# SPECIES DIVERSITY OF BEDBUGS AND ENVIRONMENTAL FACTORS INFLUENCING THEIR SURVIVAL

BY

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# DECLARATION

I hereby declare that except for references to other people's works which have been duly cited, this work is the result of my original research and that, this thesis has not been presented for a degree elsewhere, either in whole or in part.

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# DEDICATION

To my dear mother Madam Faustina Hawa Yakubu.

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| ABBREVIATION | FULL MEANING |
|---|---|
| DDT | Dichlorodiphenyltrichloroethane |
| RH | Relative humidity |
| PCR-RFLP | Polymerase chain reaction - restriction length polymorphism |
| PTSD | Post traumatic stress disorder |
| OP | Organophosphates |
| DDVP (e) | Dichlorovinyl dimethyl phosphate |
| DED | Diatomaceous Earth Dust |
| DNA | Deoxyribonucliec acid |
| COI | Cytochrome Oxidase I |
| IRB-NMIMR | Noguchi Memorial Institute for Medical Research |
| DABCS | Department of Animal Biology and Conservation Sciences |
| FW: | Width of femur |
| FL: | Length of femur |
| HL: | Length of Head |
| PTW: | Width of Pronotum |
| PTL: | Length of Pronotum |
| NS: | Not significant |
| PHL: | Length of hair on pronotum |
| 1A: | First antenna segment |
| 2A: | Second antenna segment |

| | |
|---|---|
| 3A: | Third antenna segment |
| 4A: | Fourth antenna segment |
| BP: | Base pair |

# ABSTRACT

Bedbugs are blood-sucking insects of the order Hemiptera and family Cimicidae that derive nutrition from vertebrates, including humans, their feeding is allied to the transmitting several fungal, bacterial and viral infections. A rise in international travels and emergence of insecticide resistant bedbugs within the past decade has led to renewed interest in studying these insects. Anecdotally, bedbugs exist in homes, boarding houses, hotels and other human dwellings in Ghana but have been poorly studied. The present study sought to characterize bedbugs and identify environmental factors that influence their survival in the Greater Accra Region of Ghana. Collection of adult bedbugs was carried out in six educational institutions, two tertiary and four senior high schools. Rooms and dormitories of these institutions were carefully inspected, with particular focus on mattresses, bed frames, wall crevices, carpets and furniture. Forceps, brushes, flashlights and insecticide were employed in the collection of bedbugs. Bedbug eggs were identified by their cream to white color and size. The eggs were carefully transferred into containers and colonized in the laboratory for later evaluation of the effects of environmental factors that influence their survival. Morphological identification was done with the aid of a dissecting microscope and identification keys provided by Usinger (1996). A total of 335 bedbugs were collected, of these, 230 were adults while the others were nymphs. The adults consisted of 127 (55%) males and 103 (45%) females. Three species have been identified; *Cimex hemipterus* (94%) constituted the majority of adult bedbugs, followed by *C. lectularius* (3.4%) and then *C. pipistrelli* (1.7%). Bats are host of *Cimex pipistrelli*, and to the best of our knowledge, this is the first time these bedbugs are being reported in human dwellings in Ghana. Morphometric comparisons between the three species showed significant differences in the width of the pronotum,

femur and the antennal segments of the adult samples collected. Molecular analysis also revealed nucleotide differences in regions of the COI and rDNA base pairs of the three *Cimex* species observed in this study. This is a significant find since these bugs could potentially be vectors of zoonotic diseases from bats to humans, requiring further investigations.

# CHAPTER ONE

# 1.0 INTRODUCTION

## 1.1Study background

Bedbugs have attracted much global attention in recent years, due to their presence and major resurgence in the number of infestations that has led to clinical and control problems. In Australia it has been reported that bedbug infestations have increased by five thousand percent since the year 1999 (Davies *et al*., 2012). Kemper (1936) first reported the infestation of bedbugs in England, revealing that earliest records dates back to the 1500s. Bedbugs were rare in North America and Western Europe during the second half of the 20$^{th}$ century (Ryan *et al*., 2002). However, in recent years they have increased in Europe and in many parts of the world.

The introduction of the organochlorine insecticide, dichlorodiphenyltrichloroethane (DDT) during the Second World War, reduced the population of insects including bedbugs. This success of human's inventiveness lasted only a short while, up until the late 1990's (Hwang *et al*., 2005). The organism has developed extensive resistance to the majority of modern pest control, making the challenge of elimination much greater than ever. Bedbugs have thus in recent times made an explosive comeback. Additionally, as a result of rapid increases in international travel and the adaptive pressures of nature, bedbug populations across the globe are soaring (Reis *et al*., 2010; Durand *et al*., 2012). The spread and increase in bedbugs population may also be due to the increase in human migration, especially through the tourism industry where people in other countries travel to visit other places to watch their culture and also to visit animal reserves. This may have contributed to the spread and uncontrollable increase of bedbugs as travellers carry with them either adult bedbugs, nymphs or eggs of bedbugs (Romero *et al.*, 2007). It is therefore likely that bedbugs are transported on clothes and luggage by travellers to other places (Delaunay and Pharm, 2012).

Bedbugs have been found naturally to carry about 40 human pathogens but have not been observed yet to transmit any of them (Cooper and Harlan, 2004). However, there could be mechanical transmission of hepatitis B when infested bedbugs are crushed on abraded skin (Blow *et al.*, 2001). Though bedbugs have not been observed yet to transmit infections, the potential of bedbugs for spreading diseases should not be overlooked. Bedbugs fulfil all the conditions of efficient carrier of diseases according to Harwood and James (1979). Bedbugs have been associated with direct cutaneous reaction from their bites, itching and scratching of skin due to their feeding activity, which may allow for secondary infections. Other health impacts of bedbugs include; depression, loss of appetite, insomnia (Fong *et al*., 2012). Experiences with bedbugs, may also interfere with sleeping pattern and, if severe, may result in subsequent physiological and neurocognitive health effects associated with sleep (Doggett *et al.*, 2012; Fong *et al*., 2012). Bedbugs infestation presents various issues as it has led to the close down of hospitals, threatening the provision of health services (Doggett *et al.*, 2012). Other studies have also associated bedbug infestation with anaemia in the elderly and young children living in homes that were heavily infested (Doggett *et al*., 2004).

The presence of bedbugs in a dwelling can produce a range of physical and psychological discomfort in their human hosts, as their infestations can be very difficult to eradicate. Victims of bedbug bites often experience a strong repulsive reaction to the idea of being fed on by bugs when insentient or sound asleep (Quarles, 2007). As a result, bedbug infestations are considered a public health nuisance. People experiencing infestation in their residence may feel isolated from friends and families because bedbugs can easily be transferred between people. Customers express dissatisfaction in infested hotels, hostels and guest houses which leads to loss of economic value (Hoang, 2011). Treating a residence for bedbugs can be difficult and expensive. Victims may have to dispose off infested furniture and belongings leading to both financial and psychological stress (Cassels, 2011).

## 1.2 Justification

In America, bedbugs have been collected from hotels, homeless shelters, apartments unit and community centres (Hwang *et al*., 2005). Omudu and Kuse (2010) also reported on the collection of bedbugs from hotels, hospitals and apartments in Nigeria. There have been reports in the Ghanaian mass media about the infestation of bedbugs in some senior high schools in the country (Akweteh, 2014). This situation could lead to poor sleep and loss of concentration during studies among students and may further lead to poor academic performance. Bedbug infestation can lead to property loss such as discarding and or burning infested materials like mattresses clothes and bed frames (Akweteh, 2014). Some students may also develop damaged skins due to severe itching and scratching in response to the deposition of saliva that contains anticoagulant enzymes into the host's dermis during blood feeding of the bedbugs. This leaves their skins swollen and broken

(Akweteh, 2014). Bedbugs are reported to spread through their eggs laid into bags, books, in clothing, which are carried along by travellers. Although flightless, the nymphs and adults can also fasten themselves to clothing, shoes, bags, books and other materials; these infested materials when moved from one place to another facilitate the dispersal of these insects from one place to the other.

*Cimex lectularius* is a temperate species, whereas *C. hemipterus* is subtropical and tropical in nature. However, the potential for preconditioning or adaptation may result in the overlapping of these two species in their geographical distributions. This assumption is supported by an observation from the Kwazulu-Natal region in South Africa where hybrids of *C. hemipterus* and *C. lectularius* were found (Walpole and Newberry, 1988; How and Lee, 2010). Doggett *et al*. (2003) also collected both species of *Cimex* in Australia. In recent years bedbugs have increased in many parts of the world (Krueger, 2000). This may be due to the increase in human migration especially tourism industry and development of insecticides resistance of the insects (Romero *et al*., 2007). It is likely that the bed bugs were transported on clothes in luggage of travelers (Delaunay and Pharm, 2012).With this mode of dispersal, it is possible that similar species of bedbugs may be found in Ghana. Some bedbugs such as *Leptocimex boueti* and *Cimex adjunctus* have been reported to feed on bats. These species have been reportedly found in tropical countries, particularly in Africa and they feed on humans in the absence of the original host (Robinson, 2004). The Pohle's fruit bat *Scotonycteris ophiodon* species is common in Ghana, and can be found in the urban centres (Gilbert, 2011; Badu, 2016). Thus, these bat bug species could serve as potential vectors for the spread of zoonotic diseases even in urban Ghana. Though bedbugs have not been associated with the spread of diseases, with the current health issues such as Ebola virus disease, Lassa virus disease, etc. linked with bats, the threat posed to human health is palpable;

Bedbugs may be a biological indicator of changing social and or general hygienic conditions, and might foretell the resurgence of other ectoparasites such as lice, fleas and their associated diseases (Myles *et al*., 2003). If the resurgence of bedbugs in shelters and other public facilities is not contained, there is the risk of an uninterrupted and ever-increasing growth in bedbug populations leading to larger-scale infestations, which will require more frequent and costly control efforts (Myles *et al*., 2003). It is thus prudent to properly characterize the species as differences in their biology may influence their competence as disease carriers (either passively or actively) as well as their efficient and effective control.

Identifying the species of bedbugs in a community is the first step to knowing its biological importance and how much of a problem they may be to public health, which can help to develop a better control measure. Usinger (1966) earlier identified several species of bedbugs and developed a pectoral key for their identification. Although identification of bedbugs has been carried out across the globe, especially in the Americas and Europe, there is little work done on the identification of bedbugs in Africa. Taxonomic and morphological information on bedbug species in Ghana is scarce, and there is no data that has catalogued the available species in the country. This study reports on the morphological and molecular identification of bedbugs in the Greater Accra region of Ghana, and aspects of the environmental conditions that influence the survival of these insects. This will ultimately geared towards contributing in the development of effective control tools against this insect.

### 1.2.1 Main Objective:

The main objective of the study was to identify and characterize the species of bedbugs in the Greater Accra Region of Ghana.

### 1.2.2 Specific Objectives:

1. To morphologically identify and characterize bedbugs collected from human habitations in Accra.
2. To characterize the species of the collected bedbugs.
3. To assess the impact of some environmental factors on the survival of bedbugs
4. To determine effects of the level of knowledge and perceptions of persons living with the infestation on the spread of bedbugs

# CHAPTER TWO

# 2.0 LITERATURE REVIEW

## 2.1 Background Information

Bedbugs may have undergone development from cave-dwelling ectoparasites of mammals especially bats. As humans moved from caves to tents and then into houses, they might have carried the bedbugs along which led to infestation in homes and to other people (Usinger, 1966). Kemper (1936) reports on the infestation of bedbug in England, reveals that, the first report of bedbug from England was in 1583. Bedbugs were rare in North America and Western Europe during the latter part of the $20^{th}$ century (Ryan *et al*., 2002). In recent years however, they have increased globally of the world Europe inclusive. Bedbugs have its place in the family *Cimicidae* in the insect order Hemiptera or true bugs (Yao *et al.*, 2014). They are nocturnal bloodsucking ectoparasites with piercing and sucking mouthparts to feed on plant juices, other insects, or the blood of vertebrate animals as well as humans (Usinger, 1966; Tawastsin *et al*., 2011). Bedbugs can be distinguished from other hemipterans, by the reduction of their wings to short transverse scale which renders them flightless (Carver *et al.*, 1991).

The family *Cimicidae* comprises of more than hundred (100) species, of which, only two species have specialized to feed on humans. These are the common bedbug (*Cimex lectularius*) and the tropical bedbug (*Cimex hemipterus*) (Robinson, 2004). *Cimex lectularius*, is the most cosmopolitan species and widely spread in North America and Europe. The other species (*C. hemipterus*) is found exclusively in tropical areas and widespread in West Africa (Eddy, 2011).

However, bat bugs (*Leptocimex boueti*) may feed on humans in the absence of their definite host. Bedbugs can mature to 7 mm in length and may live from 4 months up to a year, depending on the species and feeding habits (Elston and Stockwell, 2000).

Both sexes are hematophagous and can live for 12 months without feeding. In colder environments, however, bedbugs can survive for up to18 - 24 months without feeding (Delaunay *et al*., 2011). A laboratory experiment carried out by Quarles (2007) shows that, bedbugs can survive wide scope of temperatures as well as atmospheric compositions below 16.1$^{o}$C. At -10 $^{o}$C, adults bedbugs enter semi hibernation and can survive for at least five days. At a temperatures of −32$^{o}$C they can live for 15 minutes. Joelle *et al*., (2013) reports that, common commercial and residential freezers can reach temperatures low enough to exterminate most life stages of bedbug, with about 95 per cent of them dying after 3 days at −12$^{o}$C. Bedbugs show high dehydration tolerance, surviving low humidity as low as 35–40$^{o}$C range even with loss of one-third of body weight; earlier life stages are more subject to drying out than later ones (Benoit *et al*., 2007). The thermal death point for *C. lectularius* is 45$^{o}$C; all stages of life are killed within 7 minutes of exposure to temperature of about 46$^{o}$C (Quarles, 2007). Bedbugs apparently cannot survive high levels of carbon dioxide concentrations for a long time. However, exposure to pure nitrogen atmospheres appears to have little effect even after 72 hours (Herrmann *et al*., 1999).

Bedbugs are social insects and are often found in large numbers (Han *et al*., 2010). They live under congested and jam-packed living conditions; they are often in army barracks, labour and prison camps and similar situations where they may readily contact a variety of hosts (Metcalf and Flint, 1973). Beds, wooden furniture, floors, walls, cracks and crevices are places bedbugs hide during daytime and appear at night to feed on their preferred host. They may come out to feed during the day if that is when their preferred host is available

During the Second World War organochlorine insecticides such as dichlorodiphenyltrichloroethane (DDT) was introduced, which reduced the population of insects including bedbugs. This success of human's inventiveness lasted after a while. In the late 1990's, the organism has developed extensive resistance to mainstays of modern pest control, making the challenge of elimination greater than ever (Hwang *et al*., 2005). With rapid increases in international travel, and the adaptive pressures of nature, bedbug populaces throughout the world has increased immeasurably. The spread and increase in bedbug's population may be due to the increase in human migration especially through tourism. People in other countries travel to visit other places to experience their culture and also to visit animal reserves. This may have contributed to the spread and uncontrollable increase of bedbug's prevalence as travellers carry with them either adult bugs, nymphs or eggs of bedbugs (Romero *et al*., 2007). It is most probable that one of the ways bedbugs spread is by being carried on clothes and in luggage by travellers to other places (Delaunay and Pharm, 2012).

Bedbug infestation has dramatically increased so speedily that many institutions have had to close down. Fox 8 TV in the United States of America announced on its news on June 16 2015, the closure of McDonalds restaurant in Elyria due to bedbug infestation. Bedbug infestation has also led to some homeowners burning their furniture to control them (bedbugs) since the use of pesticide could not yield the desired result. The presence of bedbugs has been experienced in hospitals. Patients in these hospitals reported the presence of bedbugs, which called for immediate health and medical attention (Fox8.com).

### 2.2 Biology of bedbugs

Bedbugs are nocturnal hematophagous (Giorda *et al.*, 2013) parasite arthropods belonging to the family Cimicidae, order Hemiptera. There are six subfamilies namely: Afrociminae, Cimicinae, Cacodiminae, Haemotosiphonae, lactrociminae and Primicinae, with 24 genera and 110 species (Henry, 2009; Balvín *et al.*, 2014). Traumatic insemination occurs in the order Strepsiptera, but is more common in the true bug order Hemiptera, members of the family Cimicidae copulates via traumatic insemination (Pfiester *et al.*, 2009) where males use an intromittent organ called a paramere coupled with the aedeagus (Carayon, 1966; Pfiester *et al.*, 2009). The structural and behavioral adaptations that occur differ within the family. Whereas the evolution of such a process is vague, traumatic insemination may have evolved as a way for males to overcome pre- or post-mating female resistance (Arnqvist and Rowe, 2005). In response to traumatic insemination, female cimicids have evolved physiological adaptations that include a paragenital reproductive system, or spermalege, which consists of modifications of the abdominal wall in the region that is pierced during mating (ectospermalege) and an internal pouch attached to the body wall of the ectospermalege that receives the sperm (mesospermalege) (Carayon, 1996)

The location and development of the spermalege varies within the Cimicidae. In the genus *Primicimex* the most basal taxon of the Cimicids, females utterly lack a spermalege and have a large area of the abdomen that is pierced by males during copulation. Females of the genus *Afrocimex* have a considerable ectospermalege, but no distinct mesospermalege. In the genus

*Cimex*, the mesospermalege is a well-developed sac, therefore piercing from traumatic insemination occurs in a limited area. The mesospermalege in the genus *Stricticimex* is highly developed and attaches to the female's genital tract, preventing sperm from entering the body cavity (Carayon, 1966; Arnqvist and Rowe, 2005). Males benefit from multiple inseminations due to last-male sperm precedence, which means that the last male to copulate with the female gets to fertilize the eggs (Stutt and Siva-Jothy, 2001). Multiple traumatic inseminations from males reduce the life span of females (Arnqvist, 2003; Reinhardt *et al*., 2003) and lowered fecundity if traumatic insemination happens to occur outside of the ectospermalege (Morrow and Arnqvist, 2003).

The counter-adaptation of the spermalege aids the female in localizing the wound site during traumatic insemination (Carayon, 1966; Siva-Jothy, 2006) and reduces infection (Reinhardt *et al*., 2003) from pathogens that enter the female at the wound site (Reinhardt *et al*., 2005). “Behaviourally, unfed females have been observed to display a "refusal posture" to potential mates. They do this by flexing their abdomen, preventing male access” (Pfiester *et al*., 2009). Males therefore tend to be attracted to recently fed females, because they cannot perform the resistance stance (Siva-Jothy, 2006). Male bedbugs abuse this situation and overcome the female resistance (Reinhardt *et al*., 2009).

On the field bedbugs are found in populations at a sex ratio of 1:1 male to female (Stutt and Siva-Jothy, 2001) and occur in aggregations (Usinger, 1966) consisting of bedbugs from all stages of life, feeding status, and mating conditions (Reinhardt and Siva-Jothy, 2007). Only two of over one hundred species within the family Cimicidae feed on humans. The two species are the regular bedbug, *C. lectularius*, and the tropical bedbug, *C. hemipterus* (Robinson, 2004). Both *C. lectularius* and *C. hemipterus* species are similar and can be distinguished by the presence of an upturned lateral flange on the margin of the pronotum on the thorax of *C. lectularius*, this makes the thorax of *C. lectularius* wider than that of the *C. hemipterus*. However, in the juvenile stages this feature is less obvious (Usinger, 1966). Bedbugs feed only on the blood of mammals or birds (Usinger, 1966; Stutt and Siva-Jothy, 2001). Bedbugs are photophobic making them "secretive". Blood feeding therefore occurs mostly at night, with peak feeding taking place between 1 and 5 a.m. Although, bedbugs are nocturnal, they may adjust their feeding pattern to during the day if that is when their host is present (Tawatsin *et al*., 2011) During the day, bedbugs seek shelter in a variety of cracks and crevices and may become dormant while digesting the blood meal. Bedbugs stay in close contact with each other and discharge aggregation pheromones to help relocate their harbourage after a blood meal. This behaviour of grouping together is a means of conserving water (Benoit *et al*., 2007). Faecal spotting on surfaces is indication of bedbug presence usually, when infestation is severe in a particular place. Bedbugs also releases alarm pheromones when agitated, in most cases these pheromones are released during the course of treatment when bedbugs are disturbed (Romero *et al*., 2009).

