DIVERSITY OF HONEY BEE *Apis mellifera* SUBSPECIES (HYMENOPTERA: APIDAE) AND THEIR ASSOCIATED ARTHROPOD PESTS IN CAMEROON

BY

DAVID TEMBONG CHAM
(I80/92221/2013)

(B.Sc. UNIVERSITY OF BUEA-CAMEROON, M.PHIL. UNIVERSITY OF GHANA-LEGON)

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DECLARATION

Candidate

I, DAVID TEMBONG CHAM, Registration Number I80/92221/2013, declare that this thesis is my original work and has not been submitted for award of a degree in any other University.

Signature_____ Date _____

Supervisors

This thesis has been submitted with our approval

Prof. Paul N. Ndegwa

School of Biological Sciences, University of Nairobi, P.O. Box 30197-00100, Nairobi, Kenya

Signature _____ Date _____

Prof. Lucy W. Irungu

School of Biological Sciences, University of Nairobi, P.O. Box 30197-00100, Nairobi, Kenya

Signature _____ Date _____

Dr. Ayuka T. Fombong

International Centre of Insect Physiology and Ecology (ICIPE), P. O. Box 30772-00100, Nairobi, Kenya

Signature _____ Date _____

Prof. Suresh Raina

International Centre of Insect Physiology and Ecology (ICIPE), PO. BOX 30772-00100, Nairobi, Kenya

Signature _____ Date _____

DEDICATION

This thesis is dedicated to the Cham's family

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ABBREVIATIONS AND ACRONYMS

ABPV	Acute Bee Paralysis Virus
ANOVA	Analysis of Variance
CCD	Colony Collapse Disorder
CL	Cylindrical Log hive
CIB	Cylindrical Indian Bamboo Hive
CRP	Cylindrical Raffia Palm hive
DAWINO	Discriminant Analysis With Numerical Output
DWV	Deformed Wind Virus
GDP	Gross Domestic Product
ICIPE	International Centre of Insect Physiology and Ecology
KBV	Kashmir Bee Virus
KTB	Kenya Top Bar Hive
LDA	Linear Discriminant Analysis
MANOVA	Multivariate Analysis of Variance
ML	Maximum Likelihood
mtDNA	Mitochondrial Deoxyribonucleic Acid
OIE	Office International des Epizooties or World Organisation for Animal Health
PCA	Principal Component Analysis
PCR	Polymerase Chain Reaction
SHB	Small hive beetle
RFLP	Restricted Fragment Length Polymorphism
DPX	Mixture of distyrene (a polystyrene), a plasticizer (tricresyl phosphate) and xylene

DEFINITION OF TERMINOLOGIES

Apiary   An area where honey bee colonies are kept in beehives[1]

Beehive   A box or container where bees live[1]

Colony   The aggregate of worker bees, drones, queen, and brood living together as a social unit in a hive or other dwelling[1]

Ecotype   Regional natural bee types distinct enough to raise the need for a name[5]

Haplotype   A single nucleotide polymorphism (SNP) sequence and a representative genetic marker describing the diversity of biological organs[4]

Hive tool   A metal use for opening and cleaning the hive and lifting top bars[1]

Morphotype   Bee population with distinct morphological measurements. A statistically defined cluster[3]

Smoker   A device in which slow-burning materials (e.g. wood shavings) are used to produce smoke to subdue honey bees[1]

Subspecies   Honey bee populations with specific phynotype and mitochondrial haplotypes[2]

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[1]Collison *et al*., 2004; [2]Coroian *et al*., 2014; [3]Hepburn and Radloff, 1997; [4]Lim *et al*., 2012; [5]Meixner *et al*., 2013

ABSTRACT

Conservation of *Apis mellifera* requires detailed knowledge of the subspecies identity, diversity and factors threatening their survival. Despite the increasing reports on the spread and damage caused by both exotic and indigenous honey bee pests to bee populations in many parts of Africa, there is dearth of knowledge on the ecology of honey bees and their pests in Central Africa and Cameroon in particular. Furthermore, management practices such as use of different beehive types across Africa may affect bee health. This study was undertaken to fill these knowledge gaps and provide information to aid the conservation of African honey bees. Field surveys for honey bees and their pests were conducted in apiaries located in four major ecological zones in Cameroon in 2014 using standard sampling protocols. Morphometric analysis revealed the presence of three morphotypes of *A. mellifera* in Cameroon, namely, two savannah highland and one forest lowland populations. Genetic diversity and phylogenetic studies revealed that these morphotypes constituted five *A. mellifera* subspecies haplotypes that were adapted to different geographic locations. Three of these haplotypes represent new *A. mellifera* mtDNA haplotypes, one of which occurred in the range of the previously reported *Apis mellifera jemenitica*. The new subspecies haplotypes were restricted to the highland savannah and were genetically closely related to the lowland *Apis mellifera adansonii*, the dominant haplotype in Cameroon. This suggests that honey bee populations of Cameroon are made of distinct subspecies haplotypes with the same common ancestral origin that are adapted to distinct geographic locations. The conservation of these bee populations within their local environment is therefore recommended to maintain their genetic identities. Pest assessments of honey bee colonies revealed that these *A. mellifera* populations were associated with diverse arthropod pests such as *Varroa destructor*, *Aethina tumida*, dynastid beetles, *Galleria mellonella*, *Achroia grisella*, *Acherontia sp*, *Braula sp*, and *Megaselia scalaris*. Through molecular phylogenetic analysis this study revealed for the first time the occurrence of the Korean haplotype of *V. destructor* and *M. scalaris* in Cameroon and a unique haplotype of *A. tumida* in honey bee colonies. These pests represent a threat to bee health in Cameroon and other parts of Africa. Pest infestation levels were found to vary across ecological zones and between seasons, suggesting that environmental factors may influence their distribution and abundance. The synergistic effect of the major pests, *V. destructor* and *A. tumida* with other less frequent pests such as wax moths, were identified as contributing factors to colony losses. Beehive type was found to have an influence on colonization and infestation of pests such as *G. mellonella*. Using the right beehive type may therefore ease colonization and reduce infestation by some pest species. This study therefore revealed that honey bees of Cameroon are made of diverse populations adapted to different geographic locations and associated with diverse arthropod pests. The impact of these pests to bee health is discussed in details and available management options provided. This study therefore contributes to the existing knowledge on the ecology of honey bees and their pests in Cameroon.

CHAPTER ONE

INTRODUCTION

1.1 General introduction

Pollinators contribute greatly to both the global economy and food security. Global economic value of pollination services for example is estimated at US$ 215 billion (Gallai *et al*., 2009). This service is provided to about 35% of the global food crops (Klein *et al*., 2007). Among all the pollinator species, the honey bee, *Apis mellifera* L., is considered the most important in both the agricultural and natural ecosystems (Calderone, 2012). The pollinator services are vital especially in Africa where most economies rely largely on agriculture. For example, in Cameroon, the agricultural sector accounts for 60% of employment and contributes significantly to the gross domestic product (GDP) and foreign earnings (Fonjong, 2004). Besides their role in pollination, honey bees make useful products such as honey and wax, as well as other nutritional, medicinal and pharmaceutical products such as royal jelly, propolis, bee venom and pollen (Michener, 2007). These bee products are of great economic value. In Cameroon, for example, hive products such as honey and wax contribute over US$ 6 million annually to the economy and provide employment to beekeepers in rural communities (Ingram and Njikeu, 2011).

There are over 30 subspecies of *A. mellifera* worldwide (Bouga *et al*., 2011; Chen *et al*., 2016; Engel, 1999; Moritz *et al*., 2005; Raina *et al*., 2011). Eleven of these subspecies have been reported to occur across Africa with *Apis mellifera scutellata* and *A. m. adansonii* having the most widely distributed range (Arias and Sheppard, 1996; Engel, 1999; Hepburn and Radloff, 1997; Meixner *et al*., 2011). In Cameroon, morphometric analysis of *A. mellifera* populations led to the discovery of three distinct morphoclusters which were recognised to be the subspecies *A. m. jemenitica* in the Far North, *A. m. adansonii* in the southern parts and the *A. m.* 'monticola like' bees in the North West (Hepburn and Radloff, 1997). Recent molecular studies by Franck *et al*. (2001) on *A. mellifera* subpopulations of Africa also reported the occurrence of *A. m. adansonii* in the central region of Cameroon. This study did not however include samples from the mountainous locations where Hepburn and Radloff (1997) had previously reported the occurrence of the three morphotypes. *Apis mellifera* subspecies are sometimes called 'geographic

sub-species' (Bouga *et al*., 2011) since they are adapted to different climatic and environmental conditions (Johnstone, 2008). The conservation of these pollinators within their geographic boundaries is therefore of prime importance.

Globally, honey bees are faced with pressures from pests, parasites, pathogens, pesticides, decline of foraging resources, climate change, poor management, socioeconomic and political factors (Ellis *et al*., 2010; Goulson *et al*., 2015; Smith *et al*., 2013). These factors contribute to the decline of managed pollinators (Goulson *et al*., 2015). Pests and parasites are considered principal driving force due to their direct and indirect damage to colonies (Dainat *et al*., 2012a; Smith *et al*., 2013). Commonly encountered pests and parasites in honey bee colonies include the mite *Varroa destructor*, tracheal mite *Acarapis woodi*, the small hive beetle *Aethina tumida*, large hive beetles *Oplostomus* species, wax moths *Galleria mellonella* and *Achroia grisella*, hawkmoth *Acherontia* sp, and bee louse *Braula* sp (Fazier *et al*., 2010; Fombong *et al*., 2012; Hepburn and Radloff, 1998; Neumann *et al*., 2016; Torto *et al*., 2010a). Although most of these pests have been reported to occur in many African countries (Pirk *et al*., 2016), with varying impact on honey bee health (Dietemann *et al*.,2009; Fombong *et al*., 2012; Muli *et al*., 2014; Rasolofoarivao *et al*., 2013), there is still substantial dearth of knowledge on the health of honey bees in Central Africa including Cameroon (Pirk *et al*., 2016).

In Africa, beekeeping is mostly practiced in rural communities with enough foraging resources for the bees, and around farm lands (Ingram, 2014; King, 2014). Management practices however differ across regions and countries. These practices are influenced by traditional belief systems and educational level of the beekeepers (Ingram, 2014). Some of these practices include the use of different hive types such as Cylindrical Log hives, Cylindrical Grass hives, Pod hives, Kenya Top Bar and Langstroth hives. These hives may have an effect not only on the quantities and quality of honey (Ingram, 2014; King, 2014), but also on the health of the overall bee colony which remains poorly understood.

1.2 Problem statement

There is little information on the identity of *A. mellifera* subspecies and their associated arthropod pests in Cameroon. The first attempt to establish the identity of *A. mellifera* subspecies in Cameroon was carried out about two decades ago by Hepburn and Radloff (1997). They did not include honey bee populations from the Central, Southern and Eastern part of the country. Later studies using molecular markers by Franck *et al*. (2001) did not cover the range of the morphologically described *A. mellifera* subspecies by Hepburn and Radloff (1997). Morphometric and molecular studies are therefore needed to fully characterise the *A. mellifera* populations of Cameroon.

The parasitic honey bee mite, *V. destructor* has recently been recorded in the North, East, South, West, and some Islands of Africa (Pirk *et al*., 2016). This mite actively plays a role in the transmission and reactivation of viruses such as Kashmir Bee Virus (KBV), Deformed Wind Virus (DWV), Acute Bee Paralysis Virus (ABPV) and others (Chen *et al*., 2004; Le Conte *et al*., 2010; Rosenkranz *et al*., 2010). Feeding by the mite can also result in a weakened honey bee immune system, morphological deformities, and reduced longevity in worker honey bees. There is however, no scientific record of this mite in Central Africa including Cameroon (Pirk *et al*., 2016). Furthermore, besides the introduced pests and diseases, indigenous pests such as *A. tumida* and various *Oplostomus* species also pose a threat to honey bee health through their feeding on brood, honey and pollen (Fombong *et al*., 2012; Neumann *et al*., 2016; Torto *et al*., 2010a) and as potential vectors of diseases (Eyer *et al*., 2009). The occurrence of *Varroa* mite together with the indigenous pests such as hive beetles and wax moths can speed up colony losses (Hepburn and Radloff, 1998; Spiewok *et al*., 2008). Studies on honey bee health in Central Africa and Cameroon are therefore needed to know their health status. In addition to pests and diseases, hive types may also have an effect on the health of the bees. The impact of the different hive types used by beekeepers in Africa to keep their bees is also not well known (Ingram, 2014; King, 2014).

1.3 Justification and significance of the study

The conservation of regional stocks of *A. mellifera* requires detailed knowledge on their identity and ecology (Meixner *et al*., 2013). Knowledge on the *A. mellifera* subspecies in Cameroon is therefore vital to justify their sustainable conservation. Molecular studies are needed to determine whether the *A. mellifera* populations described by Hepburn and Radloff (1997) represent distinct subspecies or they are simply different morphotypes of the same subspecies. Studies on areas not included in previous honey bee taxonomic studies will also augment existing knowledge on *A. mellifera* subspecies and morphotype diversity in Cameroon.

Recent reports on the invasion and spread of *V. destructor*, damages and spread of other indigenous pests in many countries across Africa (Neumann *et al*., 2016; Pirk *et al*., 2016) necessitates the need for honey bee pest surveillance and documentation in Cameroon. The loss of honey bee diversity can also be facilitated inadvertently by beekeepers out of ignorance through poor beekeeping practices (Goulson *et al*., 2015). One of such practices includes the use of hive types whose impact on the health of the bees is not well known. An understanding of how different hive types affect pest population levels will lead to the identification and adoption of the most suitable hive types which promote bee health through reduced pest infestation. This study will therefore provide detailed information on the diversity of honey bee subspecies and pests as well as highlight the importance for conservation of the ecologically adapted *A. mellifera* subpopulations, ecotypes or morphotypes. It will also reveal vital information for the management of key pest species, and provide baseline information for future research.

1.4 Objectives

1.4.1 Main objective

To assess the diversity of honey bee subspecies and their associated arthropod pests in Cameroon

1.4.2 Specific objectives

The specific objectives were:

1. To identify *Apis mellifera* subspecies in Cameroon and determine their relatedness to other *A. mellifera* populations in African

2. To identify pests of honey bees in selected agro-ecological zones in Cameroon

3. To assess the impact of beehive types on honey bee colony establishment and pest infestation

CHAPTER TWO

LITERATURE REVIEW

2.1 *Apis mellifera* and related species of the genus *Apis*

The genus *Apis* Linnaeus is one of the most important in the Hymenoptera order. It contains a number of beneficial species (e.g. *A. mellifera*) that are widely distributed globally (Lo, *et al*., 2010).

2.1.1 Diversity and geographic distribution of *Apis* species

The genus *Apis* is presently known to contain 12 honey bee species (Table 2.1). Two of these species, *A. breviligula* and *A. indica,* were recently added to the list (Lo *et al*., 2010). Extinct *Apis* species that occurred during the Oligocene and Miocene epoch include *A. armbrusteri*, A. *cuenoti, A. henshawi, A. longtibia, A. miocenica, A. petrefacta, and A. vetustus* (Engel, 1998). The extant species are widely distributed all over the world and occur over a great range of habitats and climates (Table 2.1). Although most of these species share the same area, they are reproductively isolated (Koeniger and Koeniger, 2000; Wongsiri *et al*., 1990). The distribution of *A. mellifera* is allopatric to that of the other *Apis* species (Engel, 1999).

