989).

Bed bug bites were thought to be the most likely source of bacteremia, although proof was lacking (Amoateng et al., 2022). There are 45 infectious disease agents: bacteria like *Coxiella burnetii* agent of Q fever, fungi like *Aspergillus spp*, parasites like *Trypanosoma cruzi* (the causative agent of Chagas disease), *Bartonella quintana* (trench fever), and viruses like hepatitis B and human immunodeficiency virus (HBV and H.I.V.), that have been linked to the transmission of bed bugs (Lai et al., 2016). In addition, the presence of histamine in bed bug feces has also raised concerns about its possible implications on skin and respiratory health (DeVries et al., 2018). Bed bug may play a role in disease transmission. Still, more research is needed to determine if they do so directly if exposure to bed bugs indirectly contributes to infections, or due to other environmental and social determinants (Lai et al., 2016; Pietri, 2020). However, Bed bugs have not been shown to play a significant role as vectors in the epidemiology or transmission of human pathogens (Pierti, 2020; Gressier et al., 2022; Attia et al., 2023; Doggett & Lee, 2023)

Bed bug infestations can have serious psychological consequences, such as sleep deprivation, insomnia, or sleeplessness, which are commonly associated with an infestation (Goddard & Deshazo, 2009; Dogget et al., 2012). Additionally, the psychological impacts of bed bugs are significant and they can last long after the bed bugs have been physically eliminated (Goddard & Deshazo, 2009). Furthermore, it is possible for those living in infested homes to feel ashamed and social isolated. Bed bugs can have severe mental health effects on anyone, regardless of preexisting disorders (Burrows et al., 2013). The mental health repercussions of having bed bugs in one's home are mostly unknown. Bed bugs have been linked to increased rates of anxiety and depression, but no attempt has been made to document the burden on the population's mental health (Doggett et al., 2018).

Infestations of bed bugs cause financial hardship for people of all socioeconomic statuses (Mbuta et al., 2022). The disinfestation is an expensive undertaking since it requires continual inspection, quarantine of infested areas, treatments, disposal, and replacement of infested household items and other furnishings (Romero et al., 2007). Doggett et al. (2018) suggested that in a survey conducted in 2014 in the United States of America in two states, Alabama and Tennessee, 88% who participated in the survey claimed that treating bed bug infestation in their housing units had affected their budget or profitability. Similarly, in another survey in the United States of America in Virginia, 98% of respondents noted that bed bug control efforts had negatively affected their operating budget (Doggett et al., 2018).

One of the difficulties in dealing with bed bug infestation is achieving control, which makes it very costly (Doggett et al., 2018). For example, the costs of treating residential apartments heavily infested with bed bugs in Australia can reach 4,000 Australian dollars, equivalent to 2,900 US dollars (Doggett et al., 2011), likewise, Potter et al. (2013) reported that property managers in the United States of America spent close to $5,000 to treat bed bugs in their infested units. Similarly, in the United States of America, the average cost of treating a bed bug infestation range from $400 to $1,500 (Otriz, 2004; Bruno, 2010). Annually, the cost of billions of dollars are spent worldwide to get rid of this insect, which is considered a significant economic burden

(Doggett et al., 2018). In addition, more than $180 million is spent on process marketing and development for a new active insecticide (Doggett et al., 2012).

Several studies demonstrated that both the tourism and hospitality sectors were affected by bed bug infestations. (Seidel et al., 2013; Penn et al., 2017; Doggett et al., 2018; Penn & Hu, 2020). Many upscale hotels and resorts may suffer financial loss of several hundred to several thousand dollars due to guests who refuse to pay them lodging bills after being bitten by bed bugs (Doggett et al., 2018). Along with financial losses, the hospitality industry faces additional costs, such as civil litigation (Penn & Hu, 2020). Many guests avoid hotels where bed bug infestations have appeared or had a previous infestation, which is reflected in the revenues of those hotels (Martín et al., 2018). Millions of dollars have been spent reducing bed bug infestations and settling lawsuits for hotel guests who have been bitten (Morgan, 2013). Curl (2011) reported that bed bug management in the United States generated an estimated revenue of $319 million in 2010.

2.9 Prevention, public awareness of bed bugs

Preventative measures are still crucial, as bed bug infestations are challenging and expensive (Vassena et al., 2019). Nevertheless, bed bugs can be avoided by taking preventative measures, such as sealing cracks and crevices, inspecting furniture regularly, checking a hotel room before sleeping, and checking a patient's clothing before hospital admission (Delaunay, 2012). Nowadays, it's commonly known that used furniture harbors bed bugs, so many US educational institutions mandate tenants have their belongings heat-treated before moving in (Doggett et al., 2018). However, it is recommended that returning travelers who have been exposed to bed bugs use mechanical procedures (brushing, vacuuming, heating, washing, or freezing) rather than chemical methods (such as dry cleaning) to disinfect their luggage, clothing, and other items (Delaunay, 2012). In addition, barrier techniques are available for the legs of bed frames, which can prevent an infestation of bed bugs (Shipman et al., 2023).

The public's lack of bed bug knowledge, neglect of the problem, a ban on organophosphates in indoor spaces, and an increase in the number of foreign employees and new migrants from developing nations are all possible explanations for the recent resurgence of bed bugs (Siegel-Itzkovich, 2014). In addition, ineffective treatments, lack of awareness among residents, clutter, lifestyle choices, and varying degrees of cooperation from community members towards management programs, especially when reporting bed bug sightings, contributed to the continued activity of bed bugs (Romero et al., 2017). Furthermore, Copper (2006)) reported that a lack of public awareness is a major factor in the exponential increase of bed bug activity. Bed bug-infested items left on the curb are frequently picked up and brought into homes because the general population is unaware of the pest. This is a severe issue in dormitories and on university campuses. Furniture that has been previously used or rented is being redistributed. Hence, educating the public about bed bugs about how to reduce the likelihood of an infestation spreading is necessary.