Bedbugs usually live gregariously in dark hidden spaces called harbourages (Reinhardt and Siva-Jothy, 2007). Box springs, mattress seams and curtains are common harbourages (Dogget *et al*., 2012). These locations provide shelter, ample opportunity for mating, and mitigate desiccation (Reinhardt and Siva-Jothy, 2007). These harbourages are comprised of all life stages, and maintained by aggregation pheromones (Reinhardt and Siva-Jothy, 2007; Gries *et al*., 2015). Bedbugs also produce alarm pheromones when their harbourages are disturbed, which is reported to smell unpleasantly sweet or like rancid strawberries (Dogget *et al.,* 2012). *Cimex lectularius* has been observed to survive periods of starvation for months, and in temperatures near 10 $^0$C with favourable humidity they can live over a year (Usinger, 1966).

Other adaptation distinctive of cimicids, are traumatic insemination and the female paragenital system (Usinger, 1966). Rather than use the female's genital opening, male cimicids use their copulatory (intromittent) organ to puncture the female's body wall and release their sperm into the abdomen (Usinger 1966; Stutt and Siva-Jothy, 2001). It is thought that this behavior is a result of sexually antagonistic coevolution, due to female rejection of potential mates (Stutt and Siva-Jothy 2001; Reinhardt and Siva-Jothy, 2007).

### 2.2.1 Finding and feeding from hosts

Obligate hematophagy is one of the core adaptations that distinguish cimicids from their relatives, suggesting it likely only evolved once (Reinhardt and Siva-Jothy, 2007). It is not known whether this adaptation evolved from phloem sap feeding or from feeding on fur and feathers (Reinhardt and Siva-Jothy, 2007). A blood meal is required by every life stage, including adults of both sexes. Nymphs must feed within days of hatching if not they will die. Each instar must also feed before moulting. Moreover, females require a blood meal before producing eggs. In addition, C. lectularius males show sexual preference towards recently fed females (Reinhardt and Siva-Jothy, 2007). Cimicids have a narrow host range relative to other insect hematophages (Reinhardt and Siva-Jothy, 2007). The most common hosts are bats, swifts and swallows (Reinhardt and Siva-Jothy, 2007). Hence, all hosts are warm blooded and live in social groups that have relatively immobile shelters, such as caves, buildings, and nests (Reinhardt and Siva-Jothy, 2007). These hosts have also historically had overlapping habitation, a feature that lends itself to host switching by the hematophage (Reinhardt and Siva-Jothy, 2007).

Cimicids locate their host by sensing carbon dioxide, heat and kairomones though these stimuli have only been demonstrated to act as lures at distances less than a few meters (Wang *et al.,* 2009). They must crawl to their host, as they lack wings and jumping ability (Usinger, 1966). Unlike many other ectoparasites, cimicids do not live on their host and often only have contact with their host while feeding (Usinger, 1966). Cimicids, like other heteropterans, have mouthparts that are modified for piercing and sucking, their maxillary and mandibular stylets fused together to make the two separate canals; the feeding and the salivary (Usinger, 1966). While sucking blood through their food canal they inject proteolytic enzymes, anticoagulants, vasodilatory compounds such as nitrophorin, and an unidentified anaesthetic through their salivary canal (Usinger, 1966: Dogget *et al.*, 2012: Williams and Willis, 2012). When suckling on humans, bedbugs are more bent to feed on the parts that are expected to be exposed while sleeping such as the neck, face, hands, and arms. (Delaunay *et al.*, 2011: Williams and Willis, 2012). Under ad libitum conditions, *C. lectularius* adults feed approximately once every few days (Usinger 1966; Reinhardt and Siva-Jothy 2007: Dogget *et al*. 2012). Though not strictly nocturnal, *C. lectularius* generally feeds while their host is least active. It takes roughly 10 minutes for a bedbug to fully engorge once feeding commences (Dogget *et al.*, 2012). They therefore require a steady moment to enjoy feeding, when the host is least active, such as sleeping.

**2.2.2 Life cycle of bedbugs**

One life cycle from egg-to-egg is 5 weeks at 75–80% RH and 28–32 ºC. They can survive and remain active at temperatures as low as 7 ºC if held at intermediate temperatures for a few hours, but their thermal death point is 45 ºC (Harlan, 2006). If a host is available and blood meal can be accessed frequently, the female *C. lectularius* bedbug can lay 5 to 8 eggs per week for 18 weeks at a temperature of 23°C and at 90% relative humidity (Johnson, 1941), while *C. hemipterus* bedbugs will lay up to 50 eggs in their lifetime (Doggett *et al.*, 2012). Eggs of bedbugs are elongated and cream coloured measuring about 1 mm in length (Khan and Rahman, 2012) These eggs are cemented onto surfaces and may hatch within 9-12 days at a temperature of about 22ºC but under cooler temperatures, hatching may be prolonged (Doggett, 2012). The length of the life cycle is extremely variable and is dependent on ambient temperatures. Under conditions of 10°C, blood-fed adults of *C. lectularius* can live for up to 485 days, while *C. hemipterus* can live up to 300 days (Omori, 1941), These lengthy periods, however, are normally not observed under average home and hotel living conditions, this is because home and hotel normally maintains temperatures of around 22°C which may be less or more favourable for either species. The life cycle of both species take around 2 months to complete, and the adult lives for up to a maximum of 4.5 months (Busvine, 1980). Encounters with bedbugs occur mostly when people sleep in furniture that is infested. Bedbugs mostly have the ability to infest almost anywhere people live (Kells and Hahn, 2015).

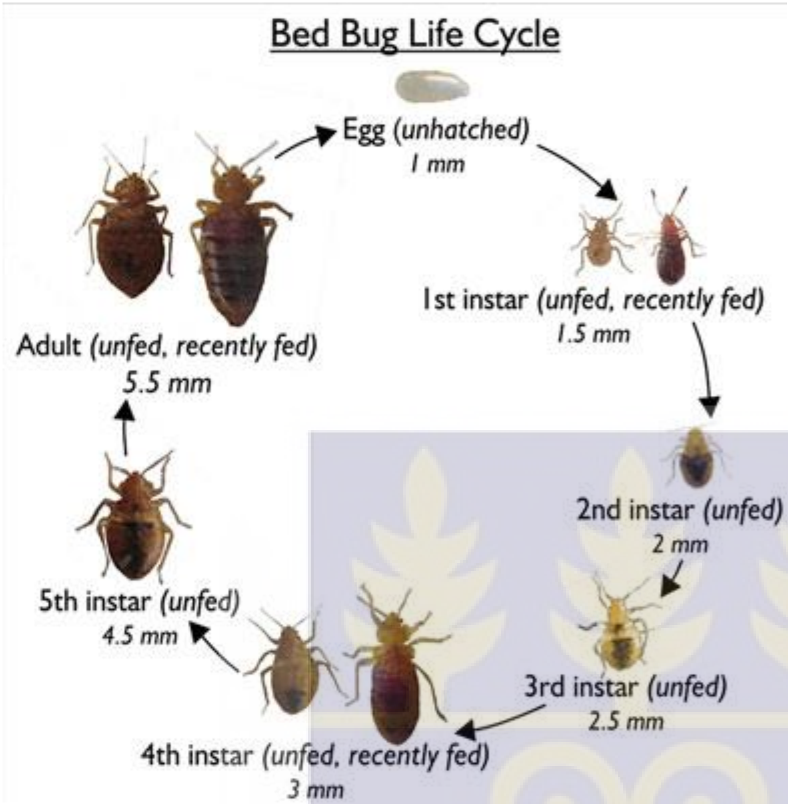


**Figure 2.1:** An illustrative diagram of the typical life cycle of *Cimex* species.

Source: http://www.pgpestcontrol.com/services/bed-bugs (2016)

During their life cycle bedbugs go through five nymphal stages before maturing as an adult with each stage looking like an adult only smaller in size and colour (Doggett *et al.*, 2012). At each instar, they require at least one blood meal to develop to the next stadium (Harlan, 2006). The first instar is about 1 mm in length, pale in colour when unfed and reddish after a blood meal. The second instar to the adult stage are brown to dark brown in colour when unfed. Adult become deep red-brown and 5-6 mm long after a blood meal (Usinger, 1966). Bedbugs at all stages will have to feed on blood for nutrition and development. During feeding it takes 3-10 minutes for complete engorgement to occur. The host attract bedbugs when they release carbon dioxide during breathing, and various compounds secreted across the skin (Doggett *et al.*, 2012). Although humans are the preferred host, bedbugs will feed on other warm-blooded animals, including cats and dogs (Clark *et al.*, 2002). It is reported that, In the United States, heavily infested poultry sheds can result in anaemia and decreased egg production (Cater, 2011). Bedbugs do not to live on the human body. The only contact with human according to Doggett *et al*. (2012) is only for a blood meal, which may occur every few days if a sleeping host is available.

The female paragenital system is thought to have evolved in response to traumatic insemination, as abdominal piercings have a deleterious effect on female survival (Usinger, 1966; Stutt and Siva-Jothy, 2001; Reinhardt and Siva-Jothy, 2007). The cimicid paragential system includes the spermalege, an organ unique to this group of heteropterans (Reinhardt and Siva-Jothy, 2007; Stutt and Siva-Jothy, 2001). It has been shown that the female directs the male's intromittent organ into the spermalege, which receives the sperm. Traumatic insemination into this organ reduces the costs of wounding and mitigates the effect of pathogens (Reinhardt and Siva-Jothy, 2007). Female cimicids still maintain their ancestral reproductive tract, which they use during oviposition (Usinger, 1966). With only two exceptions, cimicids have five nymphal instars, each one being bigger than the last (Usinger, 1966).

Environmental temperature and humidity are factors that influence how long it takes for the completion of a bedbug cycle. (Usinger, 1966). At a temperature of 23 $^{0}$C and 90% humidity, C. lectularius females lay approximately 90-150 eggs over 18 weeks, whereas C. hemipterus females lay roughly 50 eggs over their life span (Dogget *et al.*, 2012). If regular feeding is kept up and at room temperature, the life cycle of a bedbug from egg to adult will complete in approximately 50 days (Dogget *et al.*, 2012).

### 2.3 Distribution of bedbugs

Bedbugs have been shown to disperse both passively and actively that is, they may either be moved by an agent or they migrate on their own. (Pfiester *et al.*, 2009; Wang *et al.*, 2010; Dogget *et al.*, 2012). Passive dispersal is facilitated by humans inadvertently transporting them to new locations on or in their clothing, luggage, or used furniture (Delaunay *et al.*, 2011; Dogget *et al.*, 2012). Evidence for human facilitated passive dispersal is increased interceptions of bedbugs found in luggage by national quarantine inspectors in Australia (Davies *et al.*, 2012). Active dispersal of bedbugs most commonly involves individual bedbugs walking from one apartment unit to another apartment unit usually close by. Adult bedbugs has been found in doorways and hallways indicating that the bedbugs have been transported from one place to another. (Pfiester *et al.*, 2009; Wang *et al*., 2010). Female adult bedbugs are more frequently found dispersing from aggregations, suggesting they are the most likely dispersal stage (Pfiester *et al.*, 2009; Wang *et al.*, 2010)

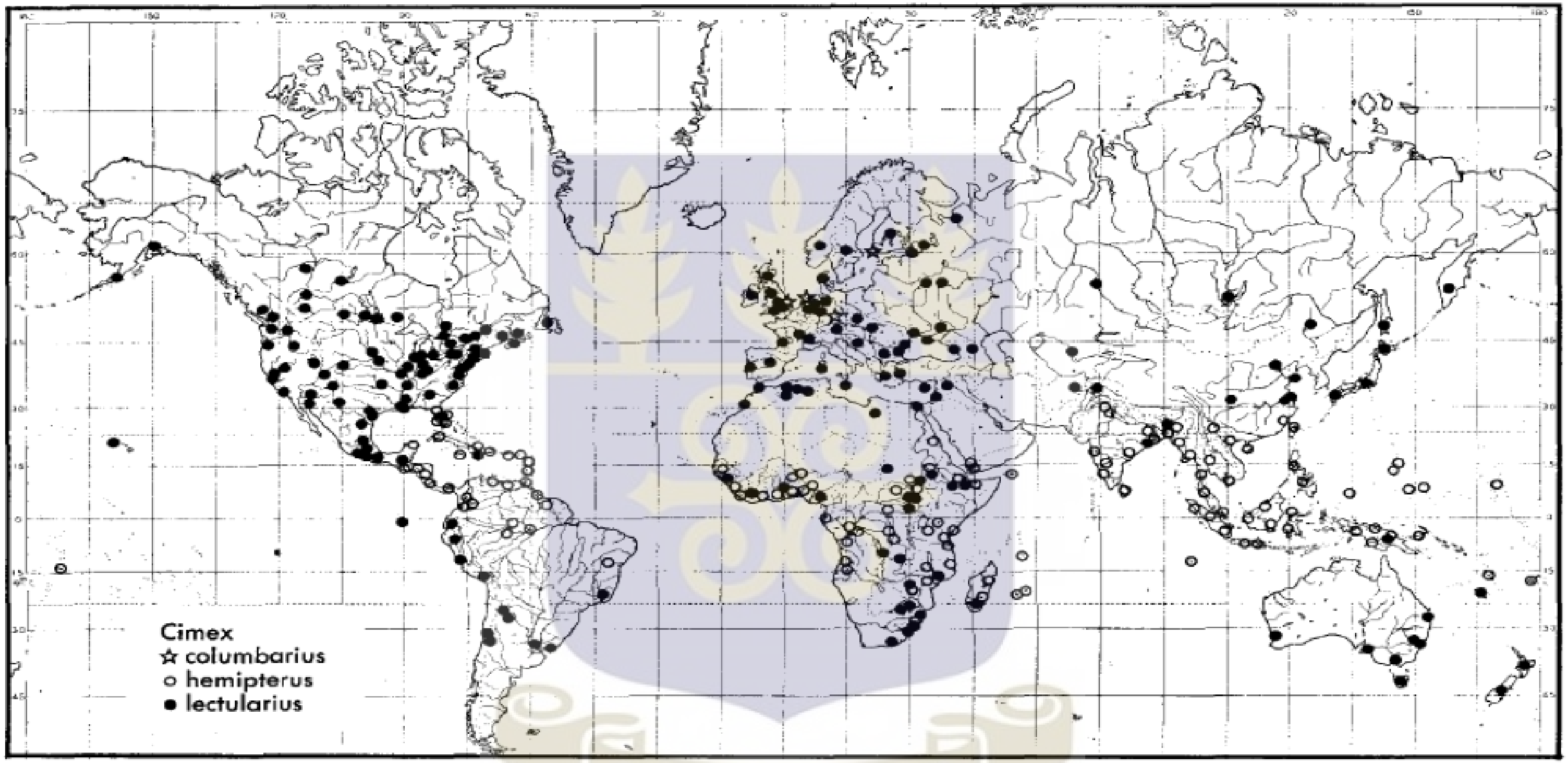


**Figure 2.2**: Global distribution of bedbug infestation in human habitations (Usinger, 1966).

Of the bedbugs displaying active dispersal, 90% of them were adults (Wang *et al.*, 2010). Common bedbugs can be found almost anywhere humans have established homes and cities. They do well in temperature and humidity that are good for humans, who also provide them ample blood meals and places to hide ( Usinger 1966: Harlan, 2005). The year 1583 was the year bedbugs were first recorded in England. (Usinger, 1966). In 1939, approximately four million people in Greater London were subjected to Bedbug infestation (Usinger, 1966). Bedbug infestations deteriorated from 1939 onwards. This decline was attributed to the introduction of residual insecticides (Busvine, 1980). Busvine (1980) reports that, the number of bedbug treatments remained stationary from the late 1960's to the early 1970's. In Africa, specifically Nigeria, bedbugs have been recorded in apartments, hotels and hospitals as reported by Omudu and Kuse, (2010). There have been reports of bedbug presence in Italy. Durand *et al.* (2012) collected a total of 198 bedbugs in 102 Italian apartments. These brings to the fore the global nature of bedbug infestation.

### 2.4 Global resurgence of bedbugs

Before the 1940s, bedbugs were near ubiquitous and generally considered an accepted pest species (Szalanski *et al.*, 2008). It was difficult to treat infestations because, safety precautions were not adhered to during application of the effective insecticides. In 1930, London had a third of its population affected by bedbug infestations (Usinger, 1966; Dogget *et al.*, 2012). By 1940s and 1950s, bedbug incidence had waned because of the use of insecticides. Despite this fact, a cosmopolitan resurgence has been made in the last twenty years (Reinhardt and Siva-Jothy, 2007). Reports of an increase in the number, distribution and intensity of infestations have been reported in Africa, Australia, Canada, Europe, Japan, Malaysia, South Korea and Thailand (Davies *et al.*, 2012).

To date, no formal scientific studies have been conducted on the prevalence of infestations. Notwithstanding, many independent reports have been observed, which point to vast increases (Dogget *et al.*, 2012). In Berlin, there were 5 reported cases of infestation in private homes during 1993, while 76 cases were reported in 2004 (Davies *et al.*, 2012). A 2006 survey of 121 pest managers in Australia revealed a 4500% increase of bedbug treatments from 2000 to 2006 (Dogget, 2007). The London School of Hygiene and Tropical Medicine, Armstrong (2013), surveyed local pest control specialists, he discovered that, the number of bedbug treatments rose an average of 24.7% every year from 2000 to 2006 (Richards *et al.*, 2009). A survey conducted in 2010 by the University of Kentucky and the National Pest Management Association found over 95% of 1000 pest management companies that operate within U.S.A. or internationally reported treating bedbug infestations that year (Davies *et al.*, 2012).

In contrast, only 25% of American respondents reported encountering infestations before 2000. Complaints about bedbugs to the New York City council increased from 537 in 2004 to 10,985 in 2009 (Dogget *et al.*, 2012). Although there can be found shortcomings in these reports, they likely constitute a somewhat accurate depiction of the rise in bedbug infestations across the world (Dogget *et al.*, 2012). One of the probable reasons for this resurgence is the increase in human global travel and the use of second hand furniture (Delaunay *et al.*, 2011; Dogget *et al.*, 2012). Additionally, infestation hot spots reported by pest control professionals are often locations where humans are highly transient (Dogget, 2007: Potter *et al.*, 2010). Some of the major locations for infestations include motels, hostels, trains, resorts, hospitals, homeless shelters, public transportation, and movie theatres (Dogget, 2007; Potter *et al.*, 2010). The shift from carbamate and organophosphate insecticides to pyrethroid insecticides (Armstrong, 2015), is another suggested cause for the rise of bedbug infestations incidence. Bedbugs had evolved to become resistant to pyrethroid so the chemical became ineffective on them (Romero, 2011b; Dogget *et al.*, 2012). Two amino acid substitution mutations that grant pyrethroid resistance due to reduced voltage gated sodium ion channels target-site sensitivity were found in bedbug populations from 17 states across the U.S.A (Zhu *et al.*, 2010). Populations demonstrating insecticide resistance through metabolic detoxification have also been identified (Romero *et al.*, 2011b: Adelman *et al.*, 2011). A third reason for the global reappearance of bedbug infestations is most probable to be an ignorance of how to detect and exterminate bedbugs (Dogget *et al.*, 2012). During the 50 years following their near extirpation, it was rare for entomologists and pest control professionals in the developed world to encounter a bedbug infestation even once (Davies *et al.*, 2012).

Roughly half of the people living in infested homes are unaware they have bedbugs (Wang *et al.*, 2010). Ignorance can also cause unintentional passive dispersal, such as taking used furniture and clothing containing bedbugs into private homes, or not thoroughly inspecting accommodations when travelling and luggage before returning home. This act of negligence may either cause one to transmit bedbugs from one accommodation to their homes. (Reinhardt and Siva-Jothy, 2007: Dogget *et al.*, 2012), Furthermore, if extermination techniques are poorly applied they don't kill the entire population them to disperse some more as they seek for refuge (Dogget *et al.*, 2012: Armstrong, 2013).

## 2.5 Clinical Relevance

The reported medical cases of bedbugs are the direct cutaneous reactions from their bite (Doggett, 2012). Bedbugs feed on blood only, this is sucked from the body of mammals including humans and birds. For nutrition, growth and reproduction, adult bedbugs need one blood meal of adequate measure. Each active instar may feed multiple times if hosts are readily available. Adults are likely to feed every 3–5 days if host is present for a period of 6–12- month (Harlan, 2006). Twenty eight (28) human pathogens have been found naturally occurring in common bedbugs. However, they are yet to be proven to biologically or mechanically transmit any of these pathogens (Usinger, 1966; Jupp *et al.*, 1991; Blow *et al*., 2001). The challenge and high costs of pest control often lead people to desperate acts such as burning away infested possessions. Bedbug infestations has led to the closing down of hospitals, threatening the provision of health services also bedbugs present various social issues including the mental health consequences of having an infestation are potentially serious (Doggett *et al.*, 2010).