Apis mellifera (sometimes refers to as the western honey bee) is the most widely distributed *Apis* species. The natural distribution range of this social bee is from Africa through the Middle East and Europe to north of the Arctic in Scandinavia (Ruttner, 1988; Ruttner *et al*., 1978). It has also been successfully introduced into the Americas and Australia (Koeniger and Koeniger, 2000). Other cavity nesting honey bees are common Asian sympatric species (Table 2.1). *Apis cerana* occurs naturally across a very wide geographical area in Asia spanning from the tropical south (Indonesia) to the temperate north (Russia and China) (Koeniger and Koeniger, 2000; Takahashi *et al*., 2002). The dwarf and giant *Apis* species are also common Asian subspecies (Table 2.1). However, the dwarf *A. florea* has been reported in Sudan and most recently in Ethiopia (Pauly and Hora, 2013) (Table 2.1). In contrast with *A. mellifera*, the dwarf honey bee colonies build a small nest comprising of a single comb about 20×20 cm suspended from a twig of a shrub or tree

in the open while the giant open nesting *Apis* species build a large single comb in the open (Oldroyd *et al*., 2008).

Table 2.1 *Apis* species and their geographic distribution

Apis species	Group	Distribution	Reference
A. mellifera	Cavity-nesting	Worldwide	(Engel, 1999)
A. cerana	Cavity-nesting	Most of Asia	(Engel, 1999; Lo *et al*., 2010)
A. nigrocincta	Cavity-nesting	Indonesia, Mindanao	(Engel, 1999)
A. koschevnikovi	Cavity-nesting	Malaysia, Indonesia	(Engel, 1999)
A. nuluensis	Cavity-nesting	Malaysia, most of Asia	(Engel, 1999; Lo *et al*., 2010)
A. indica	Cavity-nesting	India	(Lo *et al*., 2010)
A. dorsata	Giant	Borneo, Thailand, Malaysia	(Engel, 1999)
A. laboriosa	Giant	Himalayas, Nepal	(Engel, 1999; Lo *et al*., 2010)
A. d. binghami	Giant	Indonesia	(Raffiudin and Crozier, 2007)
A. breviligula	Giant	Philippines	(Lo *et al*., 2010)
A. florea,	Dwarf	Asia, Middle East, Africa (Sudan, Ethiopia)	(Bezabih *et al*., 2014; Engel, 1999; Pauly and Hora, 2013)
A. andreniformis	Dwarf	Most of Asia, Middle East	(Engel, 1999; Lo *et al*., 2010)

2.1.2 Origin and classification of *Apis mellifera*

The honey bee *Apis mellifera* is thought to have evolved around the Pleisto-Holocene epoch (Engel, 1998). The origin of this important *Apis* species has been a topic of debate for long and the debate seems to continue. *Apis mellifera* was formerly believed to have originated from western or central Asia and moved into Europe and Africa (Ruttner, 1988). Other authors however, believed that the range is large and diverse covering from Europe, Africa, and the Middle East (Engel, 1999). Later studies by Whitfield *et al*. (2006) trace the origin to Africa, from where it spread to Eastern and Western Europe. Recent studies by Han *et al*. (2012) have however contradicted this view showing through phylogenetic studies that the origin was probably out of Africa. They however did not exactly state the true origin of *A. mellifera*. Most recently, Kotthoff *et al*. (2013) using both fossils and present-day species distribution concluded

that *A. mellifera* originated from Europe and migrated to Africa and re-immigrated back into Europe and spread to other parts of the continent. The discrepancy in the origin of *A. mellifera* is synonymous with debates on the age of *A. mellifera* subspecies evolution which was suggested to be between 0.33 – 1.35 million years (Arias and Sheppard, 1996).

The classification of *A. mellifera* is based on Linnaeus 1758 as follows:

Kingdom:	Animalia
Phylum:	Arthropoda
Subphylum:	Hexapoda
Class:	Insecta
Subclass:	Pterygota
Superorder:	Holometabola
Order:	Hymenoptera
Suborder:	Apocrita
Superfamily:	Apoidea
Family:	Apidae
Subfamily:	Apinae
Tribe:	Apini
Genus:	*Apis* Linnaeus, 1958
Species:	*Apis mellifera* Linnaeus, 1758

2.1.3 Diversity of *Apis mellifera* subspecies

The honey bee species *A. mellifera* contain over 30 known subspecies (Bouga *et al*., 2011; Engel, 1999; Raina *et al*., 2011), the newest of which was reported in 2016 (Chen *et al*., 2016). This species has diversified into local races each adapted to different climatic and environmental conditions (Hepburn and Radloff, 1998). These local races or subspecies are also described as 'geographic sub-species' since their distribution corresponds to distinct geographic areas or ecosystems (Bouga *et al*., 2011; Meixner *et al*., 2013). There are about twelve *A. mellifera* subspecies in Africa with *A. mellifera scutellata* being the most widely distributed (Table 2.2). Most of these subspecies are classified based on traditional external morphological methods (Hepburn and Radloff, 1998; Ruttner, 1988).

European and Asian honey bee subspecies include the Italian bee *A. m. ligustica* which occurs in most of Eastern Europe, the Caucasian bees *A. m. caucasia*, the Carniolan bee *A. m. carnica* which are similar in color and temperature to Caucasian bees, *A. m. mellifera* which occurs in the Danish island and most of western Europe (Bouga *et al*., 2011), and the newly recorded species, *A. m. ruttneri* from Malta (Sheppard *et al*., 1997), *A. m. pomonella* from Central Asia (Sheppard and Meixner, 2003) and *A. mellifera sinisxinyuan* from China (Chen *et al*., 2016) among others (Bouga *et al*., 2011).

Table 2.2 Diversity and distribution of *Apis mellifera* subspecies in Africa

A. mellifera subspecies	African country	Reference
A. m. scutellata	Most of southern Africa including, Kenya, Tanzania, Ethiopia, South Africa.	(Arias and Sheppard, 1996; Engel, 1999)
A. m. monticola	Kenya, Sudan, Uganda, Ethiopia, Burundi, most of south-eastern Africa	(Arias and Sheppard, 1996; Engel, 1999)
A. m. 'monticola like'	Cameroon	(Hepburn and Radloff, 1998)
A. m. litorea	Kenya, Mozambique, Tanzania, and most of south-eastern Africa,	(Engel, 1999; Hepburn and Radloff, 1998)
A. m. capensis	South Africa	(Engel, 1999)
A. m. lamarckii	Egypt, most of north-eastern Africa	(Arias and Sheppard, 1996; Engel, 1999)
A. m. unicolor	Madagascar	(Hepburn and Radloff, 1998)
A. m. sahariensis	North-eastern Africa	(Arias and Sheppard, 1996)
A. m. intermissa	North-eastern Africa	(Arias and Sheppard, 1996)
A. m. adansonii	Cameroon, Mali, Sierra Leon, Ghana, Nigeria, most of western Africa	(Arias and Sheppard, 1996; Hepburn and Radloff, 1998)
A. m. jemenitica (also called, *A. m. nubi*, *A. m. sudanensis* or *A. m. bandasii*)	Chad, Cameroon, Sudan, Somalia, north-eastern Africa	(Arias and Sheppard, 1996; Hepburn and Radloff, 1998)
A. m. simensis	Ethiopia	(Meixner *et al*., 2011)

2.1.4 *Apis mellifera* lineages and their distribution

Geographic isolation and ecological adaptation has given rise to a number of honey bee subspecies and lineages (Meixner *et al*., 2013). Molecular and morphological studies have placed all the *A. mellifera* subspecies into four main evolutionary lineages, denoted as lineage A, C, M, and O. Lineage A contains most subspecies from Africa including *A. m. lamarckii, A. m. andansonii, A. m. scutellata, A. m. monticola, A. m. litorea, A. m. capensis, A. m. unicolor* and *A. m. simensis*. Lineage C consists of the subspecies from Europe and eastern Mediterranean including, *A. m. ligustica*, *A. m. carnica, A. m. macedonica, A. m. cecropia, A. m. cypria,* and *A. m. adami*. Lineage M includes the subspecies from Europe and west Mediterranean such as *A. m. mellifera*, *A. m. iberiensis*, *A. m. intermissa, A. m. sahariensis, A. m. siciliana* (Meixner *et al*., 2013) and the recently described *A. m. sinisxinyuan* from China (Chen *et al*., 2016). Most subspecies from the Middle-East and western Asia are categorized under lineage O and includes *A. m. caucasica, A. m. anatoliaca, A. m. syriaca, A. m. meda, A. m. armeniaca, A. m. jemenitica,* and *A. m. pomonella* (Arias and Sheppard, 1996; Meixner *et al*., 2013; Ruttner, 1988; Whitfield *et al*., 2006). As noted by Meixner *et al*. (2013) there is still substantial variation in these groupings based on different authors. Recent studies have led to the discovery of two other lineages namely, Y in north-eastern Africa (Franck *et al*., 2001) and Z in the Middle-East (Alburaki *et al*., 2011). The Z lineage was previously regarded as a subset of A and O lineages (Alburaki *et al*., 2011) but has recently been confirmed as a separate lineage comprising the subspecies *A. m. syriaca* (Alburaki *et al*., 2013). These new discoveries are proof that with the availability of new research techniques and tools a lot of new information will be uncovered with regards to *A. mellifera* subspecies and their lineages.

2.1.5 Methods for characterising *Apis mellifera* subspecies

The discrimination of honey bees is based on a number of techniques both morphological and molecular. The choice of a technique is dependent on the expected outcome, cost, and time.

2.1.5.1 Morphological methods

External morphological methods for discriminating honey bee subspecies range from the combination of the whole body characters suites including body size and wing angles ('classical morphometry'), to the more specific classical wing venation pattern analysis (Meixner *et al*., 2013). The characters used in morphometry can be grouped into four main categories such as body size, pilosity, pigmentation and wing venation characteristics (Ruttner, 1978). Ruttner, (1988) proposed up to 36 morphometric characters which can be used for discriminating honey bees. He also showed that above 10 characters is adequate for discriminating African honey bee subspecies. This classical method is laborious and time-consuming and can be misleading in some cases due to the environmental sensitive characters (Meixner *et al*., 2013). Therefore other techniques might be required for a more complete description of phylogenetic relationship between subspecies.

Wing venation pattern analysis is another important morphometry technique for discriminating subspecies. This technique can be performed using three main methods: classical wing morphometry based on Ruttner (1988), Discriminant Analysis With Numerical Output (DAWINO) method (Bouga *et al*., 2011), and geometric morphometry (Bookstein, 1991; Tofilski, 2008). Of the three wing venation pattern analysis, geometric morphometry is more accurate and less time consuming (Meixner *et al*., 2013). Unfortunately, data generated using this technique cannot be compared with reference data from the more widely used classical morphometry which is the technique used in the current classification of *A. mellifera* subspecies (Meixner *et al*., 2013). The classical morphometric method is therefore still recommended as the best method for describing *A. mellifera* populations since it represents the actual characters of subspecies or their ecotypes (Meixner *et al*., 2013). Generally, morphometric methods have only an intermediate power in discriminating honey bee subspecies (Francis *et al*., 2014). The use of more than one method is therefore important for increased accuracy in subspecies discrimination.

2.1.5.2 Molecular techniques

Genetic diversity of honey bees has been investigated using a number of molecular markers including mitochondrial DNA (mtDNA), nuclear markers such as DNA microsatellites and allozymes (Arias and Sheppard, 1996; Meixner *et al*., 2013).

Mitochondrial DNA (~16 000bp) is a maternally inherited circular molecule. It is one of the most widely use markers in the identification of honey bee matrilineal origin in genetic studies (Meixner *et al*., 2013). This DNA fragment has been used extensively to determine genetic diversity in honey bee subspecies using methods such as Restriction Fragment Length Polymorphisms (RFLPs), Polymerase Chain Reaction (PCR)-RFLP, and direct sequencing. The previously used enzymes in RFLP include *HinfI, AccI, AvaI, BclI, BglII, EcoRI, HincII, HindII, HindIII, NdeI, PstI, PvuII*, and *XbaI*. None of these markers have been efficient for honey bee subspecies identification (Meixner *et al*., 2013). Restriction Fragment Length Polymorphisms was therefore replaced by PCR-RFLP which unlike the RFLP that utilized the whole mitochondrial genome is specific within a PCR-amplified region. This combination is able to produce haplotypes of different evolutionary lineages even though it is still unable to correctly identify honey bees at the species level since it produces haplotypes that cannot be diagnosed (Meixner *et al*., 2013). It is however still considered an effective tool for maternal identification. The best method to determine genetic diversity using mtDNA is through sequencing which produces identical haplotypes that have a common origin as opposed to the PCR-RFLP that produces identical haplotypes by state (Meixner *et al*., 2013). Although good, this technique may only provide maternal components of the variation making it inefficient in determining introgression events. It is therefore also necessary to consider nuclear DNA markers for increased accuracy in determining colonial introgression (Francis *et al*., 2014).

Nuclear markers are also used in honey bee genetic diversity studies. Commonly used nuclear markers include allozymes, DNA microsatellites and Single Nucleotide Polymorphism (SNPs) (Alburaki *et al*., 2013; Francis *et al*., 2014; Meixner *et al*., 2013). Allozyme markers (allelic variant forms of an enzyme that codes at the same locus) have been shown to be ineffective in discriminating between honey bee subspecies since there are no fixed allelic variations between species (Francis *et al*., 2014). DNA microsatellites or short tandem repeats (STR) are

polymorphic DNA loci of between 1 to 6 bases repeated from 4 to over 100 times (Tautz, 1993). Short tandem repeats provide useful information for determining zone of introgression between subspecies and has also been useful in determining honey bee lineages (Alburaki *et al*., 2013). In combination with mtDNA this marker further improves the discriminative power between species (Francis *et al*., 2014). However, the lack of uniformity in loci and primers used in microsatellite studies makes comparisons difficult (Meixner *et al*., 2013). Another recent nuclear marker that has been used in honey bee genetic diversity studies is SNP markers. A SNP is a change of a single base, mostly by one alternative nucleotide, in a given position of a DNA sequence (Meixner *et al*., 2013). The use of SNP is promising but the technology is very expensive compared with mtDNA and microsatellites markers which are affordable and also reliable.

Molecular methods such as STR and sequencing are therefore generally advantageous over morphological methods due to their accuracy in subspecies discrimination and availability of reference sequence data for comparison. Also mtDNA is passed on from parents to offspring and has less influence on the environment as compared to morphometric characters of size, color, and pilosity (Meixner *et al*., 2013). However, morphological description of subspecies or variants is also important. In addition, morphometry can be used to determine biparental inheritance (introgression). Therefore the best way to have a full picture of the diversity of honey bee subspecies and their ecotypes is to combine morphological and molecular methods (mtDNA direct sequencing and STR). Also, since African honey bees tend to swarm more frequently (McNally and Schneider, 1992; Schneider, 1990), then a revision of the genetic and morphotype diversity is always necessary.

2.1.6 The *Apis mellifera* colony and life history

Division of labour is postulated to be the principal factor responsible for the success of insect societies including honey bees (Robinson, 1992). In *A. mellifera*, the colony is made up of three distinct members (a queen, workers, and drones) each with a unique role that contributes to the overall success of the colony. The polyandrous queen is normally the only active reproductive female responsible for egg laying in this honey bee species (Beshers *et al*., 2001). She normally mates with approximately 7 – 17 drones and uses the sperms to fertilize the eggs. Fertilized eggs

hatch into workers whereas drones develop from unfertilized eggs (Robinson, 1992). Drones are responsible for fertilizing the queen after which they immediately die. They are larger than workers, and shorter than the queen. Although the workers are females, they do not normally lay eggs (Beshers *et al*., 2001) except in the thelytokous cape honey bee *A. mellifera capensis* (Pirk *et al*., 2011; Verma and Ruttner, 1983). Worker honey bees and queens developed from fertilized eggs are diploid with a total of 32 chromosomes while drones develop from unfertilized eggs are haploid with 16 chromosomes. The workers take 21 days (3 days in the egg stage, 6 days as larva and 12 days as pupa) to complete their lifecycle, while drones complete their cycle in 24 days (3 days in the egg stage, and 6.5 and 14.5 days in the larva and pupa stage respectively). The queen has the shortest developmental time of 16 days (3 days in the egg stage, 5.5 days in the larva, and 7 days in the pupa) (Waller, 2016). Workers have a life span of between 5-6 weeks while queens on the other hand can live for up to 96 weeks and drones for approximately 13 weeks or until they mate (Waller, 2016).