Sharififard et al. (2020) suggested the concern about the spread of bed bugs may inspire more people to become aware of the problem. Raising public awareness about bed bugs might be achieved in many ways. Social media is a powerful tool to spread awareness about bed bugs, such as creating posts on identifying bed bugs, preventing them, and taking action towards suspecting a bed bug infestation. In most cases, sensationalizing the issue and frequently using fear to get people’s attention (Doggett et., 2018). However, information found online is notoriously unreliable and out- of-date, and some people's attempts at complete eradication out of panic and misunderstanding have certainly aided in the proliferation of pests in several regions (Wang et al., 2010).

Furthermore, Doggett et al. (2018) suggested the difficulties PMP (pest management professionals) face in educating the public and industry is through the spreading of inaccurate information via the internet and media. But people are still doing a lot of research online for ways to get rid of bed bugs. This means that new infestations are developing and that bed bug education is necessary (Sentana-Lledo et al., 2016).

Raising public awareness of bed bugs is possible through various forms of outreach, including presentations, sample identification, and the creation of teaching materials (Gangloff-Kaufmann, 2011). In addition, education and awareness efforts are directed toward the general public. Both governmental and non-governmental organizations need to be involved in the training of relevant parties (Emmanuel et al., 2014). In some countries, bed bug control tactics and public education can benefit from establishing a collaborative platform between academic researchers, government authorities, and pest management specialists (Olson et al., 2018).

2.10 Detection and monitoring of bed bugs

Several methods have been employed to detect bed bugs including bed bug monitors and traps, visual inspections, and canine detection to aid with early infestation detection and prevent further dispersion (Akhoundi et al., 2023). Professionals in the pest control industry typically employ a visual inspection as their primary method. However, while visual inspections can be helpful, their efficacy depends on the inspector's skill and attention to detail (Doggett, 2007). In canine inspections, dogs have been trained to look for the odors of bed bugs (Doggett et al., 2018). The dogs' accuracy varies from dog to dog and handler to handler but can reach 98% in artificial environments (Pfiester et al., 2008). However, their reliability in actual field conditions has been inconsistent (Cooper et al., 2014). Despite these drawbacks, canines are nevertheless often regarded as beneficial for fast, comprehensive inspections (Doggett et al., 2018).

2.11 Infestation dynamic of bed bugs

High-resolution molecular marker applications in recent years have revealed previously unknown information about the population structure and patterns of infestation of numerous insect pest species with public health implications (Endresby et al.,2009; Crissman et al., 2010; Pérez de Rosas et al., 2011; Booth et al., 2012). New molecular tools have made it possible to precisely determine how many populations are currently infesting a setting (Pizarro et al., 2008; Crissman et al., 2010). In addition, dynamics and characteristics are crucial to comprehend infestation patterns and history, such as the level of genetic diversity (Paupy et al., 2014). Nevertheless, the populations of pests have continued to persist even after pest control efforts have been implemented. (Perez De Rosas et al., 2007). The presence or absence of genetic mutations linked to insecticide resistance has also been identified (Yoon et al., 2008). It will be useful to have substantial data on species identification to choose effective bed bug management strategies. Unfortunately, despite the availability of powerful genetic technologies for uncovering knowledge on the biology and control of bed bugs, the infestation patterns within human dwellings remain largely neglected.

The dynamics of a bed bug infestation can be better understood by learning more about the bed bug's population genetics and breeding strategy of common bed bug, *C. lectularius* has received considerable attention in the scientific literature so far

(Szalanski et al., 2008; Booth et al., 2012; Saenz et al., 2012; Fountain et al., 2014; Alkhoundi et al., 2015; Narain et al., 2015), while information on tropical bed bug *C. hemipterus* is limited (Seri Masran & Abu Majid, 2019 ; Wan Mohammed et al., 2020).

The dynamics of *C. hemipterus* infestations have been studied by observing their dispersal behaviour (How & Lee, 2010). The authors find that postponing eradication efforts increases the likelihood of a more extensive and severe infestation. Furthermore, a recent study by Wan Mohammed et al. (2020) in Malaysia revealed a small founding population established a large infestation. Several studies that compared the genetic makeup of different bed bug populations revealed infestation started with either a single mated female or a small group of siblings (Fountain et al., 2014; Booth et al., 2015; Narain et al., 2015; Akhoundi et al., 2015).

2.12 Dispersal pattern of bed bugs

Movements of individuals or propagules that result in a spatial gene flow are referred to as dispersal patterns (Ronce, 2007). However, bed bug dispersal can be active or passive; active dispersal occurs when a bed bug leaves one host and goes to another, and passively when they hitchhike to a new area, typically on a human host's clothing or other items (Reinhardt & Siva-Jothy, 2007; Pfiester et al., 2009; Wang et al., 2010; Hently et al., 2017). Broadly, new populations are usually due to passive dispersion (Reinhardt & Siva-Jothy, 2007).

To examine the genetic structure of a population, it is necessary to understand the probable causes of dispersal. For populations found in nature, there is often a varying degree of spatial heterogeneity meaning that species are uneven across a given region (North et al., 2011) Dispersal can increase spatial synchrony while simultaneously stabilizing local populations; hence, the overall effect of this process on