### 2.5.1 Health effects of bedbug bites

Bedbug bites can cause psychological and physical discomfort to its victims (Jacob, 2015). Bedbug mouthparts are adapted for piercing the skin and sucking blood. They have extremely fine needle-like stylets that are inserted into the skin and withdrawn after feeding. During feeding, a flowing vein or capillary may not always be located by bedbugs on the first probe. This may cause it to probe again leading to multiple bites ( Doggett, 2012; Olson *et al.*, 2013). The bug injects saliva that contains various protein fractions, some of which have got anticoagulant properties. In *C. lectularius*, this includes nitrophorin, which is a vasodilator inducer (Doggett, 2012). Valenzuela *et al.*, (1996), apyrase, which inhibits platelet activation and aggregation (Valenzuela *et al.*, 1996); They also inject an inhibitor of substance, which delays blood clotting (Valenzuela *et al.*, 1996).Although their feeding usually is hardly felt, the saliva contains biologically active proteins, which may cause progressive, allergenic, noticeable symptomatic skin reactions to recurring bites

(Gbakima *et al.,* 2002; Harlan, 2006). Symptoms from bites include a raised, inflamed, reddish weal at each site bitten by the bedbug, may itch intensely for several days (Gbakima *et al.*, 2002). Immediate reactions may appear from 1 to 24 hours after a bite and may take up to 1–2 days; delayed reactions usually appear 1–3 days (or more) after a bite and may also last 2–5 days (Feingold *et al.*, 1968). After the removal of the stylets from the skin, some amounts of oozing at the bite site may occur, this is usually seen on bed sheets as small flecks of blood (Steen *et al.*, 2004). Bedbug feeding may sometimes be interrupted by the movement of the host, (Yao *et al.*, 2014) the bedbug will resume feeding after the sleeping victim settles (Doggett, 2012).

It has been found that the saliva of *C. hemipterus* contains a small amount of hemeproteins and has reduced anticlotting activity compared with *C. lectularius*, (Doggett *et al.*, 2012). Although the total protein contents of the saliva share similarities between the two species (Araujo *et al.*, 2009). Bites are often reported as occurring along the arms and legs but also will occur on any area of exposed skin. Clothing can inhibit bites because the bedbugs are denied access to the skin (Straub *et al.,* 2009). The severity of cutaneous reactions from a bedbug bite varies between individuals (Doggett *et al.*, 2012). The reaction may start out as small indistinct red macular lesions less than 5 mm in diameter (Goddard and Shazo, 2009), which may later progress into large circular or ovoid wheals (Criado and Criado, 2011), and may be as big as 2 to 6 cm in diameter, which characterizes the more standard bedbug “wheal” (Crissy, 1981).

Bite reactions can take some weeks to resolve, depending on the severity of the reaction. Patients with multiple bites or a severe cutaneous reaction may develop systemic symptoms, including fever and malaise, although this appears to be rare (Bircher *et al.,* 2005). Some individuals will not develop a reaction, even with repeated exposures. Goddard reported that he used himself as host to fifteen *C. lectularius* every three (3) weeks over a period of six (6)months, yet no cutaneous or clinical reaction developed (Goddard and Shazo, 2009). Bedbug bites may appear in a linear fashion, either individually or in a cluster. A number of authors have stated that the bites appear in groups of three (Cohen *et al.*, 2010). Bodies are kept in contact with the bedding while bedbugs are feeding. They then project the mouthparts forward into the skin (H. J. Harlan, unpublished data). A line of bites can be alluded to the number of bugs feeding on the patient during sleep over an area. The line would merely coincidental, a result of the limitation of where the bugs can reach from the bedding. This explains that, bites along the limbs and torso are directional (Doggett *et al.*, 2012). More so, the first sight of bedbugs in one's apartment or institution may not be the original encounter. Bedbugs are a highly discreet group of insects, which are active mostly at night. Many victims therefore would not notice the presence of the insect, especially if the infestation is relatively light. As a result, patients may not notice the presence of the insect depending on how light the infestation is. Patients may not develop an allergic response upon the first exposure to the bite but often do so upon a subsequent exposure. (Doggett *et al.*, 2012).

Other health effects of bedbug infestation includes lack of sleep. Bedbugs are commonly associated with sleep deprivation (Doggett and Russell, 2009: Doggett *et al.*, 2012.) Potter *et al.*, (2010) reported that, patients suffered from insomnia due to bites from bedbugs. During the night, Patients can wake due to the itch from the bite of bedbugs, and scratching can exacerbate the itching sensation, leading to greater sleep disturbance (Thorburn and Riha, 2010). Some patients have disturbed sleep from just the knowledge of having an active or past infestation in their own bed (Doggett *et al.*, 2012). Sleep deprivation is a serious medical problem that can affect neurocognitive functioning, emotional status, and various physiological factors (Doggett *et al.*, 2012). This may contribute to long-term health problems such as coronary heart disease (Waters and Bucks, 2011: Doggett *et al.*, 2012). Sleep loss is considered to have a major economic impact, and some of the most serious human-caused disasters. Phisalix (1922) reported that liquid excreted from bedbugs during feeding can induce an urticarial reaction. It was also stated that chronic bedbug infestations can cause uneasiness, lethargy, paleness and diarrhoea. It is however not clear, if these symptoms are due to the bedbug bites or the mental trauma associated with having an infestation (Ter Poorten and Prose, 2005; Doggett *et al.*, 2012). It was also reported that repeated bedbug bites may produce a severe reaction with serum sickness (Doggett *et al.*, 2012).

### 2.5.2 Social effects of bedbug infestation

Report suggest that the effect of bedbug bites are not restricted to physical symptoms alone (Jacob, 2016). They also leave psychological effects on some of the people their homes and gets infested. Socially, there can be a stigma attached with having a bed bug infestation as infested people may be teased or laughed at especially amongst children (Krinsky, 2002). This can imply that, the bedbugs are not an endorsed species in the society. Bedbugs can be a menace to the society, It can cause financial instability because infested members in a community may have to dispose furniture they wouldn't have initially due to heavy infestation. Surveys conducted, showed that, people suffering from bedbug infestation have effects similar to people suffering from post-traumatic stress disorder (PTSD) as well as delusional parasitosis (Jacob, 2016). They have been suicide case reported due to the infestation of bedbugs, There is a reported investigation, where a woman committed suicide in her apartment because she had repeated infestations of bedbugs in her apartment, even after having professionals attend to the situation (Burrows *et al.*, 2013).

### 2.6 Environmental factors that influence the survival of insects

Changes occurring the climate worldwide have been reported to be responsible for anthropogenic and environmental variation (Harrison *et al*., 2013). These climatic and weather changes not only affect the functionality of insects, but also its population dynamics, distribution, abundance and feeding behaviour (Ayres and Schineider, 2009).

Studies conducted by Shatz *et al* (2013), on various factors including anthropogenic, biotic and abiotic, were assumed to be responsible for the distribution of the Asian longhorn (*Anoplophora glabripennis*). Upper and lower thermal abiotic factors affects the reproducibility of insects. It also affects insects' emergence, flight and rate of dispersal (Yamamura and Kiritani, 1998). Biotic factors such as low and high temperatures also affects the functionality of insects.

#### 2.6.1 Temperature

Anthropogenic and natural environmental variations are affecting arthropods with each passing time. Factors such as thermal effect is changing the functionality of pest by suppressing or stimulating the genetic potential, fecundity rate and insect mortality (Regniere *et al*., 2012).

Like other Organisms, bedbugs are part of a complex ecosystem and are affected by diverse elements in their environment (Usinger, 1966). Of the various factors, temperature is the most important, since it influences all aspects of the bedbugs' activities. In caves temperatures are almost uniform, these temperatures does not affect the rate of development of bedbugs inhabiting such places. The threshold for hatching, nymphal development, and adult activity in *Cimex lectularius* is between 13 $^{o}$C – 15 $^{o}$C (Hase 1930). Although bedbugs can tolerate extreme temperature as low as -15 $^{o}$C for brief periods. The thermal death point is between 44 $^{o}$C and 45 $^{o}$C. Omori (1941) reports the ceasing of development in *Cimex lectularius* at a temperature of 36$^{o}$C. *Cimex hemipterus* has a higher threshold of development recorded at 15 $^{o}$C-18 $^{o}$C (Omori, 1941).

### 2.6.2 Relative Humidity

Rivnay (l932), reported that, relative humidity ranging from l0 to 70 were found to have a negligible effect on the rate of development of nymphs of C. *lectularius* On the contrary, Kemper (1936) noted that nymphs kept under extremely dry atmospheric conditions often died during molting. Jones (1930) and Mellanby (1935) found that unfed first instar nymphs survived for different periods, depending on the humidity.

According to Mellanby, in air at 90% RH and a temperature of 30°C, they survived 26.3 days. At lower humidity (60, 30, and 0%) survival time reduced progressively to 5.68 days and desiccation was the principal cause of death.

### 2.6.3 Influence of light on the survival of Insects

Light can affect insect behavior and development in many ways. One of the responses to light is phototaxis (Jander 1963). In the presence of light, insect may exhibit, attraction and repulsion. Attraction response to light can be employed in the trapping of insect, though effective wavelength may vary among insect species (Yang *et al.,* 2003). Repulsion can be used to prevent pests from entering an area by presenting light at a wavelength and intensities that will repel them (Kim *et al*., 2013; Reisenman *et al*., 1998).

The response to light are greatly influenced by a number of factors, including light intensity, wavelength, time of exposure, direction of light source, the contrast of light source intensity and color to that of ambient light (Antignus 2000). In addition, the impact of light on insect behaviour varies both qualitatively and quantitatively depending on the light source (Antignus 2000; Honda 2011). Many nocturnal insect species such as moths, beetles and stinkbugs are attracted to artificial light sources. These insects are attracted to light that emits strong amount of ultra violet radiations

(Cowan and Gries, 2009).During the World War II in Japan, insect light traps using blue fluorescent light were used to control rice stem borer (*Chilo suppressalis)* (Ishikura, 1950).

Aboul-Nasr and Erakey (1969) reported that the common bedbugs (*Cimex lectularius*) are able to distinguish between different wavelengths of light. Short-wavelength colors such as violet (hematoxylin 0.005% solution) and bluish-green (fast green 0.001%) were preferred compared with the other colors tested. Red (eosin 5%) had attractive qualities, while yellow (Bouin's solution) appeared to be the least attractive. Singh *et al*., (2015) have also shown that aggregations of specifically adult male bed bugs and third–fifth-instar bed bugs prefer black and red harbourages compared with other tested colors.

### 2.6.4 Crowding factors that influence the survival of bedbugs

The behavior of Cimicidae, like other animals, is largely directed to the search for food, shelter, and mates. The bedbug is not evenly distributed over its environment. Instead, bedbugs are concentrated in harborages often at some distance from the host (Usinger, 1966).

Bedbugs finds shelter in cracks and crevices in houses of man as well as in roosts of chickens, tree-holes and caves of its original hosts-bats. Bedbugs are thigmotactic, they look for places where their body surface can make contact with a rough substrate. This often leads to a cluster of bedbugs, each in contact with the other, in "brood centers" where much fecal matter, egg shells, and exuviae accumulate. Bugs return to such harborages after each meal (Kemper 1936) and remain there in a quiescent state while digestion takes place. Such clusters are formed where the substrate is dry, rough, and prevent light (Usinger, 1966).

## 2.7 Prevention and control of bedbugs infestation

Bedbugs are considered to be one of the most challenging of all insects to control as they can hide in nest, crevices, void books and mattresses ( Axtell, 1999; Omudu and Kuse, 2010). For the accommodation sector, bedbugs are problematic. They expose the facility to expensive litigation and unwanted publicity, thereby damaging brand reputations. Widespread insecticide resistance, has altered the biology of the pest (Doggett *et al.*, 2012). This has contributed to the challenge in the control of bedbugs (Doggett *et al.*, 2012). It is difficult to prevent bedbugs, however, there is the potential to minimize their impacts through risk management (Doggett *et al.*, 2012). Other forms of control could be done using either the chemical or non-chemical treatment method.

### 2.7.1 Starvation

Since every living organism needs food to sustain life, starvation has been thought of as one of the means of getting rid of bedbugs. Taking away food is a way of controlling the prevalence of bedbug infestation (Doggett *et al.*, 2012). However, keeping a room vacant to starve bedbugs may not yield the desired results because bedbugs are long-lived insects (Usinger, 1966). For instance, at 18°C, a once fed bedbug can live for up to 277.1 days (Usinger, 1966), while for a typical hotel room set to a constant temperature of 22°C, once-fed bedbugs can survive for around 135 days without a blood meal (Doggett, 2011). Another reason for its impracticality is that, to use starvation as a tool for controlling bedbugs, one would have to vacate their room for not less than 278 days to achieve desired results according to Usinger (1996).

### 2.7.2 Early detection

The key to reducing bedbug impacts for those in the accommodation sector is early detection. Early detection minimizes the risk of the infestation spreading. Early detection of bedbugs can be undertaken by various means (Doggett *et al.*, 2012). This includes the use of trained canines as practiced in the U.S.A. Regular and thorough inspections by housekeeping staff during routine room maintenance, or via pest managers are medium through which early detection can be detected (Doggett *et al.*, 2012). For effective control of bedbugs, there shouldn't be a single management option. Successful pest managers should embrace the concept of integrated pest management (IPM) (Doggett *et al.*, 2012). This is a process whereby nonchemical means of control are employed in conjunction with the judicious use of insecticides (Moore and Miller, 2008). Control process for bedbugs should first be by identification of the pest inspection of the site to determine which areas require treatment. Then, nonchemical control options should be considered which can be followed with the use of insecticide application (Doggett *et al.*, 2012). Finally, evaluation of the success of the treatment program, and risk management procedures should be undertaken to know the outcome of the intervention. (Doggett *et al*., 2012). In hotels, student dormitories, apartment complexes, and other multiple occupancy dwellings, the inspection process should include the examination of all rooms. Adjoining the room with the infestation, and ideally, risk management should be ongoing and be implemented even prior to infestation occurring (Doggett *et al.*, 2012). The failure to achieve this in an apartment complex may result in bedbugs spreading to adjoining units and subsequently increase in costs of control. Control of bedbug infestation can be placed under two broad headings namely non-chemical treatment and chemical treatment.

The control of bedbugs is made easier when bugs are detected early (Davio, 2010). Early detection as a control measure of bedbug is a strategy against the growth phase, which involve mainly identifying the presence of bedbugs in its early stages. This can be done in the training of housekeepers in bedbug detection and the educating of tenants on bedbug recognition to encourage early reporting. After bedbugs have been detected it becomes easier to limit their spread.

Restraining the spread of bedbugs can be achieved through the immediate implementation of control measures upon bedbug detection, the quarantining of infested rooms, and ensuring that infested items are bagged within the room before removal and treatment; before relocation (Doggett *et al.*, 2011) are some of the methods through which bedbugs can be controlled.

### 2.7.3 Inspection

When a bedbug infestation is suspected, a thorough inspection is required. There will be one or more primary infestation sites associated with locations where people rest, sleep, or sit for extended periods. Inspections must be performed intensively, as bedbugs are extremely flat and small (1/16" to 3/8", or 6–8 mm, long). Some idea of the tight places bedbugs can fit into puts the problem into perspective: they have been found along picture frames, between the glass and frame itself, and they have been found in the stitch holes of mattresses. Inspections not only have to be thorough, but also very methodical, starting with the identified resting sites of the person(s) and moving out to adjacent areas. All bed components and furniture should be dismantled to the point that cracks and crevices can be adequately inspected to the level of detail previously indicated. Besides dismantling furnishings, inspection and control also requires the moving and temporary relocation of furniture, equipment, and other items. An efficient work plan for determining how the room will be treated should be followed so that items are moved only once. Although saving considerable time and effort, nonchemical means do not provide residual control, and there is a risk of re-infestation if treated items are placed back in the infested area. To begin control measures, a "clean area" is set up. This is an area that initially receives both nonchemical and chemical controls (Kells *et al*., 2010). This area becomes the site where all items receiving further treatments are placed. As the clean area fills up, adjacent sections of the room will become available for treatment, and the clean area can be expanded to provide more room for treated items. Depending on the room and its contents, expanding the clean area 2–3 times usually provides enough space for everything that is treated.

### 2.7.4 Decreasing establishment

Eradication of a particular species begins with the reduction of those species until they cannot be found. This is done by trapping, among other methods. One way to reduce the establishment of bedbugs is to limit its infestation in accommodation lodgings by reducing potential harbourages. This can be achieved by ensuring that cracks and crevices are minimized in the room, Also, furniture and beds should be constructed with materials such as smooth metals and plastics rather than wood. In addition, mattress should be encased (Doggett *et al.*, 2011). Mattress encasements provides fewer hiding areas for bedbugs. The colour of encasement must also be taken into consideration as white encasement have the additional benefit of making bedbug detection easier. Furthermore, some encasement are bite-proof, encased infested mattress prevent the escape of bedbugs thereby preventing mattresses from being discarded (Cooper, 2007).

### 2.7.5 Bedbug traps

Trapping bedbugs is one of the effective techniques of controlling the number of bugs in a given place (Harlan, 2005). One of the type of traps that has been used for a long time is the Pit-Fall trap (Harlan, 2005). It has been used in research programs for the monitoring of populations (Wang *et al.*, 2009). Part of the Pit-Fall trap is the device known as the Climb-Up Interceptor. Climb-Up Insect Interceptor, acts as both a barrier and a monitor for bedbugs. This device consists of an ultra -smooth plastic bowl with an outer device like a bowl (Doggett *et al.*, 2012). It has been shown to be more effective at detecting bedbugs than visual inspection. Because of this feature, the

Integrated Pest Management (IPM) of bedbugs program has been very effective (Wang *et al.*, 2009).

### 2.8 Nonchemical control of bedbugs infestation

To achieve the reduction of the biomass of bed bugs, various nonchemical methods of control can be carried out (Doggett *et al.*, 2012). Nonchemical technologies rather have a more abrupt effect on reducing bedbug quantities (Doggett *et al.*, 2012). This technology also has the added benefit of being less hazardous than chemical technologies (Potter *et al.*, 2007). Usually, some level of insecticide application will be needed. Although an integrated method where nonchemical means of control are used to reduce the amount of insecticidal product is required (Doggett *et al.*, 2012). The simplest form of nonchemical control is the disposal of infested items as stated by Doggett *et al*. (2012). Infested items should be enclosed in plastic before removal to prevent them from becoming a contagion. Furniture set aside for throwing away should be either destroyed or rendered unusable. This will prevent others from taking the items and consequently assisting the spread of bedbugs (Doggett *et al.*, 2012). The only economically viable option for heavily cluttered premises, disposal of infested items may be. Although many infested items can be treated.

#### 2.8.1 Vacuum cleaning

Another method of reducing bedbugs in homes is vacuum cleaning. This can rapidly reduce the bedbug biomass in an infestation and even remove many eggs (Doggett, 2009). Vacuum machines are affordable, readily available, require little or no teaching to operate and does not require any operator licensing. Vacuum cleaning present a minimal risk of spreading an infestation (Doggett *et al.*, 2012). It is important that the vacuum machine has a disposable bag. This bag is immediately removed and sealed in plastic after use (Doggett *et al.*, 2012). This procedure will not allow bedbugs to move from one place to another. However, vacuuming machines comes with its own disadvantage as machines may not remove bedbugs in deep harbourages.

#### 2.8.1.2 Heat treatment

Heat is a very practical and effective means of nonchemical bedbug control. The exposure of all stages of *C. lectularius* to temperature of 45°C for one hour will kill the insect (Johnson. 1941), and at temperatures over 60°C, all bedbugs are rapidly killed (Naylor and Boase, 2010). Heat can be applied via the use of steam, through the laundering of infested clothing and bedding, via hot washing and drying (Naylor and Boase, 2010), and through the use of contained or circulated heat treatments (Paul and Bates, 2000). The heating of whole rooms comes with the risk of spreading the infestation, as bedbugs will seek cool places which has a temperature above 30°C to 35°C (Doggett *et al.*, 2012). There can be thermally protected areas which do not heat up to the essential temperatures to kill all bedbugs (Doggett *et al.*, 2012), especially in cluttered rooms (Potter *et al*, 2008). The application of heat after insecticide application was found to improve bedbug mortality (Doggett *et al.*, 2012), as the heat draws the insecticide out of porous surfaces (Nagro, 2011). Thermal control, in contrast was found to be inappropriate for bedbug control. (Doggett *et al.*, 2006). Bedbugs may not survive long in extremely cold temperature. Infested items can thus be placed into the refrigerator. Under temperatures of -17°C, *C. lectularius* will last for at least two hours (Naylor and Boase. 2010). There are various systems that employ gases to instantly freeze bedbugs; however, these can operate only under high pressure. The disadvantage being that small air currents can disperse bedbugs (Feldlaufer and Loudon, 2011: Doggett *et al.*, 2012).