There is polyethism in *A. mellifera*. However, within the worker caste it is only temporal (plastic) with younger workers performing tasks within the hive and older workers performing tasks outside of the hive (Beshers *et al*., 2001). Worker tasks generally include tending the queen and young larvae, comb construction, resource gathering (nectar, pollen, resins, and water) and defending the hive. A colony may contain 20,000 to 60,000 workers depending on age and time of the year (Beshers *et al*., 2001; Waller, 2016).

2.1.7 Ecological and economic importance of honey bees

The honey bee *A. mellifera* has been of immense socio-economic value well before the beginning of written history (Crane, 1990). It has remained an integral part of human culture with honey hunting dating back to prehistoric cave paintings (Crane, 1990). The benefit of honey bees can be grouped in to direct and indirect. Direct benefit refers to the value of the honey bee products derived from the hive, while indirect benefit is in reference to the pollination services provided by honey bees in the agricultural industry as well as their role in biodiversity conservation through the pollination of non-cultivated crops.

Honey bee hive products such as honey, beeswax, royal jelly, propolis, pollen, bee venom, and bee brood contribute enormously to food security and in economic growth (Crane, 1990; Michener, 2007). Apiculture provides full or additional family income through sales of bee products which are used not only as food but also as additives for pharmaceutical and medical products (Ingram and Njikeu, 2011; Michener, 2007). In Cameroon, in spite of incomplete and missing data hive products such as honey contribute about US$ 6 million, wax about US$ 1 million, and other products about US$ 3 thousand annually to the economy. This accounts for about 52 % of the household income of about 20, 000 beekeepers (Ingram and Njikeu, 2011). Nutritionally, honey is widely used as a sweetener, in baking, production of confectionaries, alcoholic drinks, and as an antiseptic (Crane, 1990; Ingram and Njikeu, 2011). Other honey bee products such as propolis, bee venom, bee pollen, royal jelly and beeswax also have varied uses nutritionally, medically and economically. Beeswax for example is used in making candles, models, casting and etching of objects. It is also used in the cosmetic and pharmaceutical industry in the production of ointments, soothing skin creams and lotions (Crane, 1990; Michener, 2007). Other commercial uses of beeswax include; production of furniture varnish, shoe polish, wood and paper waterproofing products and solutions (Crane, 1990). It is also used traditionally for dying cloths (Michener, 2007). Royal jelly and bee pollen are used as dietary supplements by humans. Bee products with high medicinal value include bee venom for the treatment of arthritis and rheumatism, bee pollen for the treatment of allergies, and propolis for the production of various medications and body creams. Besides classical bee products, bee brood is also an essential food source for humans (Crane, 1990; Michener, 2007).

The economic value of bees in pollination exceeds the value of the products they produce (Carreck and Williams, 1998). Honey bees are key pollinators and are essential for the pollination of many agricultural crops especially pollinator-dependent crops such as apples, almonds, blue and cranberries (Carreck and Williams, 1998; Klein *et al*., 2007). They are the most economically valued pollinators and it is estimated that approximately 35 % or one-third of human food consumption depends directly or indirectly on bee pollination (Klein *et al*., 2007). This contributes about US$ 215 billion to the global economy (Gallai *et al*., 2009). In light of the decline of wild non-honey bee pollinators as well as feral colonies of honey bees, the importance of managed bees is greater today than ever (Calderone, 2012). In spite of the ecological and

economic significance of honey bees, not much has been done on honey bee related research in many African countries (Raina *et al*., 2011) and as a consequence, the value of pollinators to biodiversity conservation and agricultural production has not been fully appreciated as is evidenced by the over US$ 150 million pollination service that Cameroon' beetle pollinators provide to oil palm plantations in Southeast Asia (FAO, 2007). Furthermore, the dearth of knowledge in honey bee related research can also be evidenced by the limited honey pest related studies especially in Central Africa including Cameroon (Pirk *et al*., 2016).

2.2 Honey bee parasites and pests

There has been a decline in honey bee health over the years, which has been attributed to several factors with pests and parasites being the main contributors (Dainat *et al*., 2012a; Smith *et al*., 2013). This is due to their ability to cause both direct (feeding on haemolymph) and indirect damage (transmission of viruses) (Chen *et al*., 2004; Fombong *et al*., 2012; Neumann *et al*., 2016; Rosenkranz *et al*., 2010). These pests and parasites have also been reported to vector a number of honey bee viruses such Deformed Wing Virus (DWV), Black Queen Cell Virus (BQCV), Acute Bee Paralysis Virus (ABPV), Israeli Acute Paralysis Virus (IAPV), Sac Brood Virus (SBV), Kashmir Bee Virus (KBV) and many others (Chen *et al*., 2004; Le Conte *et al*., 2010; Mumoki *et al*., 2014; Rosenkranz *et al*., 2010). Five of these viruses have been reported to occur in Africa (Mumoki *et al*., 2014). Parasites and pests therefore represent a threat to bee health globally.

2.2.1 Honey bee parasites

There are about 100 different parasitic mite species associated with honey bees although just three namely, *Varroa* spp, *Acarapis woodi*, and *Tropilaelaps clareae* are the major ones that threaten the survival of both managed and feral honey bees (Sammataro *et al*., 2000). Parasitic flies have also recently emerged as potential contributors to the global colony declines (Core *et al*., 2012).

2.2.1.1 *Varroa* mite

The genus *Varroa* is represented by three species of obligate ectoparasitic mites. These are *Varroa jacobsoni* Oudeman, *Varroa underwoodi* Delfinado-Baker, and *Varroa rindereri* De Guzman and Delfinado-Baker (Anderson and Trueman, 2000). Of these, only *V. jacobsoni* originally parasitic on *A. cerana* (Asian honey bee) has been associated with *A. mellifera* (Anderson and Trueman, 2000). *Varroa jacobsoni* has been redefined into two species, *V. destructor* Anderson and Trueman and *V. jacobsoni* encompassing 18 haplotypes following studies by Anderson and Trueman (2000) on the mite's mtDNA *CO1* gene sequences. Only two of the 18 haplotypes, a Korea and Japan/Thailand haplotypes have become associated with *A. mellifera* and both belong to the species *V. destructor*. Within the species complex, only the Korean-Russian type has been reported to cause damage to *A. mellifera* (Anderson and Trueman, 2000; Dainat *et al*., 2012a). *Varroa destructor* is considered the most devastating pest of honey bees worldwide. It causes varoosis, a disease of honey bee brood and adults, feeds on haemolymph of the brood and adult honey bee which weakens the immune system, induces morphological deformities, and reduces longevity in workers (Le Conte *et al*., 2010; van Dooremalen *et al*., 2012). It also transmits and reactivates a number of viruses KBV, DWV, ABPV and others (Le Conte *et al*., 2010; Rosenkranz *et al*., 2010).

The spread of *Varroa* mites is facilitated by its host the honey bee during swarming, drifting or robbing. Beekeepers can also inadvertently contribute to their spread through movement of honey bee colonies between locations (Goulson *et al*., 2015; Sammataro *et al*., 2000). Only mature female mites survive on adult bees (workers and drones and rarely on queens) while the males (do not feed) and are not found outside of brood cells (Frazier, 2011). During reproduction, the female mite enters an uncapped cell containing honey bee larvae and waits three days after capping and then begins laying eggs (one unfertilized egg which give rise to a male and three to five fertilized eggs which give rise to female mites). After emergence, the immature and adult mite then feed through a hole on the cell made by the foundress mite. The developmental time from eggs to adult takes approximately six to seven days (Frazier, 2011; Sammataro *et al*., 2000).

The geographic distribution of *Varroa* mite is comparable to that of its host *A. mellifera.* This mite shifted from its original host, *A. cerena* in Asia to the western honey bee *A. mellifera* around the first quarter of the past century (Rosenkranz *et al*., 2010). *Varroa* mite was first reported in 1952 in USSR and in other Asian countries around 1950s. In the 1960s it spread to Europe, 1970s it was reported in South America and 1980s in the United States (Rosenkranz *et al*., 2010). The first record for Africa was in 1997 (Allsopp *et al*., 1997). Today, this mite is present in almost every continent except Australia (Rosenkranz *et al*., 2010). Although *V. destructor* has been listed as a notifiable infection (infections required by law to be reported to government authorities) by the World Organisation for Animal Health (OIE) (OIE, 2016), reports on its occurrence remains scanty in Africa (Figure 2.1). After this mite invaded countries bordering the Mediterranean, it rapidly spread to many parts of Africa including Algeria, Morrocco, Lybia, Tunisia, Niger, Egypt, South Africa, Botswana, Mozambique, Swaziland, Zimbabwe, Kenya, Tanzania, Uganda, Ethiopia, Ghana, Senegal, Nigeria, Benin, and some Islands of Africa such as Madagascar (Akinwande *et al*., 2012; Allsopp *et al*., 1997; Dietemann *et al*., 2009; Fazier *et al*., 2010; Begna, 2014). Some of these countries reported drastic decline in honey bee colonies as a result of this invasion (Allsopp, 2004; Dietemann *et al*., 2009; Rasolofoarivao *et al*., 2013). At present, there exists no scientific report on the occurrence of this mite in Central Africa including Cameroon (Figure 2.1). The impact of this mite on beekeeping and thus food security and biodiversity conservation, require detailed studies on its occurrence and impact in Cameroon and other African countries.

2.2.1.2 Tracheal mite *Acarapis woodi*

The tracheal mite is an obligate endoparasite of *A. mellifera*, usually found under the flat lobe on the first thoracic spiracle (Sammataro *et al*., 2013). This mite occurs in all members of the bee colony. They are often more abundant in drones than workers or queen (Sammataro *et al*., 2013). Two other *Acarapis* species, *A. externus*, and *A. dorsalis,* are not harmful to honey bees and can be differentiated from *A. woodi* by the location on the bee (Shimanuki and Knox, 2000). Heavy infestations by *A. woodi* in Canada and North America have been reported to increase bee mortality during winter. This mite has been listed as a notifiable infection by the OIE (OIE, 2016). Bees for export therefore must undergo sanitary control of this mite. Surveillance and reporting of *A. woodi* is also a requirement by all countries exporting bees and their products

(Sammataro *et al*., 2013). This mite has been reported in Europe, United Kingdom, North and South America, parts of Asia and Africa (Pirk *et al*., 2016; Sammataro *et al*., 2013). In Africa, very few reports exist on the occurrence of *A. woodi* (Figure 2.1). Surveillance of this mite in countries not yet in the list such as Cameroon is therefore needed to confirm its absence or report its presence in order for management options to be initiated.

2.2.1.3 *Tropilaelaps clareae*

The genus *Tropilaelaps* are brood parasites of the giant Asian *Apis* species of honey bees. They were first reported in the 1960's (Anderson and Roberts, 2013). *Tropilaelaps* mites are different from *Varroa* mite both in morphology and behavior. Unlike *Varroa* mite, adult *Tropilaelaps* are generally smaller and are much longer than wider. They are light brown in color, usually hold their first pair of legs upright, and are very mobile. They depend entirely on brood for survival since their mouthparts are not designed to feed on adult bees as does *Varroa* mite (Anderson and Roberts, 2013). Like *Varroa* mite, they can be found in both worker and drone brood cells (Anderson and Roberts, 2013).

There are four species of *Tropilaelaps*. These are *Tropilaelaps clareae, Tropilaelaps koenigerum, Tropilaelaps mercedesae and Tropilaelaps thaii*. *Tropilaelaps clareae* was first recorded on the giant Asian *Apis* species *A. breviligula*, *T. mercedesae* on *A. dorsata*, while *T. thaii* and *T. koenigerum* were first recorded on *A. laboriosa* (Anderson anf Roberts, 2013). Only two of these mites, *T. clareae* and *T. mercedesae,* have switched host to *A. mellifera*. *Tropilaelaps mercedesae*, formerly *T. clareae* (Anderson and Roberts, 2013) can infests up to 90% of brood and lead to death of the entire colony if not treated (Woyke, 1984). Their damage to colonies is similar to that by *Varroa* mite (Sammataro *et al*., 2000; Shimanuki and Knox, 2000; Ritter and Akratanakul, 2006). *Tropilaelaps mercedesae* is the most widely distributed *Tropilaelaps* spp in Asia and the most likely to spread to other parts of the world (Anderson and Roberts, 2013). They are therefore a potential threat to apiculture worldwide. There is therefore the need for more research on the occurrence of these mites in African honey bee races.
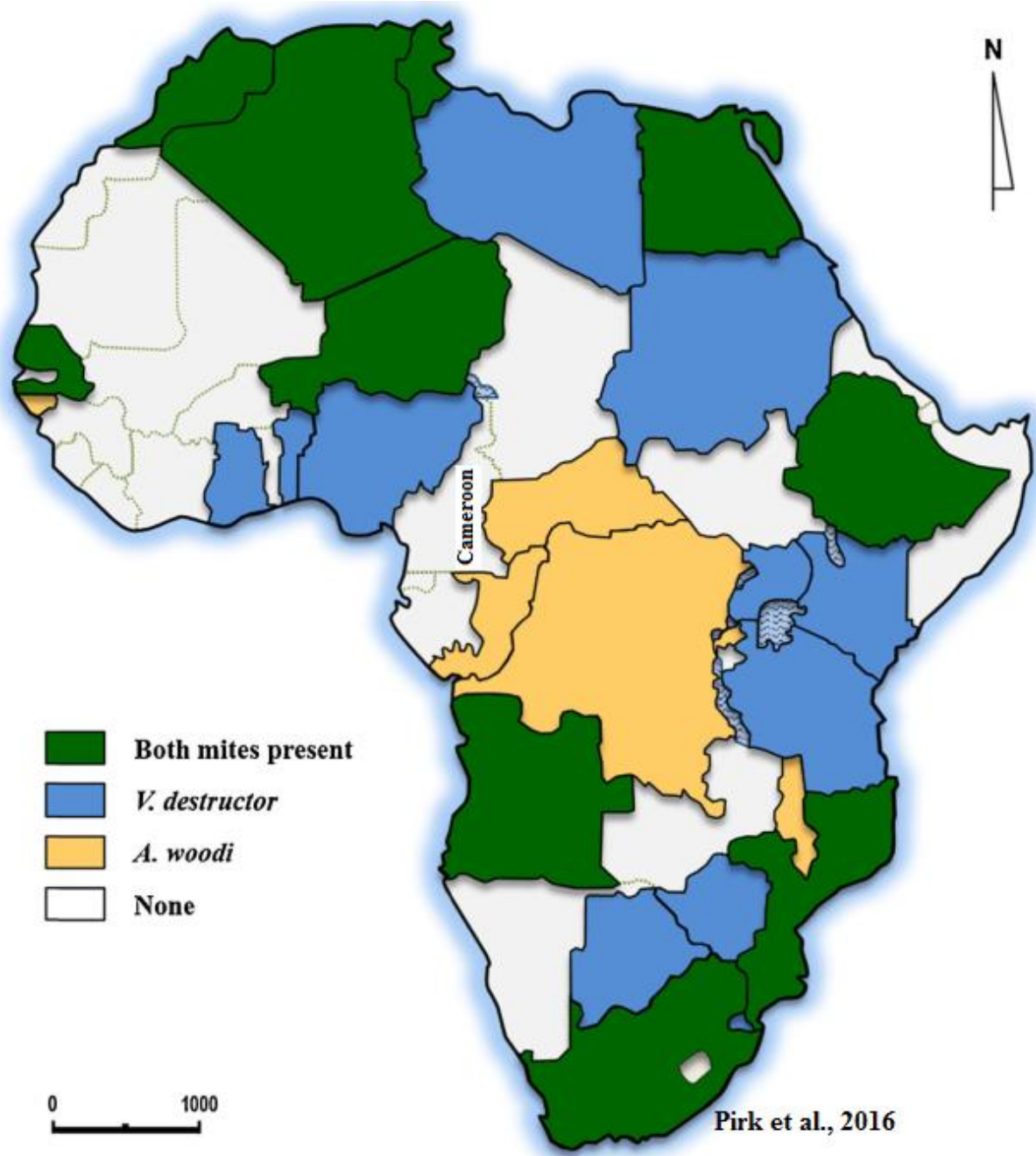
Figure 2.1 Geographic distributions of *Varroa destructor* and *Acarapis woodi* in Africa. (Source: Pirk *et al*., 2016).