#### 2.8.1.3 Use of mattress covers

Mattress covers are a good recommendation for sleeping areas that are prone to chronic infestations. Covers prevent the mattresses from tearing and from holes developing in the mattress. Mattresses that have developed holes already but not infested can be covered to prevent bedbugs from sheltering in the intricate habitat provided by the bed (Doggett *et al.*, 2012). This way bedbug control will be made simple and effective (Doggett *et al.*, 2012). If an infestation occurs, mattress covers can be replaced and the infested covers laundered for reuse. If insecticides are applied to the mattress, the covers can be used to contain this application and provide an additional barrier between the treated mattress and the person. The use of mattress covers has been perceived by many in the pest control industry as a method to further reduce any risks of pesticide to human exposure and to avoid liability associated with insecticide applications to the mattress (Kells *et al.*, 2010).

### 2.8.2 Chemical treatment

Insecticides are normally used in bedbug control, except in minor infestations. For a successful eradication to be achieved when using chemicals, the right product in its right formulation is essential (Doggett *et al.*, 2012). When the wrong product is used, regardless of the amount used, the outcome may not be as expected. In similar light, though the right product has been acquired, the directions must be followed to achieve the right formula. This will make bedbug management effective

#### 2.8.2.1 Insecticide groups for controlling bedbugs

Worldwide today, the principal group of insecticides in used against bedbugs includes pyrethroids, silicates, and insect growth regulators (IGRs). In some parts of the world, the use of carbamates and some organophosphates have continued. While more recently, neonicatinoids and arylpyrroles have begun to be active in usage (Klaehn *et al.*, 2015). The pyrethroids are the most common insecticide products in the marketplace. They make up 95% of the products registered for bedbug control in Australia (Doggett *et al.*, 2012). However, resistance to these products has been reported (Suwannayod *et al.*, 2010).

#### 2.8.2.2 Pyrethroids

The first pyrethroids were produced by chemists. They studied the structure of insecticidal pyrethrins (Davies *et al.*, 2007). These are chemicals found in the seeds of certain chrysanthemums (Eaton, 2015) Pyrethroids last longer than their natural counterparts and seem to have a similar mode of action, disrupting the normal transmission of nerve impulses by affecting the potassium or sodium ion channel in nerve cells (Davies *et al.*, 2007) . Permethrin, cypermethrin, fenvalerate and cyfluthrin are examples of pyrethroids (Eaton, 2015). The pyrethroids are categorized based on the time they were discovered (Doggett *et al.*, 2012) and are placed into four generations (Braness, 2004). Generally, the older the generation, the less effective against bedbugs. When fourth generation pyrethroid products were applied directly on adults of a resistant *C. lectularius* strain at label rates, after 10 days, only around 60% died (with the control mortality rate being 20%). When the same bedbug strain was placed on dried residuals of pyrethroids treated at label rates, the rate of mortality was reduced to 30% after 10 days (Doggett and Russel, 2009). Piperonyl butoxide (PBO), a synergist, when added, can increase topical mortality by overcoming the resistance mechanism in certain strains of *C. lectularius* and *C. hemipterus*; likewise, the addition of PBO does not always enhance residual efficacy (Lilly *et al.*, 2009). Pyrethroids generally have poor efficacy, especially when used as a left over, to battle modern resilient bedbug strains. The other disadvantage is that when exposed to sub-lethal doses of pyrethroids, resistant bedbugs can become excited (Romero *et al.*, 2009). The implication is that sub-lethal doses may lead to the dispersal of an infestation in poorly treated premises. In contrast, a susceptible strain was not found repellent (Moore and Miller, 2006). Natural pyrethrins have also been found to be ineffective against a modern resistant *C. lectularius* strain (Lilly *et al.*, 2009). Marketers, however have taken advantage of this situation to sell permethrin impregnated fabrics. A number of groups are now marketing permethrin impregnated fabrics such as mattress ticking and mattress covers (Doggett *et al.*, 2012). These marketers claim that their fabrics are able to control bedbugs. As shown, permethrin has yielded very poor results when used against resilient bedbug strains. It is almost expected that when such products have been evaluated in independent studies, they would prove to be useless against modern strains *C. lectularius* (Doggett *et al.*, 2011). Thus, there appears to be no benefit in the use of permethrin impregnated fabrics in a bedbug management program.

#### 2.8.2.3 Organophosphates

Organophosphates (Ops) interfere with the transmission of nerve impulses. Their point of action is the tiny gap between one nerve fiber and the next known as the synapse. Nerve impulses jump such gaps with the aid of chemicals called neurotransmitters. Enzymes normally destroy these chemicals immediately after the nerve impulse crosses the gap (Eaton, 2015) is one of the common neurotransmitters, Acetylcholine can be found in humans and in insects (Eaton, 2015) Acetylcholinesterase is the enzyme that breaks acetylcholine down (Tawatsin *et al.*, 2011). Organophosphate insecticides function by inhibiting the action of the enzyme acetylcholinesterase, this causes the acetylcholine to remain attached to the nerve cell, causing the cell to fire repeatedly (Eaton, 2015). This cause hyperactivity, uncoordinated movements, tremors, convulsions or paralysis (Alberts *et al.*, 2002). In Europe and the United States, organophosphates are no longer obtainable for bedbug control, except in saturated strips, although they are used in many other countries (Potter *et al.*, 2010). Pirimiphos methyl, has been assayed against a pyrethroid-resistant strain of *C. lectularius*, and no resistance was detected.

Lilly *et al.*, (2009), reported the mortality of all bedbugs within five (5) minutes when the insecticide was used. The pyrethroid-resistant strain was also exposed to aged deposits of Pirimiphos methyl with the surface treated at label rates; 52 weeks after the initial application, 100% mortality was achieved within 24 hours of exposure (Doggett and Russell, 2009). The disadvantage of these OP products is that although they are still registered, pest managers may not opt for them because of their staining and odour issues. DDVP (2,2- dichlorovinyl dimethyl phosphate) is an OP that is used as a vapour toxicant in many countries, in DDVP, the product is impregnated into plastic strips where It is used for small-scale fumigation of items infested with bedbugs(Potter *et al.*, 2009). Efficacy of DDVP can be increased significantly through the application of heat, which increases the volatility of the insecticide (Pereira and Koehler, 2011).

**2.8.2.4 Carbamates**

Like the organophosphates, the carbamates act as cholinesterase inhibitors (Calderón-Cortés *et al.*, 2010), and insects exposed to them show similar symptoms. Except that the actions of carbamates can be reversed (Eaton, 2015). Some examples of carbamates are Carbaryl, bediocarb and propoxur. There is also resistance to the carbamate group of insecticides (Lilly et al., 2009), although the degree of resistance is much lower than with the pyrethroids (Doggett *et al.*, 2012). In one of the resistance studies, bendiocarb had an LD50 that was only 238 times different between the resistant and susceptible strains of *C. lectularius* (Lilly *et al.*, 2009). In topical and residual trials against a resilient *C. lectularius* strain, bendiocarb applied at label rates performed similarly to the pyrethroids (Doggett *et al.*, 2012). Notwithstanding, when used at semi-label rate, the level of efficacy did not decrease while the pyrethroids could not to provide any level of control (Lilly *et al.*, 2009). There have been attempts in the United States to register propoxur as s bedbug control (Doggett *et al.*, 2012). However, propoxur is a carbamate, and therefore, some amount of resistance must be expected, and this has been observed in laboratory trials also in the United States, the Environmental Protection Agency has been reluctant to approve propoxur for indoor use because of its potential toxicity to children after chronic exposure (Colaizzi *et al.*, 2013). Propoxur has an unpleasant odour which makes a lot of people dislike it and wouldn't like to use it in their homes or factories. Bedbugs from Thailand has also been found to be resistant to propoxur (Doggett *et al.*, 2012).

#### 2.8.2.5 Silicates

Silicates are naturally occurring compound that is expected to be human and environmentally friendly (Sathiyamoorthy *et al.*, 2013). There are a number of silicate products available globally in an aerosol or in dust formulation. The most common silicate is Diatomaceous Earth Dust (DED) (Doggett, 2012). The silicates have a very different mode of action from those of the other insecticides. Most products disrupt the insect's physiology, but the silicates absorbs lipids on the waxy surface of the epicuticle such that the insect can no longer maintain moisture and atrophy from dehydration (Benoit *et al.*, 2007). The silicates offer a number of benefits; they have a very long shelf life. They also has very low mammalian toxicity. With its long residual life silicates are able to linger in bedbugs for some time. Finally, there is a low possibility of resistance developing due to the physical action of the product. Due to the benefits of silicates, they are one of the few products that could be used as a prophylactic insecticide (Doggett and Russell, 2008).

Their main disadvantage is that they are slow acting. One study found that DED took up to 6 days to achieve 100% mortality in adult bedbugs (Doggett and Russell, 2008). First-instar bedbugs are affected immediately when exposed to DED, most die within 3 days of exposure (Doggett and Russell, 2008). Another advantage of the slow action of DED is that, dusted bedbugs can transfer the insecticide to untreated bugs, thereby inducing a high rate of secondary mortality (Doggett *et al.*, 2012). By placing  adult bedbugs which has been dusted with first-instar *C. lectularius* insects, 80% nymphal mortality was achieved within 4 days, and all had died by day 12 (Doggett *et al.*, 2012).

### 2.8.3. Neonicotinoids

Within the Neonicotinoid insecticides, Imidacloprid has been assessed against resilient strains of *C. lectularius* and *C. hemipterus*, there has been no report on resistance to this insecticide (Lilly *et al.*, 2009). Lily *et al* (2009) reports of a 100 % mortality in 2 hours against pyrethroid susceptible strains when neonicotinoids were applied directly on bedbugs. When applied as a residual treatment, the product was not effective. It produced a mortality rate of 50 % after 12 days of exposure to the pyrethroid-resistant *C. lectularius* strain (Doggett, 2012).

### 2.8.4 Enzymes and cedar oil

There are a number of chemicals with insecticidal properties being sold for bedbug management that are do not have EPA registration in the United States (Doggett *et al.*, 2012). These include enzymes and cedar oil (Doggett, 2011). The modes of action of these chemicals are not known, and published data on efficacy are lacking (Doggett *et al.*, 2012). It has been reported that cedar oil can kill all bedbugs within a minute (Doggett *et al.*, 2012). It has a strong ovicidal effect that no nymphs can emerge from treated eggs. Despite this, it has poor residual control (Doggett *et al.*, 2012).

### 2.8.5 Insects Growth Regulators (IGRs)

Insects go through a process of growth and development called moulting (Eaton, 2015). This process allows the shedding of the skin of bedbugs and growing a new one in its place (Eaton, 2015). During moulting, a change in form may occur, for example a caterpillar changes to a chrysalis. Certain hormones control the entire process of moulting and changing of form. IGRs function to disrupt the physiology of the insect, the insect tends to die during moulting after being dosed. Methoprene, an Insect Growth Regulator, was found to be effective in laboratory trials at killing both susceptible and resistant strains of *C. lectularius* in the United Kingdom (Naylor and Boase, 2008). Under field evaluations, a 95% reduction in bedbug populations was achieved when hydropene was used together with pyrethroids (Durand *et al.*, 2012). This result was achieved although it was impossible to determine the relative contribution of the IGR to the suppression of the bedbug populations (Moore and Miller, 2006). There are, however, ethical issues surrounding the use of IGRs. When the product is applied to the nymphal stages, there are few direct adverse effects. The insect may rather have to obtain a blood meal for the insecticide to work (Doggett *et al.*, 2012). Thus, the product relies on people being bitten (Doggett, 2011). Potentially, this product could present a litigation risk to pest managers. Ideally, a controversial customer may not appreciate the use of a product that is reliant on their being fed upon.

### 2.8.6 Formulation and application methods of the chemicals

The type of insecticide formulation can influence treatment success (Doggett *et al.*, 2012). The product may be required to be applied directly onto the insects (Doggett *et al.*, 2012). Fumigation is the process of employing gaseous insecticides to control insects. This can be done on whole structures or smaller contained areas (Doggett *et al.*, 2012). The great advantage of fumigants is their ability to penetrate into all areas. (Doggett *et al.*, 2012; Miller and Fisher, 2008). Generally, for bedbug control, whole-structure fumigation is rarely carried out (Doggett *et al.*, 2012). This is because it is expensive and presents logistical problems when treating whole apartment complexes. All residents must be relocated during the treatment (Doggett *et al.*, 2012) which can become cumbersome as well. Fumigants are highly toxic to humans and require specialized training for their application (Doggett *et al.*, 2012). It is not appropriate to treat single rooms within apartment complexes (Doggett *et al.*, 2012). Gas, in its nature, is difficult to contain (Doggett *et al.*, 2012). Thus, there is a high risk of injury to others in the same building when they unknowingly breathe in the gas. There have been deaths due to the inappropriate use of fumigants (Doggett *et al.*, 2011). Off-site containment fumigation for controlling bedbugs in infested furnishings and other transportable items has been found to be effective and poses less of a human health risk, as the application can be undertaken away from residences (Walker *et al.*, 2008).

#### 2.8.6.1 Liquid application

Insecticides are pesticides that are formulated to kill insects (Insecticides, 2015). Insecticide sprays are formulated by mixing a small volume of insecticide into a large quantity of water inside a spray tank (Insecticides, 2015). The insecticides are sprayed in cracks, crevices and along baseboards where bedbugs are likely to hide (Miller, 2015). If the sprays are applied directly unto the bedbugs they will usually die from the application (Miller, 2015). However, these sprays are also supposed to leave behind active residues that kill bedbugs after the product has dried (Miller, 2015). Unfortunately, laboratory studies have found that bedbugs are not very susceptible to dried insecticide residues (Miller, 2015). Bedbugs do not typically pick up a lethal dose from simply walking across the sprayed area (Miller, 2015). Instead, bedbugs will have to sit on the dried residues, sometimes for several days, to suffer any lethal effects (Miller, 2015). If the spray is applied in cracks where the bedbugs rest, the dried residues have a much better chance of killing the bedbugs harbouring there (Miller, 2015).

#### 2.8.6.2 Insecticidal Paint

Currently, the two main vector control method for controlling the malaria transmitting mosquito, *Anopheles* species, are the indoor residual insecticide spray and insecticide treated net (Mosqueira *et al.*, 2010). In the treatment of mosquito nets, the only recommended insecticide to be used are only the Pyrethroid based insecticides (Zaim *et al.*,2000), this is due to their rapid knockdown, high insecticidal potency at low dosages and human safety (Zaim *et al*.,2000). Because of the increasing potential of mosquitoes to resist Pyrethroids, there is the need for other malaria vector controlling strategies in the fight against mosquitoes (Mosqueira *et al.*, 2010). Inesfly insecticide paint, 5A' IGR, sold under the trade name 'Inesfly' is a combination of chlorpyriphos and diazinon and an insect growth regulator (IGR) Pyriproxyfen (Mosqueira *et al.*, 2010). So far, Inesfly has proven to be effective against malaria and other insects. In Argentina, when Inesfly was evaluated against *Triatoma infestans*, it showed high mortality and long residual effects (Amelotti *et al*., 2009). Though Inesfly has proven effective against mosquitoes, there is no prove yet of its effectiveness against bedbugs.

#### 2.8.6.3 Aerosol

Aerosol products are insecticides formulated with a propellant that allows them to be sprayed out of a can into cracks and crevices (Walker *et al.*, 2008). Many types of insecticides are formulated as aerosols (Walker *et al*., 2008). The labels on these products may list very different directions regarding where the product can be applied (Walker *et al.*, 2008). For instance, one aerosol label may direct that a product be sprayed directly on an infested mattress. Whereas another product label will not direct that the spray be applied on fabric surfaces (Wang and Wen, 2011). Like the liquid insecticides, aerosols work best when the live bedbugs are sprayed unto directly with the product (Wang and Wen, 2011). However, a few aerosols leave residues that are active for several days after their application (Wang and Wen, 2011).

#### 2.8.6.4 Dust

Dusts have a higher mortality advantage over liquid insecticides when used to manage bedbugs. Bedbugs walking on dusted surfaces will become covered in the dust making direct exposure to the insecticide impossible to avoid. There are several insecticidal dusts that are labelled for bedbug control (Walker *et al*., 2008). These dusts contain some of the same active ingredients that are used in the liquid insecticide formulations. The labels for insecticidal dusts gives direction for its application in cracks and crevices. These place are enclosed thus pose very little risk of the dust drifting out into open areas. Dusts can be used in walls and voids to intercept bedbugs travelling from one apartment unit to another (Miller, 2015). They can be puffed into protected locations such as baseboards and electrical outlets where bedbugs prefer to hide (Wang and Wen, 2011). However, the dust is disadvantaged because it cannot be used in as many areas as the liquid formulations (Wang and Wen, 2011). This cannot be done because dusts are moved easily on air currents, presenting an inhalation hazard for humans (Wang and Wen, 2011)

### 2.8.7 Insecticidal Resistance

The Insecticide Resistance Action Committee (IRAC) defines insecticide resistance as a reduction in the sensitivity of an insect population to an insecticide (Palenchar *et al.,* 2015). Even when the directions of labels were adhered to, insects manifested resistivity. This was evident in the repeated failure to achieve the desired level of control (Wang and Wen, 2011) It is important to note that many of insecticide products are formulated using a specific class of insecticides that has low toxicity to mammals but high toxicity to insects (Kilpinen *et al.*, 2011).

Pyrethroids are synthetic toxicants that target the insect nerve system. Pyrethroids cause the nerves to fire continuously until the insect loses control of its bodily functions and dies (Wang and Wen, 2011). Many of the liquid spray products, aerosols, and dusts contain pyrethroid insecticides (Wang and Wen, 2011) Unfortunately, pyrethroid insecticides have been used so much throughout the world that many bedbug populations have developed resistance to them. Resistance means that the bedbugs have developed the ability to survive the pyrethroid exposure (Miller, 2015). Resistant bedbugs are also able to pass the resistance in the form of gene transfer to their offspring. The development of resistance has added to the current bedbug population explosion (Wang and Wen, 2011). This does not mean that pyrethroids will not kill bedbugs. It means that not all of the bedbugs will die. Those that survive will go on to produce resistant offspring. Therefore, these insecticides if used alone will not eliminate an infestation. In 2011, resistance to organophosphates was reported for both *C. lectularius* and *C. hemipterus* bedbugs from Thailand (Tawatsin *et al.*, 2011), and for *C. lectularius* bedbugs collected from Denmark (Kilpinen *et al.*, 2011). The introduction of OP resistant bedbugs to other places where resistant bedbugs is not present may make future bedbug control very challenging (Doggett *et al.*, 2012).

**2.8.8 Effect of water loss on bedbugs**

The common bedbug, *Cimex lectularius*, has a remarkable ability to survive 4 months to 2 years without feeding (Benoit, 2007; Usinger, 1966). A feature that presumably accounts for their incredible capacity to persist for long periods in human bedding and other locations (Benoit, 2007).

The prolonged absence of a suitable host is problematic for these obligate blood feeders due to a lack of fluid uptake to counter desiccation (Benoit, 2007). Except for cannibalism, no other attribute have been described that would account for their impressive survival capacity between sessions of blood feeding (Usinger, 1966).

All five nymphal stages and adults are mobile and require a blood meal to moult (Benoit, 2007) The adult however, requires an additional blood meal to reproduce ((Benoit, 2007; Reinhardt and Siva-Jothy, 2007). Bedbugs remain hidden during the day, and because of their small size, lack of wings, and flat body shape, they are able to crawl into tight crevices (Benoit, 2007). After feeding forays, the bugs return to these sites, resulting in the formation of dense aggregations (mixed stages), known as "brood centres (Benoit, 2007)". Aggregation helps them conserve water by reducing water loss from the body (Benoit, 2007).

Previous research, discussed in a review by Johnson, on the hydration level of *C. lectularius* indicates that this bug is particularly tolerant of drying (Benoit, 2007; Johnson, 1941). Factors influencing their unique dehydration resistance and how these factors may contribute to the recent proliferation of bedbugs have not been determined (Benoit, 2007). Female adults are capable of surviving a remarkable 16 days with no food or water, demonstrating their ability to withstand prolonged periods of starvation and desiccation (Benoit *et al*., 2007).

### 2.9 Morphological identification of bedbugs

Bedbugs have a piercing or sucking mouthpart with a sharp pointed proboscis extending from underside of the head (Martin and Webb, 1999). Hemipterans mouthpart is modified with elongated structures to form a tube through which liquid can be drawn. This tube is known as stylet. The bedbug uses the stylet to pierce and suck blood from its host (Chapman, 2013). The stylets of the mandibles are serrated whereas stylets of the maxilla are smooth (McGavin *et al*., 1999). The mandible surrounds the maxilla (McGavin *et al*., 1999). In addition, the serrated mandibular stylets are put into the feeding substrate first (McGavin *et al*., 1999). This is used for cutting and bracing against the substrate (McGavin *et al*., 1999), the smooth maxillary stylets pierces the host, saliva containing enzymes that partially digest the food source are then released (Bugs, 2015). The resultant liquid is sucked back into the insect's gut. The maxillary stylets interlock, allowing for only a sliding movement backwards and forwards into the substrate (Bugs, 2015). Hemipteran have a hemimetaboly life cycle (Bugs, 2015). Their nymphs closely resembles the adult having reduced wings (McGavin *et al*., 1999). Hemipterans include common garden insects such as aphids and cicadas. Cimicidae can be distinguished from other hemipterans by possessing a reduced wing which renders them flightless. Bedbugs are ovoid and flattened in shape, and are all obligatory blood feeders on vertebrates (Carver *et al*., 1991). Adult males have a pointed abdomen, while females have a rounder abdomen (Khan and Rahman, 2012). The dorsal side of the abdomen is sclerotized for a part of the first to third segments and partially membranous (Usinger, 1966). The ventral side of the abdomen is almost membranous (Usinger, 1966). The sixth to eight segments bears a small circular sclerotization (Usinger, 1966).

### 2.10 Molecular characteristics of bedbugs

Over one billion people currently suffer from a neglected tropical disease, which in most cases is caused by a para-site (WHO, 2010). Accurate identification of parasites and vectors is key to improving detection and monitoring and to understanding the characteristic of transmission and control of parasitic diseases. However, morphological perception of most parasite and many vector species is particularly difficult. Both parasites and arthropod vectors are often small and possess strongly different stages in their life cycles and many lack diagnostic morphological characters (Reuben, 1994). This complicates both morphological identification and understanding the relations between developmental stages found either in different host species or in the environment (Majid *et al.*, 2015). For these reasons, molecular data are widely used to complement traditional morphological approaches (Valkiunas, 2008). Because of the wide range of taxa that cause and transmit disease to humans, DNA sequences are used worldwide to identify specimens and describe species, but different markers and genes are often used for different groups of parasites and vectors (Wong *et al.*, 2014). For example, noncoding spacer regions between ribosomal subunits are often used to differentiate among helminth species, whereas cyto-chrome b is often used for haemosporidians. In some groups, dedicated online resources exist to store, compare, and analyze these data (Bensch *et al*., 2009). However, the use of these tools and related resources is predicated on a prior knowledge of the higher taxonomy of the specimens being identified (Crustacea, 2012). This situation begged the development of DNA barcoding, a large scale, and standardized approach to the molecular characterization of biodiversity to aid identification where specialist knowledge may be unavailable (Crustacea, 2012). In most eukaryotes, a DNA barcode is a sequence of approximately 650 nucleotides at the 50 end of the mitochondrial cytochrome C oxidase subunit I (COI) gene from a specimen vouchered in an appropriate collection facility (Crustacea, 2012) . The standardization implicit in barcoding a single tool applicable to all taxa, sequences linked to physical specimens is of obvious potential utility in parasitology (Crustacea, 2012).

A DNA barcode is a short gene sequence taken from standardized portions of the genome(Lyal, 2012). A DNA is used to identify species (Lyal, 2012). DNA barcoding can be used for specimens without morphological characters necessary for traditional identifications, such as roots or immature insects as explained by Lyal, (2012). The researcher, further explained that DNA barcoding being a short sequence, can be extracted from material that has not been specially preserved. It can often be extracted from standard museum or herbarium specimens. Coming from a standardized region, it allows the use of universal primers for unknown taxa. This allows rapid compilation of a global reference library. While barcoding is not intended as a tool for higher classification research or for population genetics research, sometimes the barcoding gene regions have useful information at those levels (Craft *et al.*, 2010). For over 10 years, DNA barcoding has been used to identify specimens and discern species (Ondrejicka *etal.*, 2014).

Over the years, morphological identification has been a successful tool for species identification (Han and Ahman, 2012). This method of identification however, presents its own challenges (Han and Ahman, 2012). First, both phenotypic plasticity and genetic variability in the characters employed for species recognition can lead to incorrect identifications (Han and Ahman, 2012). Then, this approach overlooks morphologically cryptic taxa, which are common in many groups (Knowlton 1993; Jarman and Elliott, 2000). Since morphological keys are often effective only for a particular life stage or gender, many individuals cannot be identified (Han and Ahman, 2012). Finally, although modern interactive versions represent a major advance, the use of keys often demands such a high level of expertise that misdiagnoses are common (Crustacea *et al.*, 2012). The limitations inherent in morphology-based identification systems and the deteriorating pool of taxonomists signal the need for a new approach to taxon recognition (Han and Ahman, 2012). Microgenomic identification systems represent one extremely promising approach to the diagnosis of biological diversity (Crustacea *et al.*, 2012). It also permit life's discrimination through the analysis of a small segment of the genome (Crustacea *et al.*, 2012). This concept has already gained broad acceptance among those working with the least morphologically tractable groups (Crustacea *et al.*, 2012), such as viruses, bacteria and protists (Hamels *et al.*, 2001). However, the problems inherent in morphological taxonomy are general enough to merit the extension of this approach to all life (Crustacea *et al.,* 2012). In fact, there are a growing number of cases in which DNA-based identification systems have been applied to higher organisms (Trewick, 2000; Vincent *et al.*, 2000; Han and Ahman, 2012).

DNA barcoding provides an important tool to improve the quality or speed of floral and faunal studies (Crustacea *et al.,* 2012). At the same time, such studies contribute to development of the global sequence library that is becoming an important community resource. The large-scale inventory of caterpillars, their hosts and their parasites in Costa Rica has provided an excellent example of how barcoding has changed the basic approach to an inventory project, starting with sampling, processing, identification, analysis, and even changing the approach to publication of results (Janzen *et al.*, 2009; Janzen and Hallwachs, 2011; Strutzenberger *et al.*, 2010; Crustacea *et al.,* 2012). In addition to the changes in work flow there have been significant impacts, from finding cryptic species to matching dimorphic males and females, which have substantially improved the quality and depth of the inventory, but also greatly multiplied the number of situations requiring further taxonomic work for resolution (Crustacea *et al.*, 2012).

Although the workflow issues differ between different habitats and taxa, other studies have demonstrated the use of barcoding in inventories of diverse taxa, including poorly known freshwater invertebrates (Zhou *et al.*, 2009; Laforest *et al.,* 2013), tropical sand flies (Azpurua *et al.*, 2010; Krüger *et al.*, 2011), bats in Southeast Asia (Francis *et al.*, 2010), are difficult to distinguish from agricultural pest moths (Roe *et al.*, 2006), pollinating insects in Africa (Nzeduru *et al.*, 2012), diverse radiations of tropical weevils (Pinzón-Navarro *et al.*, 2010a, 2010b; Tanzler *et al.*, 2012), freshwater fishes in Africa (Swartz *et al.*, 2008; Lowenstein *et al.*, 2011), amphibians in Panama (Crawford *et al.,* 2010). Perhaps there are greater opportunities for improving the speed and quality of inventories existing in the marine kingdom, where poorly larval stages are known to exist in vast quantities (Goetze, 2010; Heimeier *et al.*, 2010; Hubert *et al.*, 2010; Ranasinghe *et al.*, 2012). But for most efficient use of DNA barcoding, the appropriate sampling and data management needs to be incorporated from the beginning of the fieldwork (Leponce *et al.*, 2010; Dick and Webb, 2012; Puillandre *et al.*, 2012).

# CHAPTER THREE

## MATERIALS AND METHODS

### 3.1 Study area

The study areas were communities found in the Greater Accra region of Ghana. These include Dodowa and Oyibi. The others were Tema Senior High School (Tema), Awoshie, Pig farm, Kaneshie-Odorkor and Legon, which are suburbs of Accra. Within these communities bedbugs were sampled from human habitations that included houses, educational institutions and hospitality facilities. The Greater Accra region is the smallest in terms of land size among the ten administrative regions of the country, occupying a total land surface area of about 3,245 square kilometres, which is about 1.4 % of the total land area of Ghana. The Greater Accra region is bordered in the north by the Eastern Region, in the east by the lake Volta, in the west by the Central Region and in the south by the Gulf of Guinea. It has a coastline stretching from Kokrobite in the west to Ada in the east.

The Region is largely a low-lying undulating coastal plain with heights reaching about 250 feet above sea level with the exception of places where the topography is fragmented by hills and steep-sided monadnock. On the north-eastern part of the region are the Shai hills, which rise to about 1,000 feet. On the western end are the rounded low hills of between 400-500 feet found on the Togo series and the Cape Coast formations. A section of the Akwapim Ranges encroaches into the eastern half of the region with heights rising to 700 feet.

There are three broad vegetation zones in Accra; the shrub land, grassland and coastal land. The shrub land occurs more commonly in the western outskirts and in the north towards the Aburi hills.

It consists of dense clusters of small trees and shrubs, which grows to an average height of about five metres. The grasses are mixture of species found in the undergrowth of forests. They are short and rarely grow beyond one metre. Vegetation cover in Greater Accra is associated with landscaping, parks, urban agricultural plots, regrowth following clearing, some remnant forests, and wetland vegetation surrounding lagoons (Stow *et al.*, 2013). As a capital city, the central part of Accra consists of large government compounds that are landscaped with trees and lawns. However, the original vegetation of the metropolitan area has been altered in the more recent past century by climatic and human factors

The Accra area lies in the dry equatorial climatic zone. It experiences two rainy seasons. The first rainy season begins in the month of May and ends in the middle of July with the second season beginning in mid-August and ending in October. The region has an average annual rainfall of about 730mm, being the lowest in the country. There is little variation in temperature throughout the year. The mean monthly temperature ranges from 24.7$^{o}$C in August to 33$^{o}$C in March with annual average temperature of 26.8$^{o}$C. Relative humidity is generally high varying from 65% in the afternoon to about 95% at night

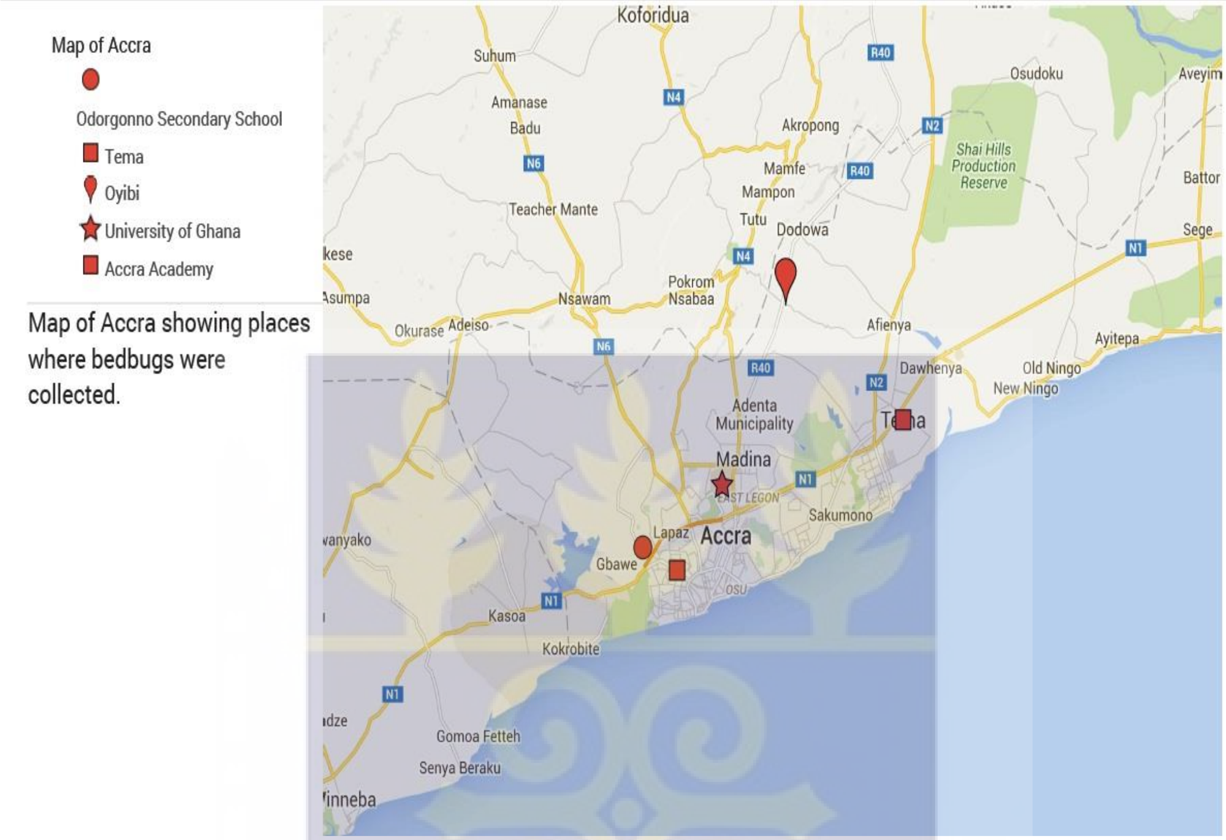


**Figure 3.1:** A map of Ghana showing the study sites in Accra. Regions highlighted red were places where the bedbugs were sampled. Source:

https://www.google.com.gh/maps/@5.7686289,0.0152298,11z (2016)

## 3.2 Study design

A multi-method evaluation study design was used to get information on the presence of bedbugs. The methods included questionnaire administration, observations, quantitative data collection and statistical text assessment. Questionnaires were used to assess the presence of bedbugs in a locality, home and institution, it was also used to seek permission to carry out inspection and collection. During collection, careful observation were made. These were compared with existing data and tested. The multi-method design was used also to investigate the knowledge people have on bedbugs, and the measures they take in the control of bedbugs.

## 3.3 Ethical clearance and study area entry

Ethical clearance was sought and the proposal approved, with permission to conduct the study being granted by the Institutional Review Board of the Noguchi Memorial Institute for Medical Research (IRB-NMIMR), University of Ghana. Introductory letters from the African Regional Postgraduate Programme in Insect Science as well as copies of the ethical clearance certificate from IRB-NMIMR were sent to the heads of the institutions seeking permission to use their premises for sampling.

## 3.4 Descriptive study on bedbug infestations

Prior to the collection of bedbugs on the field, questionnaires were administered to assess people's knowledge and perception on bedbugs as well as the preventive measures taken to control them. This was administered by the questioner-interviewer responds method. Respondents included students and homeowners. A sample of the questionnaire can be found in Appendix II.

## 3.5 Sampling techniques

Bedbugs were collected from 10 geographically distinct locations, from infested structures: rooms, dormitories, mattresses, bed frames, pillows, chairs, blankets, shoes, wardrobes, shirts, trunks and 'chop-boxes'. These were thoroughly inspected for bedbugs infestation. Signs of bedbug infestation were identified by the presence of eggs and empty egg cases, which remained in place after hatching. Black faecal spots and shed exoskeletons on surfaces around harbourage were signs signifying the infestation of bedbugs. Physical handpicking and brushing method was employed with the aid of a flashlight and a pair of forceps. Bedbugs were mostly seen hiding in holes, and crevices especially during the day where they rest and brood. Due to this behaviour, suspected holes and crevices were gently probed with a broom-stick so as not to kill the insects. They were then handpicked as they vacated their holes. Sometimes bedbugs were sited in groups making hand picking difficult; bedbugs would escape in different directions when danger is sensed in a group. A brush was used to brush bedbugs into containers when found in groups. Also the brush was used to brush off eggs glued to their sites into containers. Flashlights were used to improve visibility during inspection. In situations where holes were wide, a pair of forceps are used to gently collect the bedbug. Places from where the bedbugs were collected were noted. The collected bedbugs were then transported in specimen bottles containing 70% alcohol, to the Department of Animal Biology and Conservation Science (University of Ghana) laboratory for further processing.

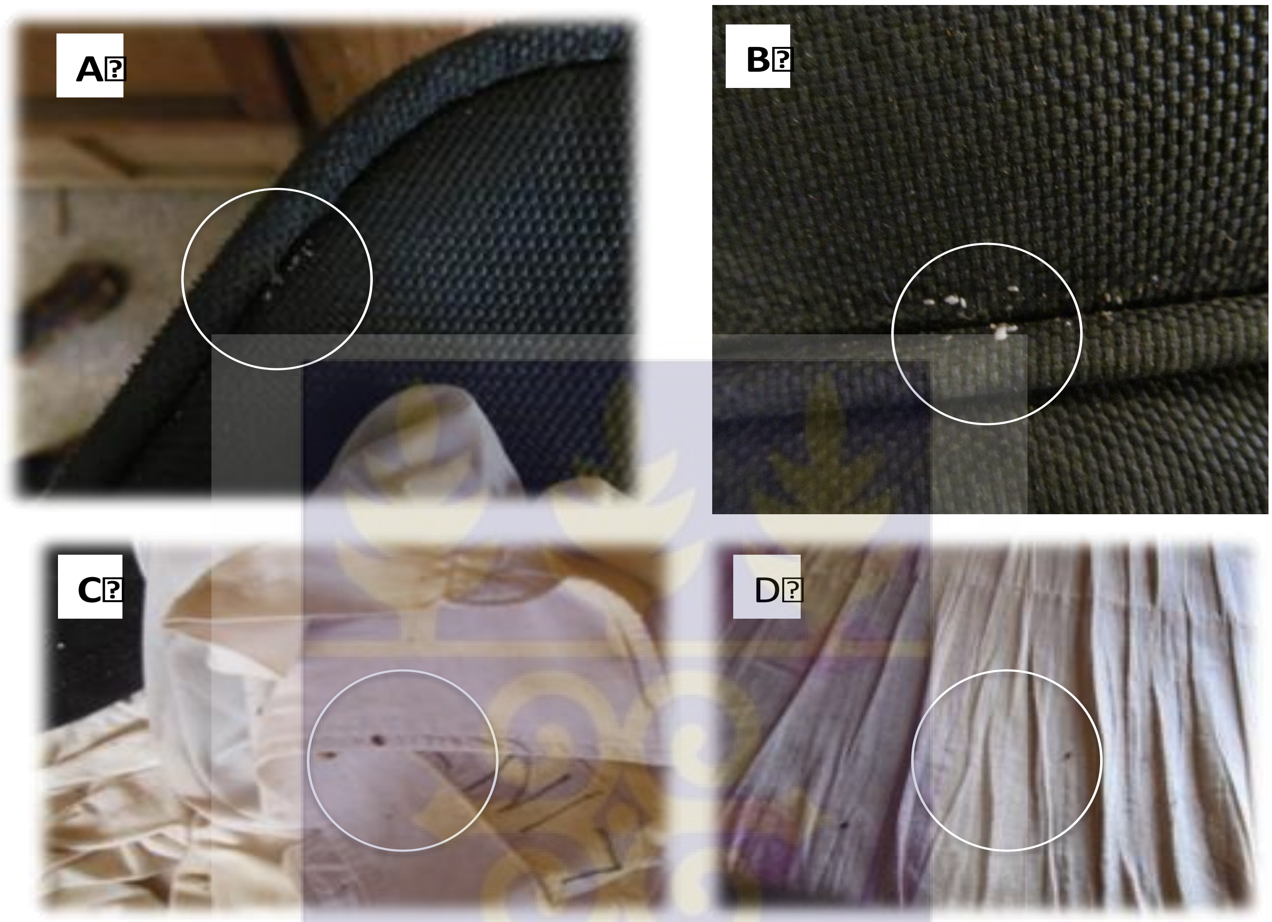


**Figure 3.2:** Sites and materials from where bedbugs were collected in human habitations during the study. White coloured ringed regions showing location of bedbugs and their droppings. A: eggs of bedbugs on a backpack; B: eggs and nymphs of bedbugs on a bag. C: Bedbugs on a shirt. D: Bedbugs on a bedding material.