2.2.1.4 Endoparasitic flies

Dipteran families such as Phoridae, Conopidae, Tachinidae, Sarcophagidae and Calliphoridae include species with larvae that are endoparasitic to honey bees (Hepburn and Radloff, 1998; Knutson and Murphy, 1990).

Conopids

Conopids, also known as the thick-headed flies, are known to parasitize both honey bees and wasps (Knutson and Murphy, 1990). They have a hymenopteran appearance and are usually black and yellow wasp mimics. Parasitoid conopids deposit their larvae on the bee which then burrow in to the abdomen and feed, pupate and emerge as adults. Of the many conopid species infesting honey bees worldwide, only one *Physocephala sp* has been reported to occur in Africa (Knutson and Murphy, 1990). It was reported from Southern Africa and Uganda (Hepburn and Radloff, 1998). There is therefore the need to expand the surveillance of these parasitoids in other parts of Africa.

Sarcophagids

The Sarcophagidae family contains approximately 2,500 species worldwide. Most sarcophagids are saprophagous while few members are endoparasitic on honey bees and wasps. Examples include *Senotainia tricuspis,* an endoparasite of honey bees while two *Sarcophaga* sp are saprophages in honey bee hives (Knutson and Murphy, 1990). Unlike conopids, endoparasitic Sarcophagids lay their eggs on the bee. The larvae burrow into the thorax where it develop in haemolymph then to the abdomen and out of the bee shell to pupate in the soil (Knutson and Murphy, 1990). *Senotainia* sp have a worldwide distribution they have been recorded in many north African countries, including Algeria and Tunisia, and in Australia (Hepburn and Radloff, 1998; Knutson and Murphy, 1990).

Calliphorids

The family Calliphoridae (blow flies), contains approximately 1,000 species worldwide (Knutson and Murphy, 1990). They are colorful and are mostly scavengers. Of the few parasitic species

only one, in the genus *Pollenia* has been recorded to parasitize honey bees (Knutson and Murphy, 1990). The development of *Pollenia* in honey bees is similar to that of Sarcophagids. Infestations by calliphorids are rare and thus they are not considered economic pests of honey bees. In Africa, they have been recorded in Egypt (Knutson and Murphy, 1990).

Tachinids

There are about 8,000 species of the family Tachinidae worldwide. Their larvae are endoparasites of a variety of insects including honey bees. The only honey bee endoparasitic tachinid is *Rondanioestrus apivorus* which has been reported to cause apimyiasis (Knutson and Murphy, 1990). Unlike sarcophagids and calliphorids, larvae of *R. apivorus* burrow in to the honey bee abdomen where they feed then emerge and pupate in the soil. *Rondanioestrus apivorus* have been reported to occur in South Africa, Uganda, and other sub-Saharan African countries. They are infrequent and require no control measures (Hepburn and Radloff, 1998; Knutson and Murphy, 1990).

Phorids

Phorids have become significant pests in apiculture recently with reports of parasitism in honey bee by *Apocephalus borealis* (Core *et al*., 2012). This parasitic fly has been implicated as a potential vector of DWV and *Nosema ceranae* and as one of the factors in Colony Collapse Disorder (CCD) in the US (Core *et al*., 2012). Another Phorid, *Megacelia rufipes* also referred to as the 'humpbacked fly' or 'coffin fly' due to its hump-backed appearance and ability to penetrate closed containers or buried carrions respectively (Disney, 2008), has also been reported infesting honey bees in Italy (Dutto and Ferrazzi, 2014). Phorid species of the genus *Phora* are also known to parasitize honey bees for example *Phora incrassate* also known as *Hypocera incrassata* or *Borophaga incrassata*. They actually belong to the genus *Megaselia* (Knutson and Murphy, 1990). This genus has been reported to attack honey bees in the Democratic Republic of Congo (DRC), Senegal, Togo and Benin (Hepburn and Radloff, 1998; Knutson and Murphy, 1990). *Pseudohypocera kerteszi,* also called *P. nigrofascipes* is also known to attack bees in South America where it feeds on pollen, bee larvae and pupae. The Phoridae genus *Melaloncha* also contains honey bee parasitic flies. More than eight species of this genus have been reported

parasitizing honey bees in Europe and in the America (Knutson and Murphy, 1990). Bees parasitized by this fly tend to fly or move with difficulty followed by subsequent death. This parasitic fly deposits its eggs into the abdomen of the honey bee where they hatch and develop in to the larvae. The larvae then move into the thorax after killing the bee where they pupate and later emerge as adults. Most *Melaloncha* spp are common in South America where they parasitize stingless bees leading to a condition called autumn disease (Knutson and Murphy, 1990). None of the phorid species reviewed here has been associated with honey bees in Cameroon. It is therefore important to ascertain their honey bee pest status in Cameroon.

2.2.2 Honey bee pests

Honey bees are attacked by a large number of arthropod pests that cause significant damage to honey bee colonies. These pests are widely distributed globally and includes hive beetles, moths and lice (Neumann *et al*., 2016; Pirk *et al*., 2016; Smith *et al*., 2013).

2.2.2.1 *Aethina tumida* and other Nitidulids

The small hive beetle *Aethina tumida* Murray (SHB) is an indigenous pest in Africa. It has been reported in over half of the African continent (Neumann *et al*., 2016). It has also recently (approximate a decade ago) invaded the United States of America and Australia causing damage to honey bees (Evans and Shimanuki, 2000; Lounsberry *et al*., 2010). The developing larvae are the most destructive stage. They feed on pollen and brood, and contaminate honey with their feces in the process (Neumann *et al*., 2016). Their eggs are usually smaller than those of bees and are laid in crevices within the hive. These beetles are known to puncture the cell capping of sealed brood or the sides of empty cells to oviposit under the pupae in adjoining cells (Ellis *et al*., 2003a; Neumann *et al*., 2013). Although generally considered a minor pest to African honey bees (Neumann *et al*., 2016) its role in the transmission of honey bee viruses and bacteria cannot be overlooked (Eyer *et al*., 2009). It is therefore listed by OIE as a notifiable infestation (OIE, 2016). This makes it one of the most important beetle pests of honey bees around the world.

Beside the SHB, other similar Nitidulid beetles infesting honey bee colonies include *Cychramus luteus* (Neumann and Ritter, 2004). This beetle is very similar to the SHB especially with freshly

emerged adults. They are similar in color, size, and basic morphology. They can however be distinguished using key features such as the shape of the ovipositor, antennae and color of the pronotum and elytra. *Cychramus luteus* is however less harmful to honey bee colonies compared with SHB. It is only occasionally present in colonies where it seeks shelter or pollen. It has only been reported to occur in Europe (Neumann and Ritter, 2004). Another Nitidulid, the dusky sap beetle *Carpophilus lugubris* has also been reported to infest honeybee colonies in Italy (Audisio *et al*., 2014). Although it is not a significant pest to honeybees, it can develop inside honeybee hives which implies that it has the potential to become a significant pest in the future (Audisio *et al*., 2014).

2.2.2.2 Large hive beetles

Large hive beetles are also becoming an increasing threat to honey bee colonies in Africa. The large hive beetle *Oplostomus haroldi* Witte (Coleoptera: Scarabaeidae) has recently been recorded in honey bee colonies in Kenya (Fombong *et al*., 2012; Torto *et al*., 2010a) and Tanzania (Njau *et al*., 2009). Another scarab beetle, the black hive beetle *Oplostomus fuligineus* Olivier a predacious African scarabaeid species that has long been recorded as pests in bee hives in Southern Africa (Donaldson, 1989), has also been recently reported to occur in honey bee colonies in Kenya (Fombong *et al*., 2012). These two scarab beetles species *O. haroldi* and *O. fuligineus* causes damage not only on pollen and honey bee comb, but also on brood through their feeding activities (Fombong *et al*., 2012; Neumann *et al*., 2016). They reproduce outside of colonies in cattle dung and are less harmful compared with SHB infestations (Neumann *et al*., 2016).

Other less harmful beetle species infesting honey bees include *Cryptophagus hexagonalis* (Coleoptera: Cryptophagidae). They are often found in low numbers and reproducing in the debris of honey bee colonies. This beetle occurs in Europe, part of Asia and North America (Haddad, 2008).

2.2.2.3 Moths

There are two main types of moths that infest and cause damage to honey bee colonies. These include wax moths and death's head hawkmoths (Ellis *et al*., 2013; Moritz *et al*., 1991).

Wax moths

There are two types of wax moths, the greater and lesser wax moth. The greater wax moth, *Galleria mellonella* L. (Lepidoptera: Pyralidae) is the most destructive pest of honeycombs (Ellis *et al*., 2013). Comb damage can also be caused by the lesser wax moth, *Achroia grisella*, and the Mediterranean flour moth, *Anagasta kuehniella* (Shimanuki and Knox, 2000). These moths are serious pests in tropical and subtropical climates, where warm temperatures favor their rapid development (Ben, 1999). The larval stage of *G. mellonella* is the destructive stage and feeds on honey, nectar, pollen and other impurities in bee wax (Ellis *et al*., 2013). Wax moths generally attack mostly weak colonies or those under heavy infestation from major pests. Their attacks can therefore speed up colony collapse or absconding (Hepburn and Radloff, 1998; Shimanuki and Knox, 2000).

Death's head hawkmoth

The death's head hawkmoth *Acherontia atropos* causes damage to colonies by feeding on honey and nectar. This moth can easily manoeuvre its way into the honey bee colony through chemical camouflage and has a thick cuticle that protects it from bee sting (Moritz *et al*., 1991). Inside the hive they use a chemical camouflage and remain still to prevent attack by bees. Their large sizes also make it difficult for bees to control (Moritz *et al*., 1991). Hawkmoths infestations can be more damaging to colonies than wax moths since they are able to deplete honey bee food stores within a short space of time if not managed. This moth is widely distributed in Europe, Africa and Asia (Ben, 1999; Moritz *et al*., 1991). *Acherontia atropos* has been reported infesting honey bee colonies in Madagascar (Rasolofoarivao *et al*., 2013).

2.2.2.3 Bee louse

The bee louse, *Braula coeca* is actually a wingless fly and feeds on honey (Frazier, 2011). Adult bee lice cause little harm to adult bees but the larvae can damage the appearance of comb honey. They can be found on adult workers and queens. Although *B. coeca* resembles *Varroa* mite in size and color (Frazier, 2011; Shimanuki and Knox, 2000) they have six legs while *Varroa* mite has eight (Frazier *et al*., 2011).

2.3 Predatory flies of honey bees

Asilids, also known as robber flies, are the only well known example of predatory flies of honey bees. About 14 genera in four subfamilies (Apocleinae, Dasypogoninae, Laphriinae and Stenopogoninae), have been recorded as honey bee predators around the world (Londt, 1993). Reports on the occurrence of predatory flies in Africa have mostly been linked to countries such as Ivory Coast, Malawi, Namibia, Kenya, Zimbabwe and most parts of South Africa (Londt, 1993).

2.4 Occasional arthropod pests and other hive invaders

There are many other honey bee pests and predators which are present occasionally and either prey on honey bee brood and/or adults or feed on their stored food. These include ants, spiders (Orb-weaver spider), dragonflies, and praying mantids. Others invade the hive and steal honey (e.g. kleptoparasites) or colonise parts of the hives (e.g. termites and carpenter bee) (Hepburn and Radloff, 1998; Ritter and Akratanakul, 2006; Shimanuki and Knox, 2000).

Ants are the most commonly encountered honey bee hive invaders in the tropics of Africa and Asia (Hepburn and Radloff, 1998; Ritter and Akratanakul, 2006). They are generalist predators that feed on brood, adult bees and food stores such as honey. Their invasion can lead to colony death or absconding if not controlled (Hepburn and Radloff, 1998; Ritter and Akratanakul, 2006). Ant species such as the weaver ant *Oecophylla longinoda*, the black ant *Monomorium indicum*, *Monomorium destructor*, *Oligomyrmes* spp, *Dorylus* spp, the fire ants *Solenopsis* spp and *Formica* spp have been reported to infest honey bees in Asia (Ritter and Akratanakul, 2006). In Africa species such as *Dorylus* sp, *Companotus pennsylvanicus* and *M. minimum* have been

reported to invade honey bee colonies (Akinwande *et al*., 2013; Kugonza *et al*., 2009). Ants are also a problem to beekeeping in Cameroon although the identity of the species is not known.

2.5 Pseudoscorpions, honey bee pests or natural enemies?

Pseudoscorpions superficially resemble true scorpions, but lack the elongated metasoma (tail) and telson (sting) (Harvey, 2002, 2007). There are over 3400 described species in over 400 genera and 26 families (Harvey, 2007; Murienne *et al*., 2008). Among these, sex genera are associated with honey bees and include *Ellingsenius* (*E. sculpturatus*, *E. hendrickxi*, *E. perpustulatus*, *E. ugandanus, E. globossus*and *E. indicus*), *Chelifera* (*C. cancroides*), *Thalassochernes* (*T. taierensis*), *Nesochernes* (*N. gracilis*)*, Heterichernes* (*H. novaezealandiae*) and *Neobisium* (*N. validum* and *N. muscorum*) (Beier, 1948; Dippenaar-Schoeman and Harvey, 2000; Donovan and Paul, 2005; Donovan and Paul, 2006; Girisgin *et al*., 2013; Read *et al*., 2014; Semmar *et al*., 2014). In spite of their mellitophilic nature, the role of pseudoscorpions in honey bee hives remains controversial with some authors considering *Ellingsenius* species as generalists, preying on both host bees and on parasites (Judson, 1990), while other authors consider them as potential biocontrol agents, preying on mites and wax moth larvae (Thapa *et al*., 2013; van Toor *et al*., 2015). In tropical Africa, reports of melittophilic pseudoscorpions occurrence have largely been from the Southern and Eastern Africa with little information about their diversity and ecological role in other parts of the continent (Dippenaar-Schoeman and Harvey, 2000; Judson, 1990). Generally, there is limited knowledge on pseudoscorpion diversity in Africa compared to Europe and the Americas (Harvey, 2007).

2.6 Management of honey bee pests

Honey bees are able to defend themselves against pests and associated diseases through various behavioural mechanisms resulting in removal or overpowering of pest and diseases and through innate genes which confer natural resistance against various diseases (Cremer *et al*., 2007; Guzman-Novoa *et al*., 2004; Spivak and Reuter, 2005). Collective defences can be both prophylactic and activated in the presence of a pest or disease. Such consist of behavioural, physiological and organisational adaptations of the colony that prevent parasite entrance, establishment and spread (Cremer *et al*., 2007; Starks *et al*., 2000). However, when honey bee

colony defensive tactics are overcome, then the infested colony faces the danger of collapse. It is at this stage that extra-colony defenses in the form of pest management tools by the beekeeper become vital in re-establishing pest-bee stability and maintaining the integrity of the colony. There are a number of management options employed by beekeepers against honey bee pests. These pest management activities may occur at different times of the year depending on location, colony requirements and type of pest, parasite or disease infestation (Rosenkranz *et al*., 2010).