**Figure 3.3:** Some sites where bedbugs were collected. A: a typical dormitory showing mattress free bed where bedbugs were collected. B: A metal bedframe showing eggs nymphs and adult bedbugs. C: A typical harbourage of bedbugs on a wooden bench

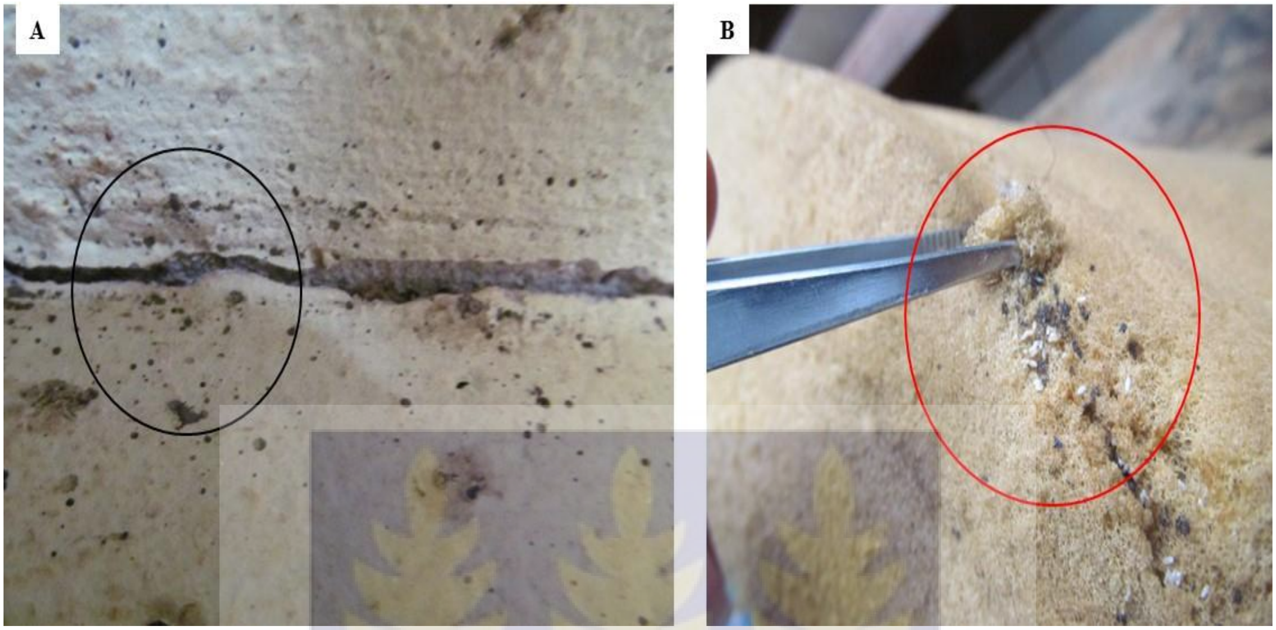


**Figure 3.4:** Typical sites where bedbugs were collected. Ringed regions show areas of bedding material with bedbug eggs and droppings. A: a typical harbourage of bedbugs in a broken wall. B: eggs and droppings of bedbugs in a mattress.

### 3.6 Morphological identification of bedbugs

Identification and sex determination was done using bedbug morphological keys provided by Usinger (1966). The physical characteristics described in Section 2.8, were used to characterise the bedbugs with the aid of a dissecting microscope LECIA ICC50 HD at 40x magnification. Morphometric data, in the form of measurements of body parts, were taken on the head, pronotum, hair on pronotum femur and antennae. These body parts were selected because they are features that have previously been used to distinguish one bedbug species from another (Usinger, 1966).

## 3.7 Molecular identification of bedbugs

Each bedbug sample was homogenized in a lysis agent with a sterile plastic pestle in an Eppendorf tube, after which the DNA was amplified for identification using, cythochrome oxidase I (COI) and rDNA 1Lep primers.

### 3.7.1 DNA extraction

Genomic DNA was extracted using kit from Zymo Research DNA extraction according to the manufacturer's instructions. To a tissue sample in a microcentrifuge tube, solutions of 95 µl distilled water, 95 µl 2x digestion buffer and 10 µl proteinase K was added. This was mixed and incubated at 55 $^{o}$C for 3 hours. Seven hundred microliters of Genomic Lysis Buffer was added to the tube and mixed thoroughly by vortexing, it was then centrifuged at 10000 x g for one minute. The supernatant was transferred into a Zymo-Spin IIC column in a collection tube and centrifuged at 10000 x g for one minute. Two hundred microliters of DNA Pre-wash Buffer was added to the spin column in a new collection tube centrifuge at 10000 x g for one minute. Four hundred microliters of g-DNA Wash Buffer was added to the spin column, it was centrifuged at 10000 x g for one minute. The spin column was transferred to a clean micro-centrifuge. Hundred microliters of DNA elution buffer was added to the spin column. This was incubated at room temperature for 5 minutes, it was then centrifuge at top speed for 30 seconds to elute the DNA. The extracted DNA samples were kept at -20 $^{o}$C for long term storage.

### 3.7.2 Genomic DNA amplification

**Table 3.1:** Sequence of primers used in PCR-RFLP for bedbug species molecular characterization

| Primer | Sequence (5´ to 3´) | Tm min\max (ºC) |
|---|---|---|
| 3BB-COI-F | AACTTAGACAAACCTGGCTCA | 58.66\58.66 |
| 4BB-COI-R2 | GTGTTGGTAAAGTACAGGATCTY | 59.2\60.99 |
| 1LepF | ATTCAACCAATCATAAAGATATNGG | 56.38\58.02 |
| 2LepR | TAWACTTCWGGRTGTCCRAARAATCA | 58.28\63.02 |

PCR was performed in 25 ml volumes containing: 5.0 µl of 5 × PCR Buffer, 1 µl of 25 mM $MgCl_2$, 0.5 µl of 10 mM dNTPs, 1 µl of 5 µM of each primer, 0.5U Taq DNA polymerase (Zymo Research), 3.0 µl each of DNA template, and 13 µl $ddH_2O$ to make 25 ml. The PCR cycling conditions were composed of an initial denaturation stage of 5 min at 95 ºC, followed by 35 cycles each consisting 1 min at 95 ºC, 1 min at 58 ºC, and 1min at 72 ºC. This was followed by a final extension stage of 72 ºC for 5 min.

### 3.7.3 Gel Electrophoresis

The PCR products were electrophoresed in 2% agarose gel dissolved in 1x TAE buffer with voltage set at 100v for 120 min after which the gel was soaked in Ethidium bromide for 30 minutes. The gel was then placed in an imaging device to view the bands of the DNA. The bands shows the separation of the DNA at the various molecular size.

### 3.8 Assessment of the effect of temperature and humidity on bedbug survival

The setup involved three conditions in monitoring daily mortality, the conditions were; outside temperatures, room temperatures and a constant temperature and humidity of 33 ± 5 °C and 70 ± 5 % respectively. Four holes of 6.5cm diameter measuring were made on the sides of the container, two holes at both sides. A hole of the same diameter was made in another container, this container was for the constant temperature setup, and all these holes were made in a 7 L container. Each container had in it four petri-dishes, each petri-dish contained five adult blood fed bedbugs of approximately equal size. The petri-dishes were loosely covered. The setup for measuring constant temperature and humidity had a 14 g cotton moistened with 150 mL of water placed on the hole. This was placed in an incubator with the temperature set to 33 °C. The incubator was monitored regularly for constant temperature and humidity, the moistened cotton is replaced when there is a rise in temperature and or humidity. The control experiment setup was kept outside in the open in front of the parasitology laboratory of the department of animal biology and conservation sciences (DABCS). Another setup was kept inside the laboratory. Data was taken after every six hours.

### 3.9 Blood feeding of bedbugs using laboratory rats

An adult Sprague dawley rat was used to provide blood meal for the bedbugs. The rat was anesthetized using a cotton soaked 15 mL chlorophorm; this made the rat docile and handling easier. With the use of a shaving stick, the rat had it abdomen shaved, it was then placed on a crucifix and held firmly to it by sticking it with a tape. Bedbugs were placed in a container covered with a cheese cloth, a slice of styrofoam was placed in the container, this is to provide a medium for the bedbugs to travel to the top of the container. The cheese cloth was tightened with a rubber band which held the cheese cloth tightly to the opening of the container. The container was then placed on the ventral side of the rat and left there overnight. Leaving it there overnight was to ensure that all the bedbugs would have access to the rat for a blood meal.

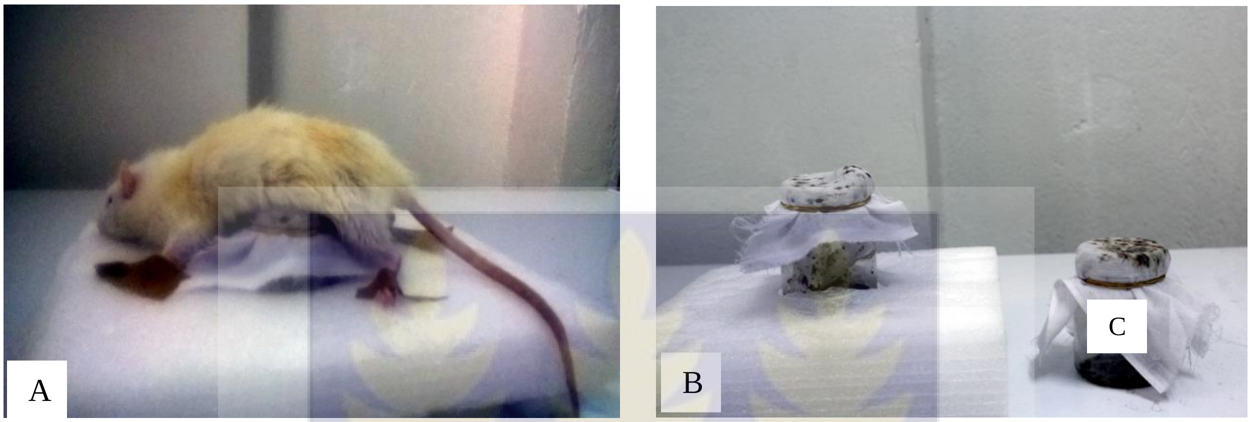


**Figure 3.5**: A setup of blood feeding of bedbugs using a rat. B: A receptacle containing bedbugs fixed into a Styrofoam crucifix. C: A container for holding bedbugs.

# CHAPTER FOUR

## RESULTS

### 4.1 Morphological description of adult bedbugs

Out of the 335 bedbugs collected, 230 (69%) were morphologically identified as adults, with 105 (31%) being nymphs. For the purposes of this study, only adult bedbugs were used, since characterization of nymphs is rather a challenging task. Out of the 230 adults, 217 (94.4%) were identified as *C. hemipterus*, 9 (3.9%) as *C. lectularius* and 4 (1.7%) as *C. pipistrelli*. One hundred and thirty-two (57.4%) of the total number of adult bedbugs used in the study were males and 98 (42.6%) were females.

**Table 4.1**: Species composition and abundance of adult bedbugs per study site during the study

| Species | Tema | Legon | Odorgonor | Kaneshie | Oyibi | Total | % Quantity |
|---|---|---|---|---|---|---|---|
| *Cimex hemipterus* | 7 | 180 | 8 | 10 | 12 | 217 | 94.4 |
| *Cimex lectularius* | 0 | 9 | 0 | 0 | 0 | 9 | 3.9 |
| *Cimex pipistrelli* | 4 | 0 | 0 | 0 | 0 | 4 | 1.7 |
| Total | 11 | 189 | 8 | 10 | 12 | 230 | 100 |

Out of the 230 adult species used for the study, *C. lectularius* was dominant in Legon, while *C. pipistrelli*, was only collected from Tema. *Cimex hemipterus* was identified among the collections from all the study sites (Table 4.1

**Table 4.2**: Number and proportion of male and female adult bedbugs used in the study

| Sex | Quantity | Percentage Quantity ( % ) |
|---|---|---|
| Male | 132 | 57.4 |
| Female | 98 | 42.6 |
| Total | 230 | 100 |

The bedbugs were oval shaped and flattened dorso-ventrally. When unfed, they look pale yellow to brown in colour; they become reddish brown after a blood meal. The average length of the adult bedbug measured 5.5 mm and the average width measured 2.5 mm (Figure 4.1).

**Figure 4.1**: A bedbug placed beside a rule

The head of the bedbug is broad and pointed at the tip having a pair of prominent compound eyes. The antennae is inserted into the head close to the eye, making it look as if the antennae, is projecting from the eye. Compound eyes were oval, black and multifaceted. The antenna is divided into four segments; the first segment being shorter than the other three segments; the 3rd and 4th segments are more slender and transparent than the first and second segments. There are fine hair like structures on all of the four segments of the antennae. Fine hairs could also be located on the head except the position of compound eyes and antennae (Figure 4.2).

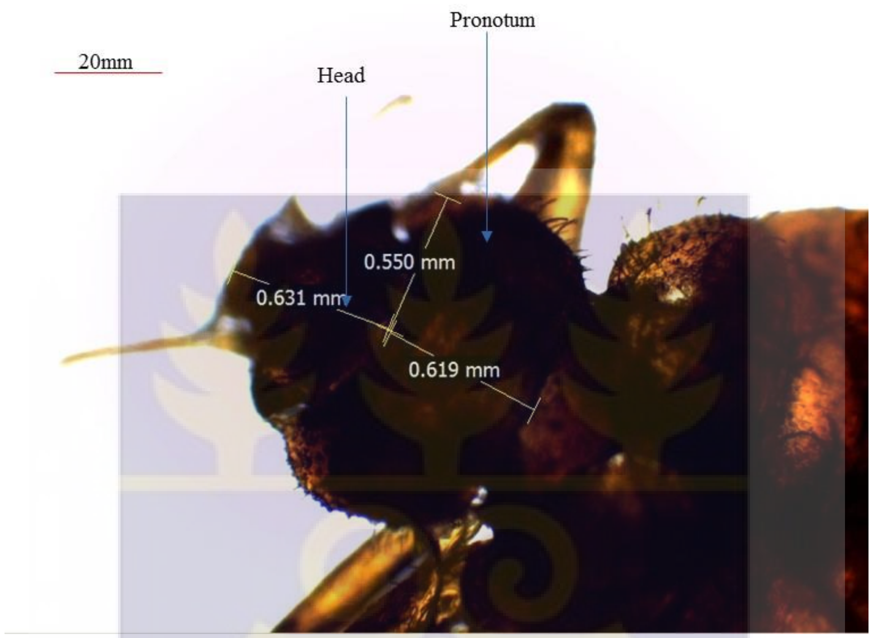


**Figure 4.2**: Anterior part of an adult bedbug showing morphometric measurements, particularly the length (0.619 mm) and width (0.550mm) of the pronotum, and the length (0.631 mm) of the head.

The mouth parts are piercing and sucking type located on the ventral side of the head. These consist of a triangular labrum, a long 3-segmented labium reaching almost the base of the prothorax, paired mandible and maxillary stylets, which were blade like, modified for piercing its' host and sucking (Morris, 1960) as shown in figure 4.3.


**Figure 4.3**: Typical head region of the bedbug showing the piercing and sucking mouthpart

The thorax is three segmented. The prothorax is much larger than the mesothorax and the metathorax, and has a distinct wing like expansion. The mesothorax is a triangular fold and the metathorax, looked more crescent shaped. The prothorax is generally twice as wide as its length. Fine hairs were found on the boarder of the prothorax. Each thoracic segment contained a pair of jointed walking legs, there were thus three pairs of legs. Each leg consisted of a linear series of segments, namely coxa, trochanter, femur, tibia and tarsus. The coxa was firm, flattened and short which connecting the leg to the thorax. The trochanter, was a small triangular structure fused to the femur, it was a broader, tubular and turgid. The tibia was slender and longer than the other leg segments. The tarsus was divided into three segments, two of the segments looked more or less equal in size; the third segment was longer. Two claws were at the tip of the tarsus. Femur and tibia were covered with fine spines called setae (Figure 4.4).

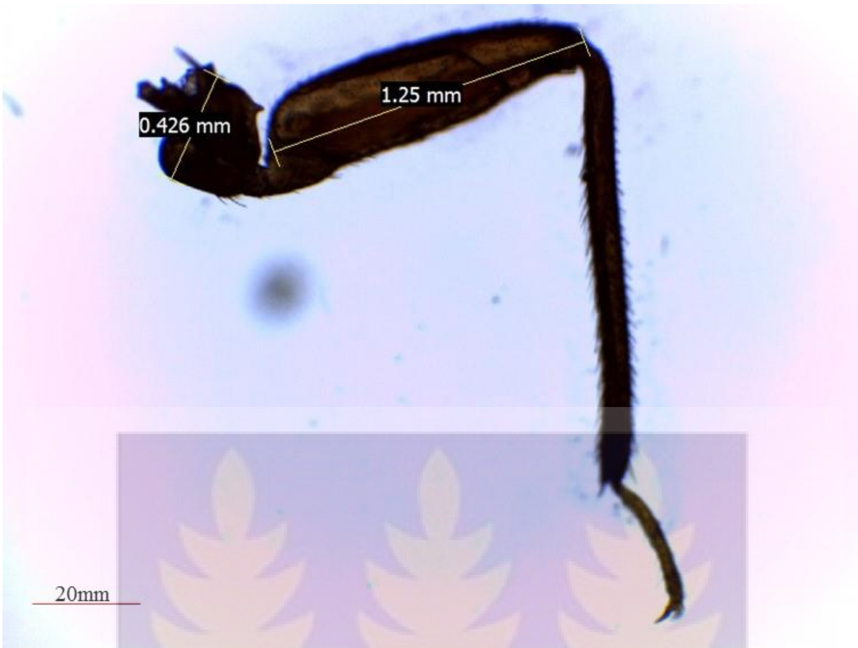


**Figure 4.4**: A typical hind leg of a bedbug showing measurements of the coxa width (0.426 mm) and femur length (1.25 mm); other parts being displayed are the tibia, tarsus and meta-tarsus.

On the ventral side of the third thoracic somite is a pair of glands. These glands produce an oily secretion that is thought to be a defence mechanism against predators. The abdomen was eight segmented. The 1st and 2nd abdominal segments were fused together. In the adult male bed bug, the abdomen was narrower and its tip was curved and slightly more pointed than in the female. An aedeagus was seen ventrally at the tip of the male abdomen curved to the left. In the adult female, the abdomen was broad and rounded (Figure 4.5).

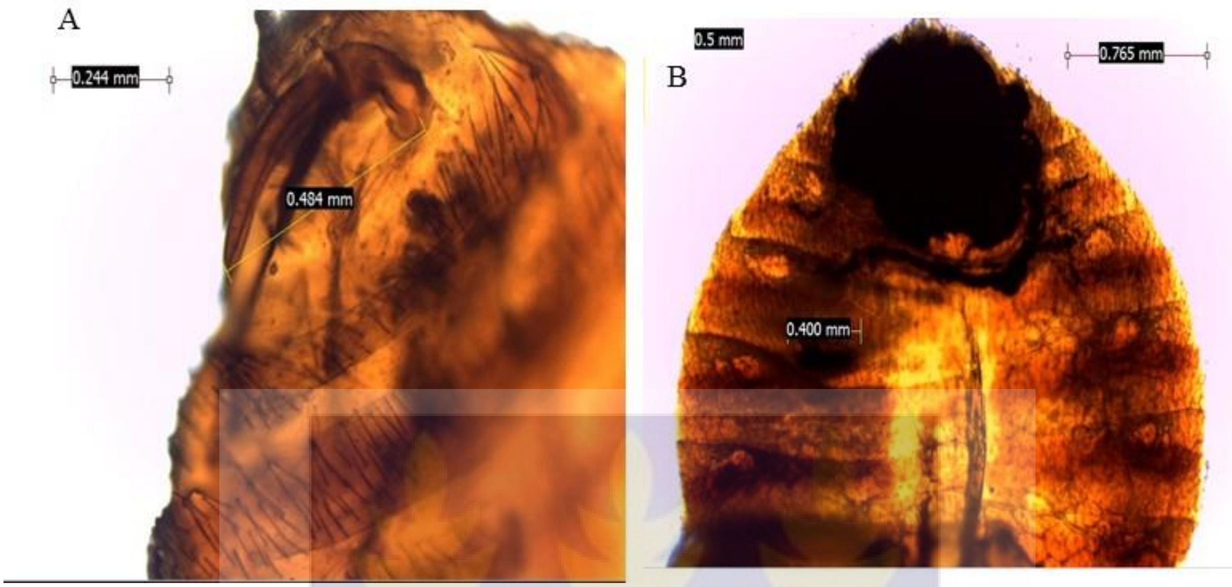


**Figure 4.5**: A typical abdominal portion of the bedbug. A: the male abdomen showing measurements of the length of the aedeagus (0.404 mm). A: B: the female abdomen showing measurements of the organ of Berlese (0.400 mm).

A small incision was seen on the left side of the 4th abdominal segment of the female when viewed ventrally (Figure 4.5). The incision is the opening of a blind copulatory pouch known as the organ of Berlese. The whole abdomen was covered with small hairs on both the dorsal and ventral sides. A cluster of relatively longer hairs was seen at the tip of the abdomen of both the male and female bed bugs.