Mite pests

Biological, chemical and cultural control methods are available for the management of *Varroa* mites. Chemically, *V. destructor* can be managed using the miticides Coumaphos, Fluvalinate, Formamidine, Cymiazole as well as formic acid, sucrose octanoate, thymol (Apiguard) and thymol+eucalyptus oil + menthol (Api-Life VAR) (essential oils) among others (Rosenkranz *et al*., 2010). Biological control of *V. destructor* involve trapping of mites in worker or drone brood and killing them using heat or other chemicals. Many other biocontrol methods including the use of *Metarhizium anisopliae* and pseudoscopions are still under investigation (Rosenkranz *et al*., 2010; van Toor *et al.*, 2015). The application of powdered sugar treatment, requeening and breeding for *Varroa*-sensitive hygienic behaviour has also been used as management options (Harris *et al*., 2010; Spivak and Reuter, 2005). Many of the control methods against *Varroa* are effective against *Trapilaelaps* mites (Anderson and Roberts, 2013). Tracheal mites can be controlled chemically by applying formic acid or menthol during periods of non-nectar flow. Other management options include the use of vegetable oil, grease patties and resistant honey bee stocks (Anderson and Roberts, 2013).

Endoparasitic flies

Endoparasitic flies are generally considered pests of non-economic importance, thus development of control measures has not been necessary (Knutson and Murphy, 1990). However, recent reports on *A. borealis* as a potential vector or reservoir of DWV and *Nosema ceranae* and also as one of the factors in Colony Collapse Disorder in the U.S.A. (Core *et al*., 2012) have led to increased attention on the role of endoparasitic flies on bee health.

Hive beetles

The SHB can be controlled using a combination of chemical, biological and cultural (mechanical) methods which can be applied either inside or outside of beehives. Inside hives, methods include manual removal of adults (using as aspirator), use of kitchen cleaning wipes to trap adults beetles, bottom board and top board traps with killing agents, interception traps for wandering larvae and modified entrances to limit SHB colony infestation (Neumann *et al*., 2016; Torto *et al*., 2010a). The organophosphate Coumaphos and Permethrin can also be used to control SHBs (MAAREC, 2008). Outside of the beehive, baited pole traps can be use to attract and kill adult SHBs while UV light can be use in honey houses to attract SHB wandering larvae (Neumann *et al*., 2016). Early extraction of harvested honey and comb storage in less humid areas may also limit SHB damage (MAAREC, 2008). Other methods such as sterile insect techniques, use of soil concrete around the hive to prevent pupation, soil treatment around hives with pesticides, application of entomopathogenic fungi or nematodes in the soil around hives, removal and treatment of top soil around the apiary are less efficient methods for control (MAAREC, 2008; Neumann *et al*., 2016). Large hive beetles are most commonly managed using cultural methods (active killing of adult beetles).

Moths

Stored honey bee wax of five or fewer stacked supers without honey can be protected from moths using paradichlorobenzene crystals, while hives and equipment can be fumigated using aluminium phosphide (MAAREC, 2008). *Bacillus thuringiensis* (Bt) and parasitic wasps e.g. the pupae parasitoid *Brachymeria intermedia* (Nees) (Ellis *et al*., 2013; MAAREC, 2008) are the available biological control options. Cultural management of moths includes freezing comb honey and storage of equipment in dry or well-lit areas with good air circulation (MAAREC, 2008). However, the key to protecting honey bee colonies from wax moth, is maintaining a strong colony (Ellis *et al*., 2013; Hepburn and Radloff, 1998).

Minor, nuisance or opportunistic pests and predators

Minor and nuisance pests of honey bees such as robber flies, bee louse, and predators such as ants, wasps, earwigs, mice, birds, lizards, amphibians and other mammals are usually given less

attention and as such, there is no or very few management options (Hepburn and Radloff, 1998; MAAREC, 2008; Ritter and Akratanakul, 2006). Most of these minor pests are best controlled using cultural methods that aid the development of a strong colony that can defend itself and by putting in place mechanical barriers such as traps and fences (Ritter and Akratanakul, 2006).

2.7 Honey bee hives and beekeeping practices

A box or container where bees live is called the beehive (Caroll, 2006). There are a number of different hive types used by beekeepers across Africa. The choice of the hive and beekeeping practices is governed by a number of factors including traditional beliefs or culture, education and convenience (Ingram, 2014; King, 2014). Hive types can generally be classified in to two main groups; fixed comb hives and moveable comb hives.

2.7.1 Fixed comb hives

Fixed comb hives are made from materials such as tree barks, hollowed out logs, basketwork, calabashes, clay, grass and bamboo (Caroll, 2006). These hives are generally frameless and honey bee combs are attached to the walls of the hives, thus the name fixed comb hives. Commonly used fixed comb hives in Cameroon include the Cylindrical Indian Bamboo (CIB) and the tapered Cylindrical Grass hive (CG). Other fixed comb hives such as the bark and clay pot hives are no longer in use while log hives are less frequently used (Ingram, 2014). These hives are affordable, easy to construct by the beekeepers themselves and are mostly installed on trees. The disadvantage of these hives is that they are less durable and difficult to manage. Honey harvesting and processing is also difficult while the quality and quantity of the harvested honey is relatively low compared to that of moveable comb hives (Ingram, 2014; King, 2014).

2.7.2 Moveable comb hives

The movable-comb hives, also called modern hives include the Langstroth hives (L) and Kenya Top Bar hives (KTB). These hives are more complex than the traditional fixed comb hives (King, 2014). Langstroth hives for example contain a bottom board, two hive bodies (brood chambers), honey supers (or boxes) of various depths (full, medium, and shallow), and a cover.

Each box contains wooden frames that hold the wax combs which serve as the nest substrate for the colony. Other hive components include an outer telescoping cover, inner cover, queen excluder, entrance reducer, and a hive stand (King, 2014; Westendorp, 2006). Although these hives are more expensive than the traditional hives, they are easier to manage and they produce honey of high quality and quantity (Ingram, 2014; Westendorp, 2006). The most commonly used modern hive type in Cameroon is the KTB hive and costs about US$ 35. It is about half the cost of the Langstroth hive and about three times more expensive than the traditional hives (Ingram, 2014).

2.7.3 Honey bee hive design and pest infestation

Although beekeepers in Africa use a combination of both traditional hives (fixed comb hives) and modern hives (moveable comb hives) (Ingram, 2014; King, 2014), the impact of these hives on colonization and pest infestation is not well known. The only attempt to document such impact in Africa was by Kugonza *et al*. (2009) in Tanzania where they reported that fixed comb grass hives were able to colonize faster and were less frequently infested by pests compared with the modern hives (Langstroth and KTB). Besides this attempt, little is known on the influence of hive types on the infestation levels of honey bee pests. In Cameroon, the predominant hives used by beekeepers are the CIB, CG, and KTB (Ingram, 2014). No information exists on the impact of these hives on pest infestation and establishment. It is therefore imperative to fully understand their influence on pest infestation for improved bee health.

CHAPTER THREE

GENERAL MATERIALS AND METHODS

3.1 Description of study agroecological zones

This study was carried out in apiaries located in four major ecological zones in Cameroon. These zones include, the highland Sudan savannah of the North West (NW) and West regions, Guinea Savannah zone of the North (NRD) and Adamawa regions, lowland dense evergreen forest of the South West (SW) and South regions, and degraded evergreen forest of the East (East) and Central regions (Figure 3.1). These areas are known to be the major honey and hive product producing areas (Ingram and Njikeu, 2011).

In the NW and West, apiaries were located at altitudes of between 1191 to 2208 mm (Table 3.1). This zone is cool and humid with annual temperatures of about 21 °C and rainfall of about 1700 mm. The rainy season is divided into the long rainy season (April/May to November), and short rainy season (March to April/May). The dry season occurs between November to March (Pamo, 2008). The hills of this savannah are dominated by grass fields with sparse stunted trees adapted to its harsh dry season (Pamo, 2008). Raffia palm bushes are commonly found in the valleys (Hughes and Hughes, 1992). Many economically important crops such as *Coffee arabica*, *Zea mays* (maize), *Solanum tuberosum* L. (Irish potatoes), *Colocasia esculenta* (cocoyam), *Dioscorea alata* (yam), *Phaseolus vulgaris* (beans), and many varieties of vegetables are also common (Pamo, 2008).

Most of the Guinea savannah zone of the North and Adamawa has similar ecology to the Sudan savannah with some subtle differences between them. The zone is warmer and drier with average annual temperature of 25 °C and low humidity. The dry and rainy seasons are similar to those of the Sudan savannah (Pamo, 2008). The vegetation of this savannah region is a mixture of thick woodland and grassy undergrowth. Commonly grown crops in this zone include *Manihot esculenta* (cassava), cocoyams and plantain, with robusta coffee (*Coffea robusta*) being the main cash crop. Most of the *Raphia* sp. found in the Sudan savannah also occurs in this region (Hughes and Hughes, 1992).

In the SW, sampling was done in apiaries located at low altitudes ranging between 239 to 614 mm (Table 3.1). The climate of the lowland dense forest zone is generally characterized by high temperatures with an average of 26.4 °C in the coastal lowlands and 23.5 °C in the rain forests. This zone has an annual rainfall of > 2000 mm and high relative humidity. There are three seasons; a dry season from December to March; a short rainy season from March to June and a long rainy season from September to November/December (Pamo, 2008). This forest zone consists of a continuous canopy of leaves with very thin undergrowth. It is unique for its huge plantations of economic crops such as *Theobroma cacao* (cocoa plant), papaya, bananas, oil palm, and rubber plants (Pamo, 2008). Many trees of economic importance such as *Milicia excelsa* (Iroko), *Entandrophragma spp.* (Mahogany), *Diospyros sp* (Ebony) and *Triplochyton scleroxylon* (Obeche) can also be found in this forest zone (Pamo, 2008).

The degraded forest zone of the East and Centre lies mid-way between the NW and SW in terms of altitude and vegetation cover. Apiaries in this zone were located at altitude ranging between 631 to 668 mm (Table 3.1). The climate of this forest zone fluctuates between that of the SW and NW as temperature and rainfall decreases from the coastal evergreen forest towards the degraded forest zones (Pamo, 2008). Compared with the SW, this zone consists of very thin undergrowth where a lot of human activities have led to deforestation.
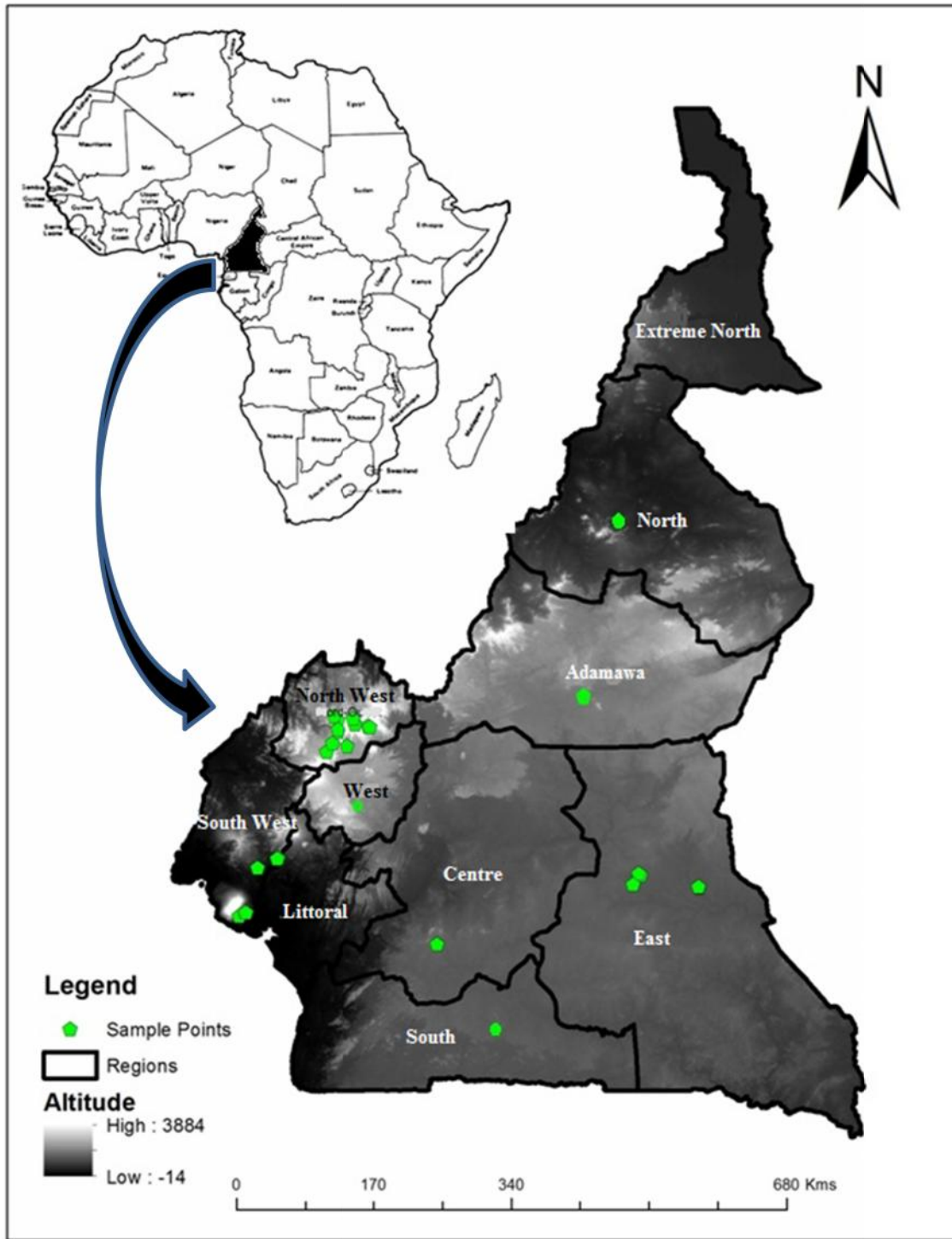
Figure 3.1 Map of Cameroon showing study sites

Table 3.1 General honey bee subspecies and pest survey information showing the ecological zones, location of apiary sites and number of colonies sampled during each season

Ecological zone	Apiary site	Apiary ID	Coordinates	Altitude (m)	Hive type	Colonies assessed in D	Colonies assessed in W	Colonies assessed in EW	Colonies used for HB races survey
Sudan Savannah of the North West (NW)	Belo 1 (BE1)	1	N 06.18002°, E 010.33402°	1203	KTB	10	9	9	5
	Belo 2 (BE2)	2	NA	NA	KTB	2	0	0	
	Laikom (LK)	3	N 06.28586°, E 010.34344°	1957	KTB	10	10	10	5
	Ekuijua (EJ)	4	N 06.33059°, E 010.29085°	1836	KTB	6	0	5	5
	Bamenda-Nkwe (BN)	5	N 05.93303°, E 010.20830°	1710	KTB and	0	7	0	5
	Kumbo (KBO)	6	N 06.20772°, E 010.69884°	1714	KTB	4	4	4	
	Bamoka Ndop (ND)	7	N 05.99665°, E 010.44055°	1191	KTB	0	3	0	
	Menda-Nkwe (MN)	8	N 05.89871°, E 010.19455°	2208	KTB and CIB	0	0	4 KTB,1 CIB	
	Kumbo1 (KBO1)	9	N 06.22320°, E 010.68106°	1714	CIB and CRP	7 CIB,1 CRP	6	9	2
	Kumbo 2 (KBO2)	10	N 06.20112°, E 010.69774°	1729	CIB	5	6	6	3
	Oku (OKU)	11	N 06.24181°, E 010.53170°	2192	CIB	8	9	4	5
	Njinikijim (NJ)	12	N 06.15760°, E 010.34155°	1429	CL	7	0	0	
	Bambili (BL)	13	N 06.02741°, E 010.27069°	1443	CL	2	0	0	
West	Bamenyam (BYM)	14	N 05.77412°, E 010.31653°	1197	KTB	0	5	0	5

Table 3.1 Continue

Ecological zone	Apiary site	Apiary ID	Coordinates	Altitude (m)	Hive type	Colonies assessed in D	Colonies assessed in W	Colonies assessed in EW	Colonies used for HB races survey
Degraded evergreen forest zone of the East (East)	Bertoua 1 (BT1)	15	N 04.58325°, E 013.68123°	668	KTB	4	4	8	5
	Bertoua 2 (BT2)	16	N 04.56307°, E 013.70180°	673	KTB	10	8	7	5
	Kaigama (KMA)	17	N 04.45830°, E 013.62112°	667	KTB	8	7	7	5
	Batouri (BTR)	18	N 04.42582°, E 014.35288°	648	KTB	7	8	8	3
	Batouri 1(BTR1)	19	N 04.46832°, E 014.36560°	631	KTB	3	3	2	2
Evergreen forest zone of the South West (SW)	Meveo village (MV)	20	N 04.11218°, E 009.23868°	614	KTB	5	5	9	5
	Buea 2 (Molyko) (BU2)	21	N 04.15185°, E 009.29668°	539	KTB	5	5	7	3
	Bomaka, Buea 1(BU1)	22	N 04.14539°, E 009.31357°	484	KTB	4	5	2	2
	Kumba (KBA)	23	N 04.64715°, E 009.44385°	236	KTB	7	7	4	5
	Tombel 1 (TOB1)	24	N 04.74998°, E 009.66188°	457	KTB	3	3	3	3
	Tombel 2 (TOB2)	25	N 04.75303°, E 009.67373°	522	KTB	6	5	7	2
	Other Zones/Localities where honeybee samples were collected for morphometric and molecular analysis								
Degraded evergreen forest of the Centre	Azom-Yaounde (YD)	26	N 03.67631°, E 011.45515°	742	KTB	0	5	0	5
Evergreen forest zone of the South	Ebolowa (EWA)	27	NA	NA	KTB	1	5	0	5

Table 3.1 Continue

Ecological zone	Apiary site	Apiary ID	Coordinates	Altitude (m)	Hive type	Colonies assessed in D	Colonies assessed in W	Colonies assessed in EW	Colonies used for HB races survey
Guinean Savannah zone of Adamawa	Ngaoundere (ADA)	28	NA	NA	KTB/Grass hive	0	5	0	5
Guinean savannah North	Garoua (GA)	29	NA	NA	Grass hive	0	5	0	5

KTB, Kenya Top Bar hive; CIB, Cylindrical Indian Bamboo hives; CL, Cylindrical Log hives; CRP, Cylindrical Raffia Palm hives; D, dry season; W, Short rainy season; EW, long rainy season; NA, not available.

3.2 Beehive types encountered during field surveys

A total of six hive types were encountered during field surveys for honey bee races and their associated arthropod pests (Chapter 4 and Chapter 5, respectively). These are the Kenya Top Bar (KTB), Langstroth (L), Cylindrical Indian Bamboo (CIB), Cylindrical Raffia Palm (CRP), Cylindrical Grass (CG) and Cylindrical Log (CL) hives. Of these, KTB, CIB, and CL were commonly encountered during field survey (Figure 3.2). The KTB hive is the most widely used hive type across Cameroon while the CIB and CL are commonly used by beekeepers in some localities in the North West (Table 3.1).

The Langstroth hive had similar dimensions (length x width x height: 50.5 cm x 41.3 cm x 24.5 cm) to the standard langstroth hive (King, 2014) except for the shape, presence of fixed bottom board and absence of queen excluders between brood boxes. The KTB hive varied slightly in size from one locality to another with the hives possessing a frame capacity of 19 - 25 top bars. The three traditional hive types (CIB, CRP, and CL) were all cylindrical in shape, without any frames or crossbars and differed in their construction material (Figure 3.2). The CIB was made predominantly from Indian bamboo (*Bambusa* sp.), the CRP from the frond stalk of raffia palms, and the CL from the trunk of oil palms (*Elaeis guineensis*). The CRP and CIB hives were wrapped externally with grass and the inner surfaces divided into two compartments (using pieces of Indian bamboo). These include a narrow 'brood chamber' close to the hive entrance and a large 'honey chamber' at the rear (Figure 3.2 D). This inner modification minimises destruction of brood during harvesting. The CL on the other hand had no inner partitions (Figure 3.2 F).
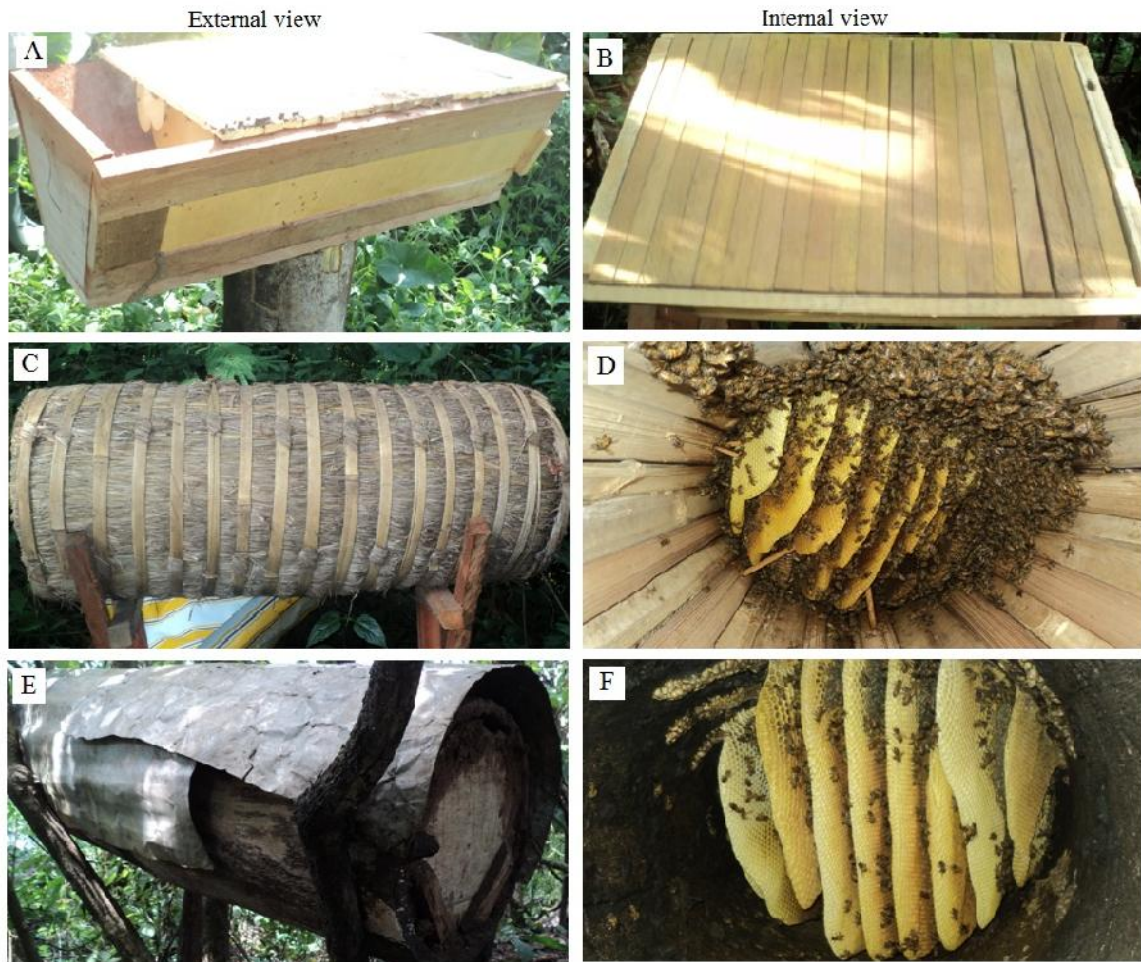
Figure 3.2 Internal and external view of the common beehive types encountered during honey bee races and pest survey in Cameroon. The hive types include Kenyan Top Bar (A and B) Cylindrical Indian Bamboo (C and D) and Cylindrical Log (E and F).

3.3 General sampling procedures

The honey bee races and pests assessment took place during the dry season, short rainy season and end of the long rainy season. This covered the period from January 2014 to December 2014. Within each of the major ecological zones, a minimum of 4 apiaries were selected for sampling from at least 4 different districts. In each apiary, about 60 and 100 % but not more than 10 colonies were randomly selected for sampling. Sample size was therefore depended on the available number of colonies. Each colony was assigned a unique number, which were mixed and picked one at a time. Over 350 honey bee colonies were assessed in 24 apiaries from various hive types throughout this study (Table 3.1). All collected pests and honey bee samples with the exception of adult wax moths and other lepidopterans (that were preserved dry), were preserved in 95 % ethanol in separate tubes

according to species and colony number for subsequent morphological and molecular analysis.

Sampling of pests and honey bees from the modern hives (e.g. KTB) was carried out following standard procedures (Ellis and Macedo, 2001; Torto *et al*., 2010b; Neumann *et al*., 2013; Meixner *et al*., 2013). Sampling of *Varroa* mite and bee louse for example was carried out using the standard sugar roll assay (Ellis and Macedo, 2001). In this assay, worker bees were collected from the brood comb in to a jar. The bees were then mixed with sugar dust and shaken on a white surface where the mites and louse were collected. All available combs were further inspected for the presence of beetles and moths. Small hive beetles were collected using an aspirator, while moths and large hive beetles were collected using the hive tools and/or hand. In traditional frameless hives, there were some modifications of the standard sampling procedures. In assessing colonies in these cylindrical hives, the rear of the hives was gently opened and the inner surfaces thoroughly checked for the presence of pests, which were collected when present. Other escaping pests around the edges were collected. After the initial inspection, entrance of the hives were gently smoked for a period of between 30 to 40 seconds and a brief pause of between 60 and 120 seconds was observed to allow the smoke take effect and repel the bees to the rear of the hive. The rear was then reopened and bee samples collected for use in the honey bee races diversity study (Chapter 4) and in the sugar roll assay (Chapter 5). The assessment of colonies for the presence of mites and bee lice in honey bee colonies in the Central, South, West and Northern regions was done by collecting ~ 350 bees in to 95 % ethanol which were later examined for infestation in the laboratory (Chapter 5).

3.4 Hive type and apiary sites selection for the hive experiment

Three apiary sites were selected for the hive type experiment (Chapter 6). Two of these sites, Bamenda and Oku were located in the savannah zone of the North West, and the third sites, Buea in the forest zone of the South West (Table 3.1). The 3 hive types selected for use in this study include, KTB, CIB and L hive types. The KTB and CIB beehives are the most commonly used hive types in Cameroon while the standard L hive type was introduced since it is the most manageable and acceptable hive type in most areas around the world.

CHAPTER FOUR

APIS MELLIFERA SUBSPECIES AND MORPHOTYPE DIVERSITY IN CAMEROON

4.1 Summary

Knowledge on the morphotype and genetic diversity of *A. mellifera* populations is vital for the conservation of regional stocks of this important pollinator. In Cameroon, although some information exists on *A. mellifera* morphotypes, little is known on their genetic diversity. To bridge this knowledge gap honey bee samples were collected from four different geographic locations across Cameroon including those where *A. mellifera* morphotypes had previously been reported and locations not included in previous studies. Morphometric analysis was used to determine the morphotypes of *A. mellifera* while molecular markers (mtDNA) were used to determine whether the morphologically defined honey bee populations represent distinct subspecies or whether they are simply morphotypes of the same subspecies. Morphological analysis revealed the presence of three morphotypes of *A. mellifera* in Cameroon. These included the larger and darker bees of the highland Sudan savannah, the smaller and lighter bees of the lowland forest zones, and the smaller and darker bees of the Guinean savannah. Although the three morphotypes were morphologically similar to previously reported *A. mellifera* morphotypes, this study further expanded the range of the described bee populations of Cameroon by including samples from the lowland forest zones of the East and Central and showed further that most of the lowland bee populations were morphologically similar. Genetic diversity and phylogenetic studies revealed the existence of five *A. mellifera* subspecies haplotypes. Two of these haplotypes have previously been reported and three were new ND2 mtDNA haplotypes. *Apis mellifera adansonii* and *A. mellifera scutellata* were the known subspecies haplotypes. The *A. m. adansonii* haplotype was the most dominant and widespread while *A. m. scutelata*, the East and Southern African subspecies was detected in only a few colonies. Two of the new subspecies haplotypes were restricted to highland areas while one occurred in both the highland and nearby lowland areas. Genetic diversity analysis showed that all the highland subspecies haplotypes were more closely related to the lowland *adansonii* populations than to each other. This seems to suggest that they all descended from the same common lowland *adansonii* populations. However, none of these highland haplotypes were recorded in the lowland forest zones of the

East, Central, and South. This study therefore shows that honey bee populations of Cameroon are made of distinct subspecies haplotypes that are adapted to different geographic areas.

4.2 Introduction

There have been increasing global concerns on the conservation of both wild and managed pollinators of which the honey bee *Apis mellifera* are of primary concern due to their role in pollination and food security (Gallai *et al*., 2009). Efforts in the conservation of honey bee diversity and identity have been hindered inadvertently by beekeepers. This is usually out of ignorance through the movement of colonies between apiaries and across geographic locations (Goulson *et al*., 2015; Meixner *et al*., 2010). Conservation efforts can therefore actually be boosted by knowledge on the identity of the diverse honey bee subspecies and their ecotypes or morphotypes across the different geographical locations.

It is known that genetic diversity can improve the fitness and productivity of honey bees (Mattila and Seeley, 2007; Tarpy, 2003). Unfortunately information on bee diversity is not well known in Africa (Meixner *et al*., 2013). In Cameroon in particular, information on genetic diversity of honey bees is limited. The first attempt to study the diversity of *A. mellifera* subspecies in Cameroon was carried out about two decades ago by Hepburn and Radloff (1997). Their study was based entirely on external morphology and limited to mostly highland areas of the Northern and Western parts of Cameroon. The study led to the identification of three morphocluters of *A. mellifera.* A detailed study of the honey bee populations on other localities such as the Central, East and South which were not covered by Hepburn and Radloff (1997) are needed. Also, since almost two decades have passed since the study took place and the fact that African honey bees are made up of feral populations that tend to migrate often (McNally and Schneider, 1992) a revision of the honey bee subspecies diversity is necessary.

Although studies by Franck *et al*. (2001) on the molecular diversity of African honey bee subspecies recorded *A. m. adansonii* in Cameroon, their samples were collected from just one location around the central region and did not include localities where Hepburn and Radloff (1997) had earlier reported the occurrence of two different *A. mellifera* morphotypes. Molecular studies are therefore necessary to confirm whether the subspecies reported by Hepburn and Radloff (1997) are actually distinct *A. mellifera* subspecies or they are simply

morphotypes of the same *A. melifera* subspecies. Such information is vital for the conservation of locally adapted honey bee populations in reserve areas and to limit the loss of the local genetic bee stocks (Meixner *et al*., 2013). The aim of this study was therefore to identify the *A. mellifera* subspecies and their morphotypes in Cameroon and to determine their relatedness to other *A. mellifera* populations across Africa.

4.3 Materials and methods

4.3.1 Honey bee sampling

Honey bees (n = 30) were collected from 54 colonies in 24 apiaries located in 21 localities within the four different ecological zones (Table 3.1) as described in section 3.1 and 3.4. The sites were selected to ensure broad geographical coverage that represents the diverse ecological zones and the beekeeping areas of Cameroon. The honey bee samples were collected from the honey bee comb, preserved in 95% ethanol and taken to the laboratory for further analysis. A total of 520 individual honey bee samples (10 worker bees per colony) were processed for morphometric analysis. The dissected thoraces of 90 individual worker bees (54 individuals from colonies used in morphometric analysis and 36 more individuals from colonies in other apiaries) were used for molecular analysis. The samples were sorted to expand the range of sites sampled by Hepburn and Radloff (1997).