**Table 4.3**: Measurements of some characteristic features of three *Cimex* species examined in the study, represented is the mean and the standard deviation shown in brackets

| Species | FW | FL | HL | PTW | PTL | PHL | 1A | 2A | 3A | 4A |
|---|---|---|---|---|---|---|---|---|---|---|
| *Cimex. pipistreli* | 0.29 (±0.007) | 1.21 (±0.031) | 0.59 (±0.007) | 0.51 (±0.015) | 0.55 (±0.016) | 0.16 (±0.011) | 0.19 (±0.004) | 0.59 (±0.019) | 0.57 (±0.011) | 0.49 (±0.013) |
| *Cimex. lectularius* | 0.35 (±0.015) | 1.29 (±0.024) | 0.69 (±0.012) | 0.61 (±0.017) | 0.60 (±0.028) | 0.15 (±0.012) | 0.21 (±0.008) | 0.65 (±0.007) | 0.61 (±0.008) | 0.53 (±0.032) |
| *Cimex. hemipterus* | 0.36 (±0.007) | 1.26 (±0.015) | 0.62 (±0.012) | 0.57 (±0.025) | 0.54 (±0.004) | 0.15 (±0.008) | 2.36 (±1.446) | 0.64 (±0.019) | 0.61 (±0.040) | 0.43 (±0.037) |
| LSD ($P \leq 0.05$) | 0.02 | 0.07 NS | 0.03 | 0.05 | 0.05 | 0.02 | 2.42 NS | 0.04 | 0.07 NS | 0.08 NS |

FW = Fermur width, FL = Fermur length, HL = Head length, PTW = Pronotum width, PTL = Pronotum length, PHL = length of hair on pronotum, 1A = first antennal segment, 2A = second antennal segment, 3A = third antennal segment, 4A = fourth antennal segment LSD = Least Significant Difference.

Morphometric measurements of some distinctive morphological features of some parts of the bedbugs showed significant differences between the species. There was however, some parts that did not show any significant differences between the three species. Following the Least Significant Difference (LSD) post-hoc analyses of the morphometric data, the width of the femur, head length, width of pronotum and the second antennal segment all showed significant differences between the three *Cimex* species. The length of femur, length of the pronotum, the first antennal segment, the third antennal segment and the fourth antennal segment did not show any significant difference (Table 4.4).

### 4.2 Molecular identification of bed bugs

Of the 230 adult bedbug samples obtained from the six locations in Greater Accra, 88 were used in the genome analysis with 80 exhibiting positive COI amplicons. Amplicons of the COI gene from *C. hemipterus*, *C. lectularius* and *C. pipistrelli* were approximately 420 bp in size.

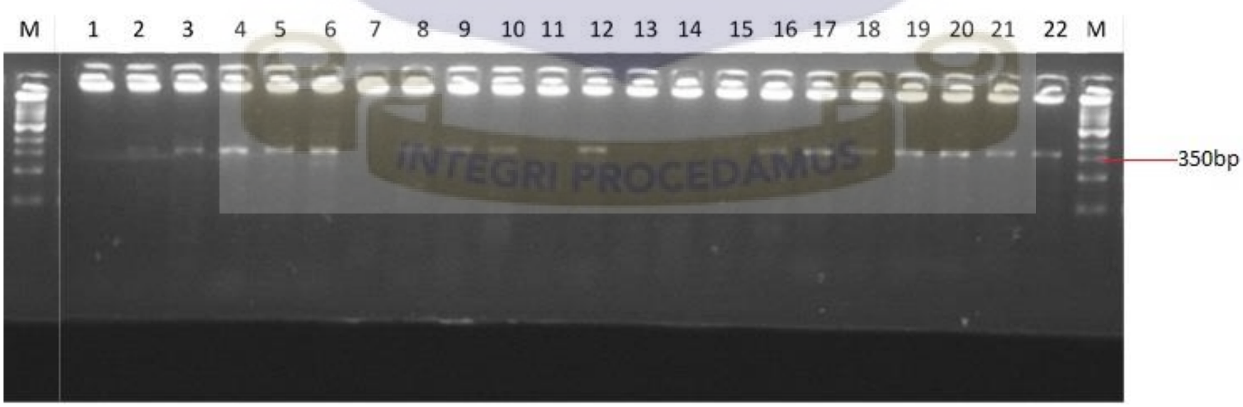


**Figure 4.6**: Ethidium bromide stained 2.0 % agarose gel electrophoregram of PCR amplified DNA of *Cimex* species. Lane M: 100 bp ladder; lane 1 -2: amplified DNA of *Cimex* species.

### 4.2.1 Identification of *Cimex* Species Molecular Forms

DNA samples were eluted from 88 bedbugs, these DNA samples were further analyzed by PCR-RFLP to determine their molecular forms. The COI primers amplifed 80 DNA out of the 88 DNA extract used. All showed band sizes at 420 bp except for DNA extract of *C. lectularius* which showed multiple band size at 420 bp and 250 bp. The 1Lep primers, amplified 24 bedbug DNA extracts out of the 88 bedbug DNA extracts used. Identification was done by comparing their band sizes with that of the molecular wight 100 bp marker (Figure 4.6).

## 4.3 Influence of temperature and relative humidity on the survival of bedbugs

Bedbugs kept in the open were exposed to the outside temperature and humidity, with an average maximum temperature of 28.9 $^{o}$C, average minimum temperature of 26.4$^{o}$C, average maximum humidity of 77.4% and average minimum humidiy of 70%, 2.5 % of the bedbugs died after within 6 hours, 10 % mortality was recorded after 18 hours. Bedbugs kept under room conditions, recorded 5% mortality after 12 hours within 18 hours, under an average maximum temperature of 27.6 $^{o}$C and at an average minimum temperature of 26.5 $^{o}$C, the average maximum relative humidity was recorded at 77% and the minimum average relative humidity was at 74%. Bedbugs that were exposed to temperatures of 33 ± 5$^{o}$C and relative humidity (RH) of 70 ± 5 %, after twelve (12) hours recorded, 65 % mortality. At the same temperature for eighteen hours, the mortality was at ninety-five (95) %.

**Table 4.6:** The number of hours within which bedbugs died at a given temperature and relative humidity over a 72 hour period

| Time (hours) | RH% of control setup | Temp. of control setup | Mortality of bedbugs placed under control setup | RH% of Room setup | Temp. of Room setup | Mortality of bedbugs placed under in room setup | RH% in incubator | Temp. in Incubator | Mortality of bedbugs placed in incubator |
|---|---|---|---|---|---|---|---|---|---|
| 0-6 | 65.9 | 27.8 | 0 | 78.9 | 26 | 0 | 70±5 | 30±5 | 0 |
| 6-12 | 74.75 | 27.5 | 1 | 77.5 | 26.5 | 0 | 70±5 | 30±5 | 26 |
| 12-18 | 73.65 | 26.5 | 0 | 75.65 | 27.55 | 0 | 70±5 | 30±5 | 12 |
| 18-24 | 68.15 | 3.5 | 4 | 74.25 | 27.6 | 1 | 70±5 | 30±5 | 0 |
| 24-30 | 75.1 | 25.9 | 0 | 74.85 | 28.2 | 0 | 70±5 | 30±5 | 0 |
| 30-36 | 77.4 | 26.85 | 0 | 75.75 | 27.15 | 0 | 70±5 | 30±5 | 0 |
| 36-42 | 68.85 | 25.15 | 0 | 73.7 | 26.4 | 0 | 70±5 | 30±5 | 0 |
| 42-48 | 67.8 | 29.6 | 0 | 75.85 | 27.55 | 0 | 70±5 | 30±5 | 0 |
| 48-54 | 79.65 | 26.15 | 0 | 73.6 | 27.55 | 0 | 70±5 | 30±5 | 0 |
| 54-60 | 73.75 | 28.4 | 0 | 72.9 | 26.1 | 0 | 70±5 | 30±5 | 0 |
| 60-66 | 78.75 | 28.25 | 0 | 76.9 | 27.2 | 0 | 70±5 | 30±5 | 0 |
| 66-72 | 75.5 | 27.9 | 0 | 75.35 | 26.8 | 0 | 70±5 | 30±5 | 0 |
| 72 | 79.5 | 29.5 | 0 | 77.4 | 27.55 | 0 | 70±5 | 30±5 | 0 |

### 4.4 Descriptive study on bedbug infestation

Sixty questionnaires were administered in total of which 35 (58%) were females and (42%) were males (Figure 4.7). Fifty individuals (83%) were students from educational institutions and ten (17%) were home owners.

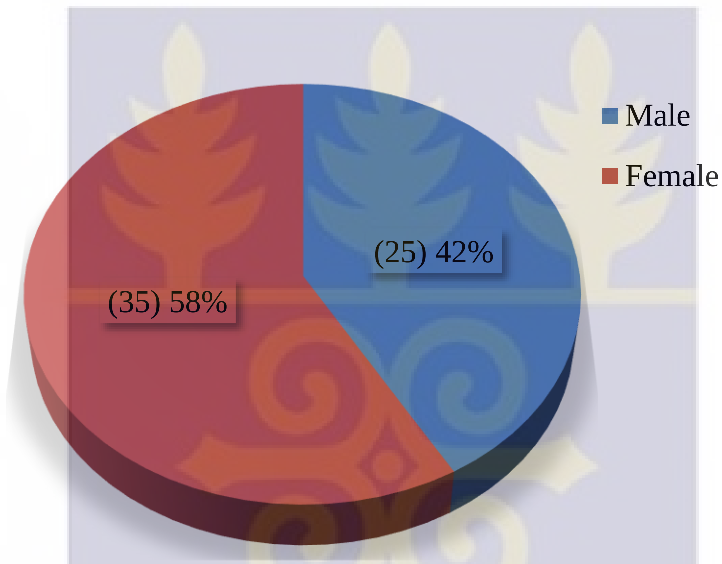


**Figure 4.7:** Gender composition of respondents to the administered questionnaires during the study

About seven per cent (6.7%) of the respondents had Junior high school education, 73.3% had senior high school education and 20% had access to tertiary education.

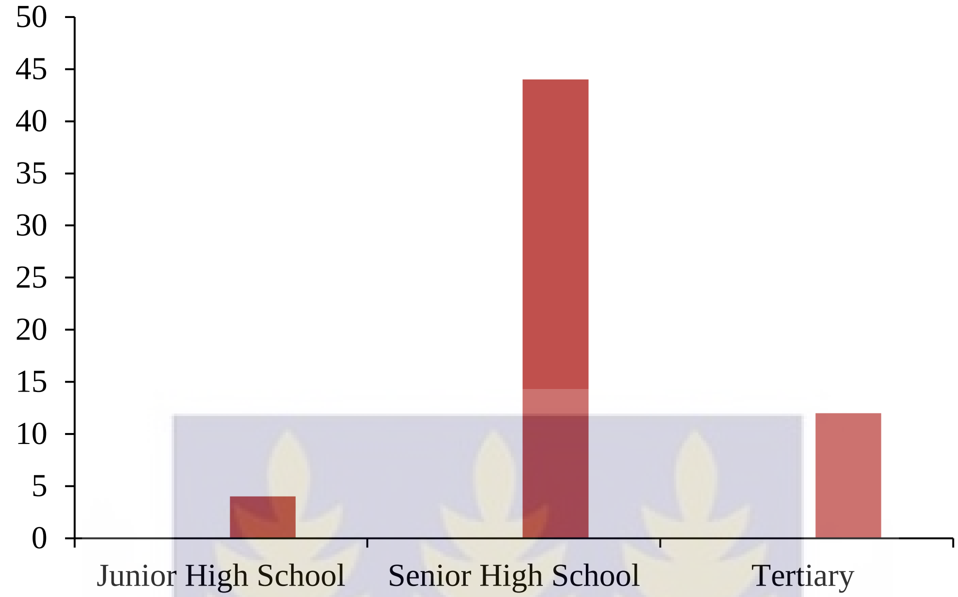


**Figure 4.8:** Level of education of respondents in the questionnaire study

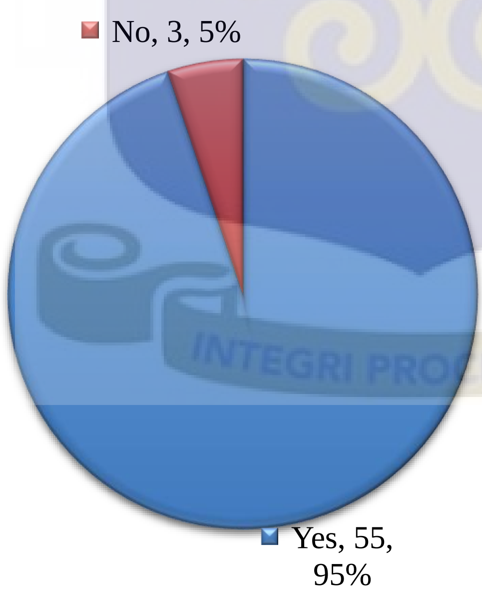


**Figure 4.9:** Percentage of respondents who exhibited knowledge of bedbugs

Fifty-five of respondents had knowledge on bedbugs and have seen one before. Ninety-eight percent of the respondents agreed they needed help in controlling bedbugs (Figure 4.9), effort to exterminate bedbugs in their rooms has proven futile. All of the respondents uses, one of the various insecticides sold on the market to help in controlling insects found in their rooms including bedbugs.

**Table 4.5**: Insecticides sold under various trade name and their active ingredients.

| Trade name | Active Ingredients |
|---|---|
| Sasso | 60% Propane\n-butane, 0.20% Tetramethrin, 1.15% Permathrin |
| Killit | 0.135% d-Tetramethrin, 0.06% d-Allethrin, 0.45% Cypermethrin |
| Heaven | 60% Propane\n-butane, 0.16% Bioallethrin, 0.06% Permathrin |
| Raid | Imiprothrin, Prallethrin, Cyfluthrin |
| ORO | 0.20% Tetramethrin, 0.25% Permathrin, 0.01% d-fenothrin |
| Dursban | Chloripyrofos |
| Trigger | Lamda Cyhalothrin |


**Figure 4.10:** Depiction of some commercial household insecticides used by correspondents.

Their active ingredients are displayed in Table 4.5.

**Figure 4.11**: Some commercial insecticides and how it is used by some institutions to control bedbugs. A: Chlorpyrifos sold under the trade name Dursban; B: Trigger, with cyhalothrin as the active ingredient. C: knapsack sprayer used in spraying.

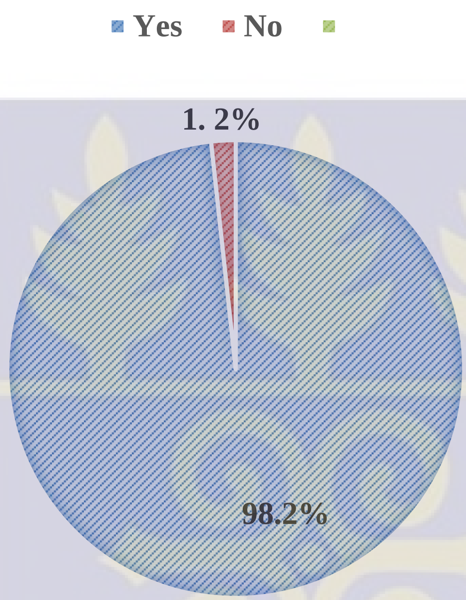


**Figure 4.12:** Proportion of respondents needing support in their efforts to control bedbugs

The results showed that all the respondents shared their room with others, either a friend or a family member. These respondents also visited other people, sometimes spending days with a friend or a family member.

All the respondents reported that they do see insects in their room. Some insects seen by some of the correspondents were cockroaches, ants, spiders, mosquitoes and bedbugs. About 91% of the respondents reported they get bitten by insects' every day from mostly mosquitoes, ants and bedbugs. Bites are received on arms, legs, back and around the neck. In their quest to control these insects in their rooms, the use of conventional insecticides were employed. The various insecticides used are sold under the brand names; Sasso, Killit, Heaven and Raid amongst others (Table 4.1). Other respondents also employed the use of nonconventional insecticides or synthetic chemicals such as kerosene, turpentine and Camphor.

# CHAPTER 5

# 5.0 DISCUSSION

Three species of Cimicidae were morphologically identified and genetically characterized from among human dwellings in southern Ghana. Of the three species identified, one was *C. hemipterus. Cimex hemipterus* is distributed broadly throughout the tropical and sub-tropical regions of the world. *Cimex lectularius*, the common bedbug, is widely distributed in the temperate and sub-tropical regions, both north and south of the equator (Bonnefoy *et al.*, 2008); this species has been confirmed in Ghana by this study. *Cimex pipistrelli*, an ectoparasite of bats (African Chiroptera Report, 2014), was for the first time identified in this study to be present in human residence. Although the presence of pipistrelle bats in Africa is in contention, Bedford (1936) found *C. pipistrelli* while sampling for mammalian ectoparasite in Cape Town, South Africa. In South Africa, sightings of pipistrelle bats have been reported in (Benda *et al.*, 2004) Furthermore, during the periods where temperatures drop in the temperate zones, bats migrates hundreds' of kilometres to warmer areas (Denny, 2003). It is therefore possible that the presence of *C. pipistrelle* in Ghana could have resulted from migrant pipistrelle bats visiting Ghana with them, or these bats coming into contact with other more native bat species thereby transferring the ectoparasites to them. During migration, bats travel through caves, tunnels, mines and underground sites (Fenton, 2012). With increasing interest in tourism and travelling, it is possible that there has been some habitat invasion either by the bats or humans, leading to the transfer of these particular bedbugs from bats to humans.

*Cimex hemipterus* was the predominant bedbug species sampled followed by *C. lectularius* and *C. pipistrelli.* Bats are the original and most common hosts of members of the family *Cimicidae* (Balvin *et al.*, 2012). *Cimex pipistrelli* was one of the species of bat ectoparasites identified from the samples collected. The presence of a bat bug among human dwellings could be attributed to several factors. These factors could include, but not limited to the rise in travel and tourism. Someone may have visited a place where bats brood, such as a cave or a forest area (Denny, 2003; Balvin *et al.*, 2012). Eggs of bedbugs have sticky substances that allow them to adhere to surfaces without falling off (Dogget *et al.*, 2012). This allows the eggs to be transferred from one location to another with much ease and could have contributed to their spread in this instance.

Bat habitat loss due to increasing population and forest degradation could also be a factor in the increasing spread of bedbug infestations; the Ghanaian population is increasing at rate of 2.7 % with a current population of 24.2 million (National Population Council, 2011). This increase in population has resulted in the competition for space between human, plants and animals. More of the woodland and forest areas are being cleared for farming, housing and industrialization leading to loss of habitat for some bat species (Stow *et al.*, 2013). These acts of clearing the forest for human activities has probably sent man closer to the animals and vice versa as their habitats overlap, coupled with competition for resources. Bats losing their habitats and or food source would have to fly long distances in search of food and or shelter, this situation could lead to the dispersal of bat bedbugs into human dwellings, which may have fallen off during flight or feeding. With the emerging public health concerns relating to bats as reservoirs of zoonotic diseases (Calisher *et al.*, 2006; O'shea *et al.*, 2014) and the potential of bedbugs as vectors of some of these diseases, the presence of this particular bedbug species (*C. pipistrelli*) in human dwellings call for peculiar attention as well as further investigation.

*Cimex lectularius* are bedbugs of the temperate regions, mostly found in the Americas and the European countries, adapted to cold areas. From this study, *C. lectularius* was identified in Ghana. Trade in used furniture especially beds, mattresses and chairs, in many markets in Ghana could also be facilitating the transfer of these bedbugs from the temperate regions of the world to our local communities. Most of these second hand furniture come into the market from different places including the European countries and other African countries; these countries have their own share of bedbug problems (Dogget *et al*., 2012). This could be one of the possible media through which the bedbug, *C. lectularius* was introduced into Ghana. In addition to this, there are people who travel outside for business and tourism who could serve as avenues for the passive dispersal of bedbugs from other countries, in this situation the temperate countries into Ghana.

The study confirms the presence of bedbugs in Ghana, primarily found in some senior high schools and tertiary educational institutions in Ghana. This is rather disturbing since it implies students in such institutions would have poor quality of sleep, coupled with its attendant negative effect on teaching and learning, as well as the general wellbeing of students. The actual cause of this sudden surge in bedbug infestations in these places is not yet known. But this could be attributed to a multitude of factors, including resistant to pyrethroid base insecticides by these bedbugs. Bedbugs, due to their haemtophagous feeding habit are believed to have coevolved with one of their primary host, humans (Warnet, 2015). Hence they are closely associated with human habitation, particularly those housing dense populations with standard hygienic conditions, such as boarding house accommodation facility. Furthermore, the congestion and relatively poor hygienic conditions observed in the dormitories, especially in some of the senior high schools, provides ideal hiding and breeding places for these bedbugs to thrive. According to Omudu and Kuse (2010), the rise in bedbug infestation in Nigeria was partly due to overcrowding and deteriorating sanitary conditions within apartments. Unkempt rooms and congestion facilitates the spread of ectoparasites, including bedbugs. This could explain in part the sudden surge in bedbug infestation in the educational institutions.

Most of the wooden bedframes encountered during the study, were very old and majority of them had broken parts and holes in them. These holes provide breeding and hiding places for the bedbugs. Also some of the mattresses were rather very old, with holes and torn parts in them facilitating bedbug habitation. According to United State of America (U.S.A) department of defence (2012), bedbugs and their eggs are unknowingly carried in or on pieces of furniture and luggage. In addition to this, human migration could be a major contributory factor, to the levels of infestation being observed in the second and third cycle institution. The cyclical nature of vacations and reopening of schools, means students travel frequently to and from various parts of the country, and possibly other countries. The activity of human, traveling from one place to the other can be a passive way of transferring a bedbug or its egg from one infested place to another that may not have recorded any incidence of bedbugs.

The collection of bedbugs was done from rooms, which serve as sleeping places for people, with most of the bed frames made of wood and a few metal frames. Majority of the samples were collected from rooms with wooden bed frame as compared to rooms with metal bed frames. However both materials served as a nest for eggs and hiding places for nymphs and adult bedbugs. Contrary to the current finding, Potter (2011) had recommended the use of metal framed beds over beds with wooden frames as a means of controlling bed bugs. Omudu (2010) attributed high bedbug infestation to poor hygiene and housekeeping practices. This was evident in this study, and the site with the highest infestation of bedbugs had poor hygiene and housekeeping practices. The rooms were stuffed with dirty clothing and wooden boxes and broken walls, however, this is not to refute the fact that some clean and relatively spacious rooms also harboured bedbugs.

Prior to sample collection, it was made known to our team that these institutions in their quest to control the bedbugs sprayed the dormitories at the end of every academic year, yet students complain of bedbug bites. Investigations revealed that most of the insecticides used for fumigation in these institutions contained formulations of D-Tetramethrin, D-Allethrin, Propane, Butane, Cypermethrin, Lamda-cyhalothrin, Cyfluthrin and Phenothrin as active ingredients in conformity with the report of Davies *et al*. (2011). In some situations, students resorted to the use of kerosene to control bedbugs, which was relatively effective (student personal communication).

These insecticides were effective only against the adults and nymphs but not the eggs as evidenced by bedbug bites and wheals seen on skin few weeks after insecticide application. Unlike the resistance of bedbugs to pyrethroids in the U.S. A. (Romero *et al*., 2007; Moore and Miller, 2009), the use of pyrethroids is seemingly an effective way of controlling adult bedbugs in Ghana, as shown by the number of dead bugs seen minutes after application. Also most of the insecticide sold on the market have pyrethroids as active ingredients. Eggs of bedbugs collected after insecticide application kept in the laboratory gave rise to nymphs one to two weeks after insecticide application. This suggests that the insecticides had no effect on the eggs of bedbugs but was good for the control of nymphs and adults.

Metal bed frame do not prevent the incidence of bedbug infestation. Bedbugs were collected from rooms furnished with metal-framed beds, with these bedbugs seen around and in loosed screws, screw holes and other open holes within the metal frames. Whereas Omudu (2010) attributed the infestation of bedbugs in Nigeria to poor hygiene and housekeeping practices, bedbugs in this present study were seen and collected in rooms that were well kept and newly occupied. Bedbugs can be transferred from one person to the other through the carrying of an egg or adult infested material from one place to the other. When an egg or an egg laying adult is introduced in a new place a colony can be established and when not detected early there can be an invasion. That notwithstanding, poor unhygienic conditions and overcrowded places influences the rapid multiplication of bedbugs as crowded and unhygienic places provide ideal places of shelter for bedbugs to hide and come out at night to feed.

The trade in second-hand household furniture and electronic goods has reached an all-time high in many sub-Saharan African countries. This could continue to facilitate the spread of bedbugs or their eggs over long distances. The common practice of moving infested household materials outdoors in order to expose them to sunlight has been reported to have little or no impact on bedbugs because they are quick to hide away from sunlight into dark protected areas (Olson *et al.*, 2013). This was witnessed in one of the Senior High Schools where the bedbugs were seen hiding in the other half of the mattress as the other surface was exposed to sunlight. The use of insecticides to control bedbugs could have severe public health consequences either by increasing human exposure to indoor pesticide residue or increase the levels of resistance in bedbugs to pyrethroid insecticides resulting from ineffective application techniques (Durand *et al*., 2012).

Bedbugs have setae on their legs which allows them to climb and hold on to surfaces without falling off (Usinger, 1966), an infested clothing, shoe, books or bags (Figure 3.2) can provide means of transportation and dispersal of bedbugs as bedbugs can cling onto these materials or hide in them.

Usinger (1966), revealed that species of bedbugs can be distinguished by examining the first segment of the thorax, which expands more laterally and of which the extreme margins are more flattened *in C. lectularius* than *C. hemipterus*. These features are difficult to use in bedbug identification since it requires a great deal of expertise to carefully use these features to distinguish one species from the other. Therefore, taxonomic identification is important with necessary specialized taxonomic expertise. Usinger (1966) further mentioned the use of the femur, the length and width of the femur to help in delineating bedbug species. From the current analyses, femur width significantly differed between the species (Table 4.5) and could be used as a characteristic feature for morphological species identification.

Molecular techniques are commonly used in research laboratories worldwide for species identification; these include gene sequencing, phylogenetic tree analysis and PCR-RFLP, which helps to reliably and practically distinguish one species from the other. This study used DNA-based identification techniques, employing the cytochrome oxidase I gene (*COI*) and Lep genes for molecular differentiation of bedbugs. Cytochrome oxidase I (*COI*) is a mitochondrial gene, which is conserved in most arthropods; it is species specific and has relatively high degree of genetic variation. The value of PCR to differentiate two bedbug species was demonstrated in this study. This approach could help the quick survey of bedbug species, when only cast exoskeletons or eggs or damaged carcass of bedbugs are found in sample sites. The *COI* PCR revealed a diagnostic DNA band size of 450 bp for the *C. hemipterus* while that of *C. lectularius* showed double DNA bands at 450 bp and 250 bp. *Cimex hemipterus* having a diagnostic DNA band size of 450bp concurs with the findings of Apiwat *et al*., (2013). The diagnostic band sizes that were not successfully detected may be due to failed PCRs. Failure to amplify targeted DNA may be attributed to various aspects of the PCR conditions, such as concentration of $Mg^{++}$, buffer pH, primer design, DNA template quality, difficulty of target and cycling conditions (Davies and Gray, 2002; Jones, 2002; Roux, 2009). The primers used had nonspecific regions which probably accounted for why they did not amplify DNA segments where the other researchers (Apiwat *et al.*, 2013; Robinson *et al.*, 2015) found them to amplify. This suggests that the species found in the study could perhaps not be exactly the same as those reported by Robinson *et al.* (2015) and Apiwat *et al.* 2013) but probably sub species or sibling species of what they reported, requiring further investigations.

Bedbugs exposed to temperatures of 30 ± 5$^{o}$C and relative humidity of 70 ± 5 % recorded 95 % mortality in less than 24 hours. This confirmed Omori (1942)'s submission that when bedbugs are exposed to temperatures as high as 30$^{o}$C their survival was affected negatively. There was however, a 5% survival within the set temperature and humidity range, suggesting that high temperatures limit bedbug numbers and could play a vital role in reducing the abundance of bedbugs. Though bedbugs are susceptible to heat, they often hide in insulated locations, such as in the holes and crevices of materials (Benoit, 2007). The study has also shown that, under room temperature, bedbugs may live longer than bedbugs exposed to a heated environment

All the respondents who volunteered to take part in providing answers to the questionnaire had attained some level of formal education and could express themselves clearly in the English language, they also could read and write in English. The study revealed that majority of the respondents had knowledge of bedbugs. Ninety-eight per cent of the respondents who volunteered have knowledge on bedbugs and had seen a bedbug before. This further supports the confirmation of the presence of bedbugs in the country and among human dwellings. Only a few had not seen bedbugs before and thought they were insects of past and do not exist in the 21$^{st}$ century. It is therefore important to create awareness of bedbug infestations through education on possible ways of detecting and identifying them.

# Chapter Six

# 6.0 Conclusion and Recommendation

## 6.1 Conclusion

Three species of bedbugs were identified in the study, *C. hemipterus*, *C. lectularius* and *C. pipistrelli*. This is the first time *C. pipistrelli*, a bat bedbug has been reported in human dwellings in Ghana. This could have other consequences for disease transmission from bats to humans and hence require further attention. The bedbugs were found mainly in the Senior High School dormitories; halls and hostels of some tertiary institutions and in some homes. The insects were found in mattresses, bed frames, wooden boxes books and crevices in walls. Currently, different methods of control including the use of insecticides, are employed in the control of these insects. However, the development of more effective control tools is required since the infestation seems to persist post-control efforts.

## 6.2 Recommendation

It is recommended that genomic sequencing be carried out on the DNA extracted to enable comparison with other sequences that other researchers have found. This will clarify as to whether the species found in this study could be insipient or sibling species or different species altogether from what others have previously reported.

It is further recommended that studies on bedbugs be carried out where samples would be collected from hotels and human habitations other than boarding houses across the country. This will help in building a database of bedbugs, which in the long run will help in finding solutions to the bedbug menace and also compare the difference in bedbug infestation in slums and elite areas.

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# APPENDICES

## APPENDIX I

**Preparation of reagents**

**Preparation of Tris-Acetate-EDTA**

10ml of 1M Tris hydrochloride (Tris Hcl) and 2ml of 0.5M EDTA was prepared and diluted with distilled water to 1000 ml.

**Preoaration of 10ml of 1M Tris hydrochloride**

0.5M EDTA (pH 8.0)

186.1 g of disodium EDTA (Na2EDTA) was dissolved in 800 mL of dH2O the solution was stirred using a magnetic stirrer, NaOH was added to bring the pH 8.0 where the EDTA will dissolve. After HCl was added to adjust the pH to 8.0 was adjusted to 8.0 with HCL.

The solution was stored at room temperature.

**1 M Tris Hcl**

In preparing 1M Tris Hcl, 121. g Tris Base was dissolved in 700 ml $dH_2O$

The volume was filled up to 1L with dH20 and stored at room temperature.

# APPENDIX II

A sample of the questionnaire used for the study

**Department Animal Biology and Conservation Sciences, University of Ghana.**

**Characterization of bedbugs and assessment of environmental factors that influence their survival in Ghana.**

This research seeks to evaluate the incidence of peculiar insects and their level of infestation in human dwellings, especially our bedrooms, towards shedding light on their health effects and the possible designing of effective control. As part of this study, questions will be asked about the incidence of these peculiar insects in your room. The findings of this study will contribute in the correct identification and the development of effective ways of controlling these insects found in our rooms.

Participation in this work is completely voluntary and you are at liberty to opt out whenever you feel so. The study is being carried out for academic purposes only. Confidentiality is ensured. Your response shall not be divulged to anyone other than what the study is meant for and your anonymity is ensured

Date ………………………………. Location/Service site ……………………

**General information**

| Question | Proposed answer |
|---|---|
| 1. Sex | [ ] Male [ ] Female |
| 2. Age | |
| 3. Education Level | [ ] Primary [ ] J.H.S [ ] S.H.S [ ] Tertiary |
| 4. Marital status | [ ] Married [ ]Single [ ] Widow |
| 5. House size | |
| 6. Do you share your room with others? | [ ]Ye [ ] No |
| 7. House hold size? | |
| 8. How long have you been staying in the house | |

**Insect information**

1. Do you often see insects in your room? [ ] Yes [ ] No
2. What type of insect have you observed in your room? [ ] Cockroaches [ ] Mosquitoes [ ] Spiders [ ] Ants [ ] Flies [ ] Bedbugs [ ] others (please specify)
3. How frequent do you see these insects in your room? [ ] Everyday [ ] Once a week [ ] Once a month [ ] others (please specify)
4. Are you or your roommates experiencing any bites from any of these insects [ ] Yes [ ] No
5. If your answer to question 4 above is Yes, how often do you get these bites? [ ]Everyday [ ] weekly [ ] fortnightly [ ] monthly

6. Which insect(s) do you frequently receive bites from? [ ] [ ] Mosquitoes [ ] Ants [ ] Cockroaches [ ] Flies [ ] Bedbugs

7. On average, how many insect bites do you receive each night? [ ] 1-5 [ ] 6-10 [ ] 10 – 20 [ ] many

8. Where were you bitten? [ ] In your room [ ] outside your room

**Knowledge on insect bite**

1. Do you have problems with insect bites at home? [ ] Yes [ ] No

2. Indicate specific areas where bites are located. [ ] All over body [ ] legs [ ] Arms [ ] back [ ] face [ ] other (specify)

3. Have any old/new items been brought into your room/apartment, such as , TV computer, clothes, shoes, furniture, bedding materials, any used items?
[ ] Yes [ ] No

4. Have you seen spots/ blood stains or marks of any kind on your bed sheets? [ ] Yes [ ] No

5. Do you often spray your room with any type of insecticide before and or after you first noticed the bite mark/insects? [ ] Yes [ ] No

6. If you answer yes to question 5 above, then state the most frequently used insecticide.[ ] Sasso [ ] Raid [ ] Kill it [ ] Heaven [ ] other (specify)…………………………..

7 How frequently do you wash your bedding materials? [ ] weekly [ ] fortnightly [ ] monthly [ ] other (specify)

8. Have you made contact with anyone who is also experiencing similar insect bite? [ ] Yes [ ] No

9. Have you spent any time outdoors in grassy/wooded areas that corresponds with the time frame that you first noticed the bite marks? [ ] Yes [ ] No

10. If you answer yes to the above question, where did you spend your time? [ ] park [ ] farm [ ] other (specify)

11. Which of these insects may transmit diseases? [ ] Mosquitoes [ ] Ants [ ] Cockroaches [ ] Flies [ ] Bedbugs [

12. What diseases can be transmitted? [ ] Malaria [ ] Elephantiasis [ ] Dengue fever [ ] other (please specify)……………………………………………………………………….

13. In what ways can the transmission of diseases be interrupted or prevented? [ ] Proper sanitation [ ] Use of Insecticides [ ] Use of treated nets [ ] Integrated Vector Management (IVM) [ ] other (please specify)………………………………..

**Insecticide use**

1. Do you use insecticides against insects in your room?
[ ] Yes [ ] No

2. If you answered No in the above, why do you not use them?
………………………………………………………………………………………
What alternative vector control tool(s) do you use against these insects? [ ] personal hygiene [ ] Environmental hygiene [ ] other please specify
3. If you answered yes to 1 above, what type of insecticide do you use [ ] plants [ ] Sasso [ ] Raid [ ] kill it [ ] Heaven [ ] others (specify)…………
4. How often do you use this insecticide? [ ] Everyday [ ] Once a week [ ] Once a month.
5. When was the last time you used an insecticide? [ ] today [ ] this week [ ] this month

**Knowledge on Bed bugs**
1. Do you know about bed bugs? [ ] Yes [ ] No
2. Have you seen one before? [ ] Yes [ ] No
3. When was the last time you saw one? [ ] Today [ ] this week [ ] this month [ ] this year
4. Where did you see it? [ ] in your room [ ] in a friend's room
5. Do you need help in controlling these insects? [ ] Yes [No]
6. If you answered No to question 5 above, give reasons.
7. If you answered yes to question 5 what type of help would you require? [ ] Advice from an expert [ ] Spraying [ ] collection

APPENDIX III

Data Sample.

| species | FW | FL | PTW | PTL | HL | PHL | 1A | 2A | 3A | 4A |
|---|---|---|---|---|---|---|---|---|---|---|
| A | 0.313 | 1.24 | 0.598 | 0.636 | 0.558 | 0.139 | 0.19 | 0.622 | 0.581 | 0.414 |
| A | 0.311 | 1.23 | 0.521 | 0.576 | 0.621 | 0.187 | 0.203 | 0.622 | 0.621 | 0.496 |
| A | 0.268 | 1.09 | 0.496 | 0.507 | 0.597 | | 0.172 | 0.503 | 0.533 | 0.528 |
| A | 0.318 | 1.36 | 0.461 | 0.513 | 0.601 | 0.105 | 0.208 | 0.649 | 0.573 | 0.499 |
| B | 0.388 | 1.33 | 0.631 | 0.579 | 0.67 | | 0.207 | 0.641 | | |
| B | 0.377 | 1.26 | 0.647 | 0.571 | 0.743 | 0.2 | 0.218 | 0.687 | 0.638 | 0.513 |
| B | 0.421 | 1.37 | 0.641 | 0.571 | 0.684 | 0.142 | 0.235 | 0.668 | 0.59 | 0.448 |
| B | 0.31 | 1.33 | 0.605 | 0.86 | 0.68 | 0.145 | 0.229 | 0.649 | 0.633 | 0.521 |
| B | 0.37 | 1.119 | 0.663 | 0.582 | 0.785 | 0.166 | 0.229 | 0.637 | 0.629 | 0.809 |
| B | 0.331 | 1.28 | 0.494 | 0.576 | 0.671 | 0.114 | 0.183 | 0.672 | 0.639 | 0.555 |
| B | 0.301 | 1.23 | 0.56 | 0.561 | 0.667 | 0.161 | 0.169 | 0.688 | 0.628 | 0.441 |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| B | 0.375 | 1.33 | 0.639 | 0.554 | 0.67 | | 0.162 | 0.617 | 0.573 | 0.514 |
| B | 0.359 | 1.39 | 0.691 | 0.596 | 0.694 | 0.192 | 0.233 | 0.649 | 0.633 | 0.531 |
| C | 0.392 | 1.43 | 0.666 | 0.502 | 0.726 | 0.133 | 0.178 | 0.648 | 0.6 | 0.526 |
| C | 0.34 | 1.32 | 0.65 | 0.643 | 0.67 | | 0.212 | 0.627 | 0.581 | 0.499 |
| C | 0.395 | 1.29 | 0.55 | 0.619 | 0.631 | 0.139 | 0.212 | 0.627 | 0.581 | 0.499 |
| C | | | 0.586 | 0.624 | 0.768 | 0.105 | 0.22 | 0.613 | 0.561 | 0.511 |
| C | 0.363 | 1.31 | 0.513 | 0.62 | 0.574 | 0.154 | 0.186 | 0.51 | 0.464 | 0.382 |
| C | 0.388 | 1.33 | 0.631 | 0.579 | 0.67 | | 0.207 | 0.641 | | |
| C | 0.377 | 1.26 | 0.647 | 0.571 | 0.743 | 0.2 | 0.218 | 0.687 | 0.638 | 0.513 |
| C | 1.31 | 1.14 | 0.611 | 0.59 | 0.618 | 0.693 | 0.161 | 0.584 | 0.55 | 0.487 |
| C | 0.32 | 1.31 | 0.58 | 0.554 | 0.555 | 0.117 | 0.165 | 0.691 | 0.891 | 0.513 |
| C | 0.281 | 1.18 | 0.71 | 0.551 | 0.556 | 0.105 | 0.203 | 0.683 | 0.595 | 0.483 |
| C | 0.248 | 1.21 | 0.622 | 0.507 | 0.554 | 0.122 | 0.132 | 0.648 | 0.639 | 0.467 |
| C | 0.381 | 1.33 | 0.583 | 0.552 | 0.555 | 0.138 | 0.186 | 0.665 | 0.633 | 0.494 |
| C | 0.327 | 1.29 | 0.996 | 0.551 | 0.591 | 0.128 | 0.178 | 0.693 | 0.611 | 0.517 |
| C | 0.377 | 1.37 | 0.484 | 0.529 | 0.602 | 0.156 | 0.161 | 0.665 | 0.636 | 0.566 |
| C | 0.323 | 1.33 | 0.621 | 0.517 | 0.566 | 0.163 | 0.255 | 0.631 | 0.618 | 0.555 |
| C | 0.33 | 1.1 | 0.599 | 0.558 | 0.672 | 0.105 | 0.175 | 0.591 | 0.421 | 0.43 |
| C | 0.374 | 1.27 | 0.462 | 0.528 | 0.671 | 0.16 | 0.152 | 0.62 | 0.575 | 0.44 |
| C | 0.355 | 1.12 | 0.607 | 0.561 | 0.75 | 0.104 | 0.198 | 0.622 | 0.56 | 0.48 |
| C | 0.3 | 1.14 | 0.636 | 0.554 | 0.67 | 0.149 | 0.219 | 0.584 | 0.421 | 0.331 |
| C | 0.33 | 1.31 | 0.534 | 0.596 | 0.574 | 0.153 | 0.19 | 0.716 | 0.636 | 0.503 |

A: *Cimex pipistrelli*. B: *Cimex lectularius* C: *Cimex hemipterus.*