4.3.2 Morphometric analysis

The right forewing of each worker bee was dissected, rinsed consecutively in an ethanol series (95%, 70%, 50% and 20%), distilled water and carefully mounted following the methods described by Meixner *et al*. (2013) and Francis *et al*. (2014). Unlike in Francis *et al*. (2014) where they mounted the wings directly on microscope slides after removing from distilled water, in this study, the wings were mounted on microscope slides using DPX mountants. The slides were allowed for about 24 hrs to air-dry before measurements were taken. Other mountants such as gum Arabic, Euparal and Canada balsam can also be used (Meixner *et al.,* 2013). Other parts such as tergites 3, 4 and 5, and sternite 3 were also dissected and adhering tissues carefully removed using blunt forceps and then mounted as described above. Using the Leica microscope and LAS EZ software version 1.5.0 (Leica Application Suit, Switzerland), images of each of the body parts were captured and measurements taken. For measurement of wing morphometric characters, images of each

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wing was captured using the LAS EZ software and imported in to the tps Util program version 1.60 (Rohlf, 2013). The images were then build on tps Util and later imported in to tps Dig2 version 2.18 (Rohlf, 2015) for further analysis. A total of 18 Ruttner (1988) morphometric characters were measured per worker bee. These comprise nine measurements related to size, four angles, five characters related to pigmentation and one character for pilosity (Table 4.1; Figure 4.1).

The morphometric data of all the 18 characters were summarized and subjected to Principal Component Analysis (PCA) and Linear Discriminant Analysis (LDA) to determine possible separation of bee populations from the different geographic locations and to determine the linear combination of characters that best described the separation. Multivariate and univariate analysis of variance (MANOVA and ANOVA respectively) followed by Tukey HSD (honest significant difference) post hoc test to determine whether the bee populations differed from each other and in the measured characters. All data analyses were performed using the R statistical software (R Core Team, 2015).

Table 4.1 Morphometric characters used in honey bee morphotype delineation

Morphometric character	Category
Pigmentation of scutellum, B and K (36)	Pigmentation
Pigmentation of scutellum, cupolla (35)	Pigmentation
Pigmentation of tergite 2 (32)	Pigmentation
Pigmentation of tergite 3 (33)	Pigmentation
Pigmentation of tergite 4 (34)	Pigmentation
Tergite 3, longitudinal (9)	Size
Tergite 4, longitudinal (10	Size
Sternite 3, longitudinal (11)	Size
Wax mirror of sternite 3, longitudinal (12)	Size
Wax mirror of sternite 3, transversal (13)	Size
Fore wing length (17)	Size
Fore wing width (18)	Size
Cubital vein, distance a	Size
Cubital vein, distance b	Size
Wing angle J10 (26)	Angles
Wing angle N23 (30)	Angles
Wing angle B4 (22)	Angles
Wing angle O26 (31)	Angles
Length of cover hair on tergite 5 (1)	Pilosity

Ruttner (1988) character numbers are indicated in parenthesis
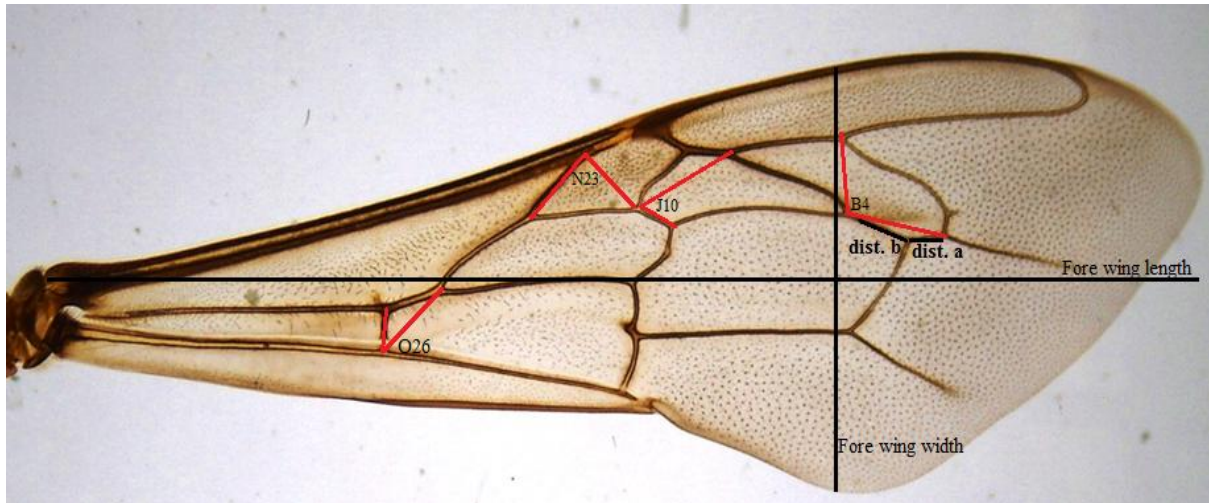
Figure 4.1 Honey bee wing showing wing angles and distance characters used in morphometric analysis.

4.3.3 Molecular analysis

Genomic DNA was extracted from the thoracic muscle of each worker honey bee using the ISOLATE II Genomic DNA kit (Bioline, UK) according to the manufacturer's instructions. Extracted DNA was stored temporally at -20 °C until further use. A 688 bp region of the mitochondrial DNA (mtDNA) encompassing tRNA ILE and part of the ND2 gene was amplified in a T100™ thermal cycler (Bio Rad) using primer pairs ILE: 5'-TGATAAAAGAAATATTTTGA-3' and L1: 5'-GAATCTAATTAATAAAAAA-3' (Arias and Sheppard, 1996). Amplification was performed using the Mytaq HS Mix 2x kit (Bioline, UK). Polymerase Chain Reaction (PCR) was carried out in a 25 µL final reaction volume containing 1 µL DNA template, 0.5 µL primers (20 µM each), 10.5 µL RNase free water, and 12.5 µL of Taq DNA polymerase. The thermal profile consisted of an initial denaturation step of 95 °C for 1 min, followed by 35 cycles of 95 °C for 20 s, 45 °C for 20 s, 72 °C for 15 s and a final extension step of 72 °C for 5 min. The PCR products were verified on a 1 % agarose gel stained with ethidium bromide against a 100 bp DNA Hyperladder (Bioline, UK) in a 1X TAE buffer and purified using the ExoSAP purification kit (Thermo Fisher Scientific, USA) adhering to the manufacturer's protocol. The PCR products were subsequently sequenced in both directions at Inqaba Biotech (Pretoria, South Africa).

The obtained DNA sequences were visually inspected and edited using Geneious version 8.1.8 (Biomatters, 2015). Edited sequences in Geneious were imported in to MEGA version 6.0 (Tamura *et al.*, 2013) for further analysis. Multiple alignments of the consensus ingroup

sequences together with sequences of representative honey bee subspecies extracted from GenBank were performed using the MUSCLE method within MEGA (Tamura *et al.*, 2013). The initial 634 bp ingroup sequences were trimmed to 579 bp after aligning to exclude the initial portions with insertions and for comparison with reported sequences. Representative haplotypes of honey bee populations from each geographic location where samples were collected together with sequences from reported *A. mellifera* subspecies were used for phylogenetic analysis. Phylogenetic relationship of the subspecies sequences was inferred using the neighbor-joining method employing the Kimura 2-parameter model (K2) with 1000 bootstrap replications (Nei and Kumar, 2000; Tamura *et al.*, 2013). Evolutionary divergence over sequence pairs within and between groups was estimated using the K2 model.

4.4 Results

4.4.1 *Apis mellifera* morphotypes

Principal component analysis of the morphometric data revealed the existence of three morphotypes of *A. mellifera*, 1, 2, and 3 (Figure 4.2) in Cameroon. Morphotype 1 comprise of honey bee populations from the highland savannah of the North West and West, morphotype 2 comprise of honey bees from the South West, Central and East; and morphotype 3 of honey bees from the Guinean savannah of the North in Adamawa. There was, however, minimal overlap of populations. The first three principal components (PC1, PC2 and PC3 respectively) accounted for about 59.1 % of the variation (PC1: 31.8 %, PC2: 17.3 %, and PC3: 10 %). Linear discriminant analysis (LDA) plot of the first and second discriminant function (DF1 and DF2 respectively) clearly confirmed the separation of the three morphotypes and explained 95.5 % of the variation in the data (DF1: 68.1 %, DF2: 27.4 %) (Figure 4.3). Multivariate analysis of variance (MANOVA) of all the measured characters simultaneously indicated that the three morphotypes were significantly different from each other (Wilks Lambda = 0.009; P = 0.001). The morphotypes differed significantly in 10 of the 18 morphometric characters subjected to the discriminant analysis ($P < 0.001$) (Table 4.2).
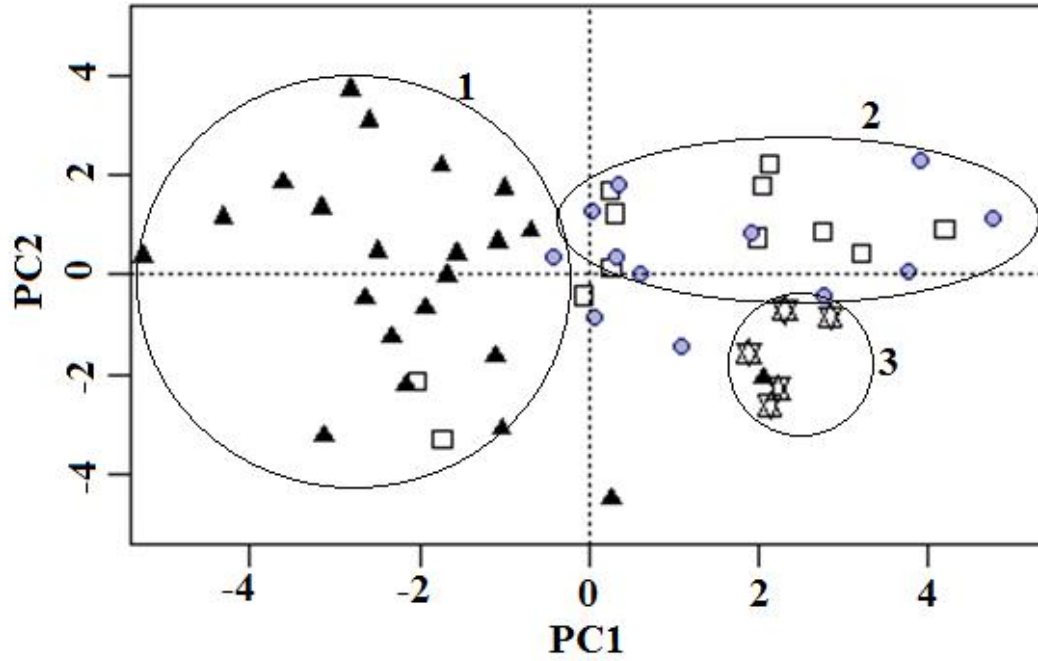
Figure 4.2 Principal component analysis (PCA) plot of *Apis mellifera* morphometric data showing the morphotypes across different geographic locations. The morphotypes include bee populations from: (1) North West and West (▲), (2) South West (□), East and Central (◉) and (3) Adamawa (𝕏).
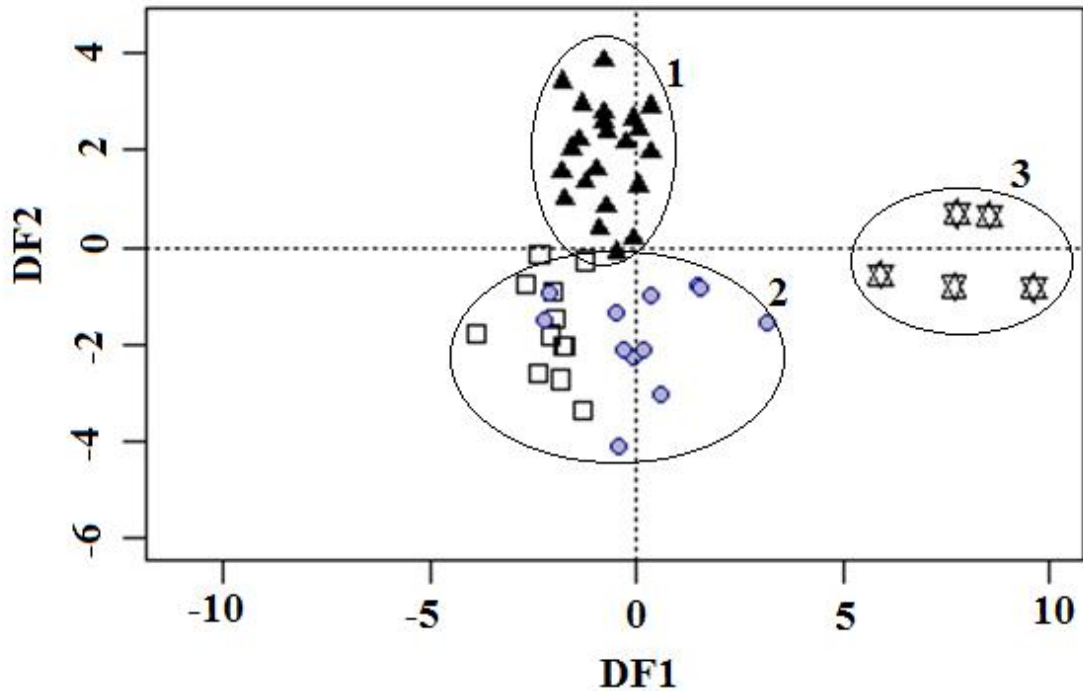
Figure 4.3 Discriminant analysis (DA) plot of *Apis mellifera* morphometric data showing the morphotypes across different geographic locations. The morphotypes include bee populations from: (1) North West and West (▲), (2) South West (□), East and Central (⬤) and (3) North in Adamawa (✡). DF 1 and DF2 = Discriminant Function 1 and 2 respectively.

Table 4.2 Mean ± SD of morphometric characters of *Apis mellifera* populations

Morphometric character	Morphotype 1 (n = 22)	Morphotype 2 (n = 24)	Morphotype 3 (n = 5)	F statistic	df	*P* value
Pigmentation of scutellum, B and K (36)*	1.08 ± 0.91^a	4.35 ± 2.62^b	0.28 ± 0.08^a	19.95	50	<0.001
Pigmentation of scutellum, cupolla (35)*	7.25 ± 0.74^a	8.38 ± 0.62^b	6.88 ± 0.37^a	16.67	50	<0.001
Pigmentation of tergite 2 (32)	6.61 ± 0.92^a	7.06 ± 0.73^a	7.24 ± 0.45^a	2.36	50	0.105
Pigmentation of tergite 3 (33)	5.77 ± 1.00^a	6.25 ± 0.66^a	6.16 ± 0.45^a	2.34	50	0.108
Pigmentation of tergite 4 (34)	3.73 ± 0.85^a	3.86 ± 0.85^a	3.94 ± 0.64^a	0.27	50	0.766
Tergite 3, longitudinal (9)*	2.04 ± 0.04^a	2.00 ± 0.04^b	1.99 ± 0.01^b	10.76	50	<0.001
Tergite 4, longitudinal (10)*	2.01 ± 0.03^a	1.95 ± 0.04^b	1.97 ± 0.02^b	15.24	50	<0.001
Sternite 3, longitudinal (11)*	2.49 ± 0.06^a	2.44 ± 0.04^b	2.35 ± 0.02^c	16.39	50	<0.001
Wax mirror of sternite 3, longitudinal (12)	1.16 ± 0.12^a	1.14 ± 0.03^a	1.09 ± 0.02^a	1.26	50	0.294
Wax mirror of sternite 3, transversal (13)	2.06 ± 0.24^a	2.07 ± 0.04^a	1.97 ± 0.02^a	0.76	50	0.471
Fore wing length (17)*	8.54 ± 0.10^a	8.30 ± 0.09^b	8.23 ± 0.03^b	44.98	50	<0.001
Fore wing width (18)*	2.93 ± 0.05^a	2.85 ± 0.04^b	2.81 ± 0.02^b	30.61	50	<0.001
Cubital index	2.37 ± 0.25^a	2.38 ± 0.19^a	2.49 ± 0.07^a	0.644	50	0.53
Wing angle J10 (26)*	50.60 ± 1.30^a	50.80 ± 1.02^a	48.30 ± 0.49^b	10.57	50	<0.001
Wing angle N23 (30)	91.41 ± 1.11^a	91.44 ± 1.69^a	90.68 ± 1.05^a	0.638	50	0.533
Wing angle B4 (22)*	102.27 ± 2.46^a	102.64 ± 3.21^a	96.44 ± 2.26^b	10.3	50	<0.001
Wing angle O26 (31)	39.65 ± 2.30^a	39.73 ± 2.16^a	41.64 ± 0.28^a	1.89	50	0.162
Length of cover hair on tergite 5 (1)*	0.28 ± 0.02^a	0.25 ± 0.02^b	0.21 ± 0.01^c	27.01	50	<0.001

Measurements are in millimeters, angles in degrees and pigmentation is based on Ruttner (1998) codes. Ruttner (1988) character numbers are indicated in parenthesis. Morphotype 1= Honey bees from the North West and West; Morphotype 2 = honey bees from the South West, Central, and East; and morphotype 3 = honey bees from the North in Adamawa. The morphotypes are ranked according to their power of discrimination. n = number of colonies, SD = standard deviation, * Indicate characters that are significantly different between morphotypes (ANOVA and Tukey HSD, = 0.05).

4.4.2 *Apis mellifera* genetic diversity

Results from the alignment of the honey bee ingroup consensus sequences revealed the possible existence of five *A. mellifera* haplotypes in Cameroon (Cam1 to Cam5) (Table 4.3). The five haplotypes showed large variation at multiple sites (26 sites) when compared with ND2 mtDNA sequences from reported subspecies across Africa and Europe (Arias and Sheppard, 1996). The number of variable sites was reduced to 15 when compared with available sequences from African subspecies only (Table 4.3). These variations were in the form of T   C and A   G type transitions (80%, n = 12 and 13.3% n = 2 respectively) and A   T type transversions (6.7%, n = 1). The average percent divergence over sequence pair between *A. mellifera* sequences from Cameroon and other African countries was found to range from 0.2% to 1.6% while divergence within the haplotypes from Cameroon ranged from 0.2% to 0.9%. With the exception of the haplotype Cam5 that diverged largely from the others (0.5 to 0.9%), all the other haplotypes (Cam2 to Cam4) were more closely related to Cam1 (0.2% divergence) than to each other (0.3% divergence).

Table 4.3 ND2 mitochondrial DNA variable regions among *Apis mellifera* haplotypes from Cameroon and subspecies from across Africa

A. mellifera subspecies /haplotypes	ND2 mitochondrial DNA variable sites															location
	3	24	156	162	165	240	387	396	425	456	498	516	549	555	576	
adansonii 1*	C	C	T	C	G	T	C	T	A	T	C	A	T	T	T	Nigeria
adansonii 2*	.	.	.	.	A	.	T	.	.	.	.	.	.	C	.	Senegal
capensis	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	South Africa
intermissa	.	.	.	T	.	.	T	.	.	.	T	.	C	.	.	Morocco
sahariensis	.	.	.	.	.	.	T	.	.	.	T	.	C	.	.	Morocco
lamarckii	T	T	.	.	.	C	T	.	G	C	T	.	.	.	.	Egypt
monticola	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	Kenya
*scutellata*1*	.	.	C	.	A	.	T	.	.	.	.	.	.	.	C	Kenya
scutellata 2*	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	South Africa
Cam1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	Cameroon (East, C, SW,NW)
Cam2	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	Cameroon (NW,W, SW)
Cam3	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	Cameroon (NW, NRD)
Cam4	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	Cameroon (ADA)
Cam5	.	.	C	.	A	.	T	.	.	.	.	.	.	.	C	Cameroon (SW, NW)

Dots = conserved sites; *Apis mellifera* subspecies from GenBank as reported by Arias and Sheppard, 1996; NW = North West; W = West; C= Centre; NRD = North; ADA = Adamawa.

4.4.3 Phylogenetic relationship and haplotype distribution

Phylogenetic analysis of the honey bee mtDNA sequences supported the existence of the 5 subspecies haplotype groups (Figure 4.4). These groups represented 2 known and 3 unknown *A. mellifera* haplotypes. The haplotype Cam1 matches perfectly with the previously reported *A. m. adansonii*. This *adansonii* haplotype was the dominant and most widely distributed. It occurred in approximately 48% (n = 43) of the total colonies sampled. It was the only recorded haplotype in the lowland forest zones of the East, Central and South. It also occurred in some apiaries in the SW and the savannah zones of the NW and West. The haplotypes Cam2, Cam3 and Cam4 were all unique and did not cluster with any previously reported ND2 mtDNA subspecies haplotype (Figure 4.4). Cam2 was recorded only in samples from the NW and SW, Cam 3 was restricted to the highland savannah of the NW and North, and Cam4 occurred exclusively in samples from the Nord in Adamawa. Cam5 was a very rare haplotype that was recorded in only two apiaries and in three colonies in total. This haplotype clustered with *A. m. scutellata* (Figure 4.4).
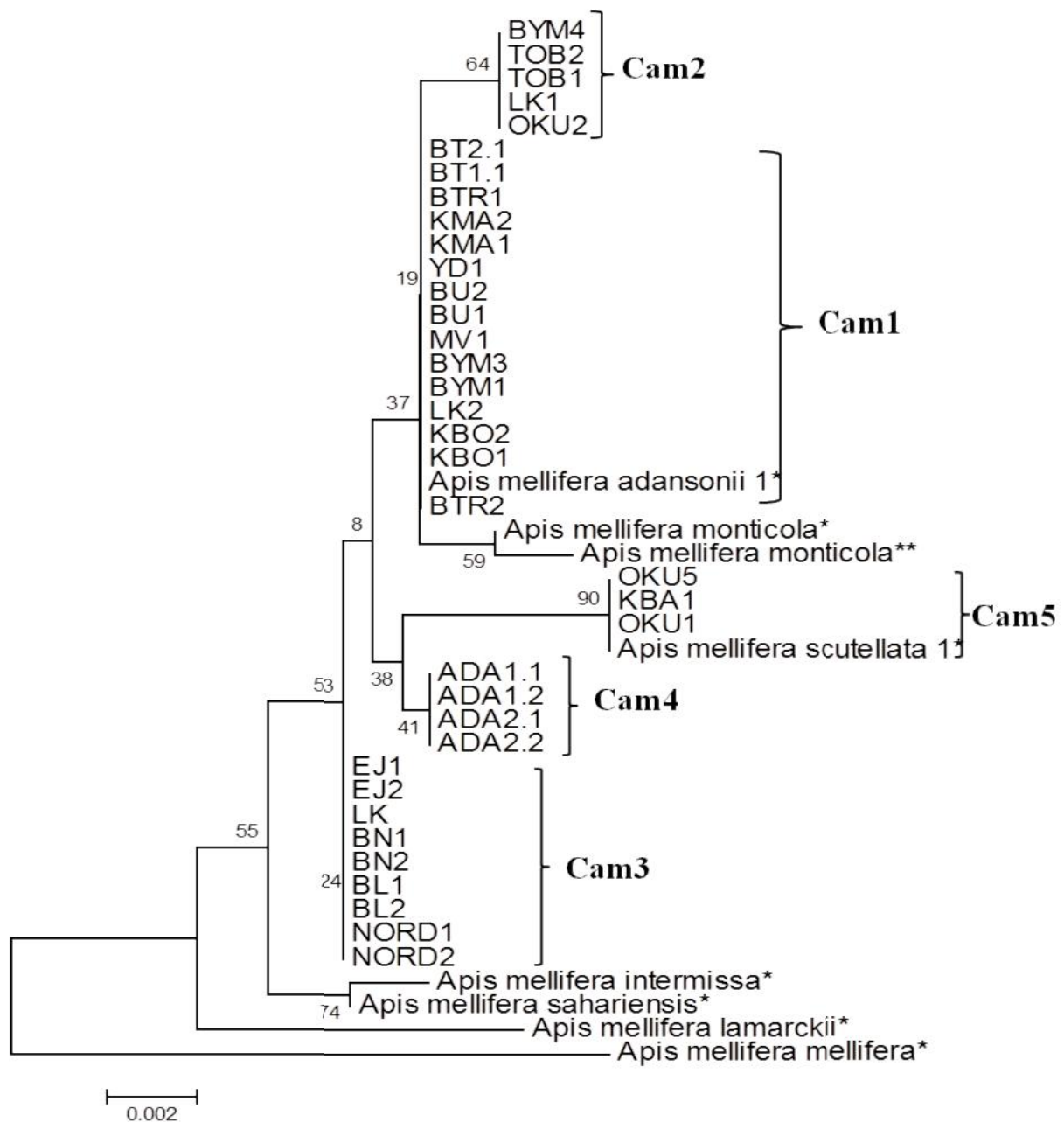
Figure 4.4 Neighbor joining phylogenetic tree of *Apis mellifera* subspecies haplotypes from Cameroon. Cam1 to Cam5 represent *A. mellifera* haplotypes from Cameroon, asterisk (*) denote *A. mellifera* subspecies from GenBank, while abbreviated names follow that in table 3.1 above. Bootstrap support values are indicated above each node.

4.5 Discussion

This study represents the first attempt to fully describe *A. melifera* populations of Cameroon using a combination of both morphological tools and molecular markers. It also assesses the relatedness of the honey bee populations to previously described morphotypes and other *A.*

melifera subspecies across Africa. The three morphotypes of *A. mellifera* from the morphological analysis supports previous findings by Hepburn and Radloff (1997) where they showed that mountain honey bees of Cameroon were distinct from the lowland and Northern populations. However, unlike previous studies, this study further describes the populations from the South, Central and East. The resulting morphotypes recorded in this study are likely the result of geographic isolation (Ruttner, 1988). The highland zone is quite distinct from the lowland areas or from the Far-North, in terms of altitude, temperature, rainfall, vegetation, and other environmental variables (Pamo, 2008). These morphological variations in the bee populations are likely link to adaptations that allow them to cope and thrive in the different ecological zones.

Molecular markers further supported the distinction of the mountain and lowland populations of *A. mellifera* by showing that almost all the lowland populations were *A. m. adansonii* and that there exist at least two unique subspecies haplotypes of the mountain bees. Therefore, this was the limitation of the morphometric analysis. It however supported the uniqueness of the morphologically described Northern populations which were formerly suggested by Hepburn and Radloff (1997) to be *A. m. jemenitica*. The occurrence of *A. m. scutellata* in some few colonies could not really be explained since this subspecies is the dominant subspecies haplotype in the east and southern parts of Africa (Arias and Sheppard, 1996). However, Arias and Sheppard (1996) did not find any clear distinction between ND2 *scutellata* 2 from South Africa and *adansonii*1 from West Africa which seems to suggest that the ND2 *scutellata*1 that was recorded in this study might be a possibility. There is therefore possible mixing between these two subspecies.

The three highland subspecies haplotypes recorded in this study seem to have a common ancestral origin. The fact that all the highland subspecies haplotypes were genetically closely related to the lowland *adansonii* haplotype than to each other is an indication that these highland populations probably descended from the lowland *adansonii* populations. Also, they were more closely related to the lowland *adansonii* population than to mountain bees from other parts of Africa. This finding therefore seems to support the hypothesis that mountains bees should be considered differentiated populations of the subspecies surrounding the mountains (Hepburn *et al*., 2000). Thus mountain bees of Cameroon should belong in the same lineage as the lowland populations (in this case lineage A). Detailed molecular studies would however, be needed to fully determine whether the *A. mellifera* haplotypes detected in

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the North represent a different lineage, O (represented by *A. m. jemenitica*) as previously suggested by Hepburn and Radloff (1997).

The uniqueness of the subspecies haplotypes of the mountainous and lowland zones is an indication that there is minimal exchange of genetic material between these *A. mellifera* populations. None of the highland haplotypes for example was detected in the Central, East or South of Cameroon. The occurrence of one of the highland *A. mellifera* subspecies haplotype (Cam2) in the lowland forest zone of the SW was not a coincidence. This zone is quite close to the highland zone of the NW and thus natural migration of bee swarms between these zones is likely to facilitate introgression. In addition, Cam2 was recorded close to Mount Cameroon, which represents the highest point of the Country with characteristics similar to the Western highlands of the NW and West (Pamo, 2008). The occurrence of these subspecies haplotypes within limited boundaries is also probably aided by the lack of movement of bee stocks between these ecological zones by beekeepers. Also, breeding of local bee populations is not yet practiced in Cameroon since beekeeping is still largely based on natural bee swarms. This is an advantage to the country since the natural *A. mellifera* genetic pools are still preserved and diverse. This may be of great significance in terms of fitness (e.g. resistance against diseases) (Tarpy, 2003) and productivity (e.g. honey and wax) (Mattila and Seeley, 2007). It is unlike in many areas in Europe where constant movement of bee stocks has led to a reduction in genetic diversity or introgression of regional strains (Meixner *et al*., 2010).

4.6 Conclusion

Three morphologically distinct *A. mellifera* populations are present in Cameroon. These morphotypes constitute at least five different subspecies haplotypes that are adapted to distinct geographic locations. The unique highland subspecies haplotypes may represent the previously described *A. mellifera* monticolar-like bees in the NW and *A. m. jemenitica* bees in the Nord. Besides the *A. m. scutellata* haplotype, all the highland subspecies haplotypes are closely related to the lowland *A. m. adansonii* haplotype which is also the dominant honey bee subspecies in Cameroon. This study therefore showed that although the *A. mellifera* populations of Cameroon can be said to belong to the same evolutionary lineage, A, they are made of unique subpopulations that are adapted to distinct geographic locations. Three unique ND2 mtDNA *A. mellifera* subspecies haplotypes are recorded in this study, which

therefore adds to the existing knowledge on honey bee subspecies diversity in Africa. Proper management and conservation of this honey bee subspecies diversity within their geographic boundaries is therefore recommended. Further studies using a combination of both mtDNA and nuclear makers are required to fully described introgression between the subspecies and to resolve taxonomic uncertainty regarding the northern *A. m. jemenitica* populations.

CHAPTER FIVE

DISTRIBUTION AND SEASONAL ABUNDANCE OF HONEY BEE PESTS IN SELECTED AGRO-ECOLOGICAL ZONES IN CAMEROON

5.1 Summary

Honey bees are faced with threats from both biotic and abiotic factors with pests as one of the key players of the current global honey bee colony decline. In some parts of Africa, a number of both invasive and indigenous pests have been recorded with differential impact on honey bee health. However, there still exists very limited information on honey bee health and on the ecology of honey bee pests in Central Africa and Cameroon in particular. To fill this knowledge gap, field surveys of honey bee colonies for pests were undertaken across four ecological zones in Cameroon during the dry season, short and long rainy seasons in 2014. Honey bee pests were sampled using standard protocols and identified based on morphology and DNA barcodes. This study revealed the presence of eight honey bee pests in Cameroon. These are the parasitic mite *Varroa destructor*, the small hive beetle *Aethina tumida,* dynastid beetle, wax moths *Galleria mellonella* and *Achroia grisella*, hawkmoth *Acherontia sp*, bee louse *Braula sp* and the phorid *Megaselia scalaris*. Other arthropods such as ant species including three deleterious ones, *Monomorium cryptobium*, *Oecophylla longinoda* and *Dorylus molestus* were also recorded. The findings of this study showed for the first time the occurrence of the Korean haplotype of *V. destructor* in Cameroon and Central Africa, the occurrence of a unique haplotype of *A. tumida* for the first time in honey bee colonies, and the occurrences of *M. scalaris* in honey bee colonies in Cameroon. Studies on pest distribution and seasonal abundance clearly showed that *V. destructor* and *A. tumida* were the major pests of honey bees and may be contributing factors to the colony losses recorded. *Varroa destructor* occurred in high numbers in the savannah zone, highland located apiaries and during the dry season. In contrast, *A. tumida* occurred in high numbers in the forests zones, at lowland located apiaries and during the rainy season. The incidence and levels of the other pests (e.g. wax moths) although low, could be used as indicators of colony health status since their high incidence in the forest zone was correlated with high colony losses. This study therefore provides new information on the diversity of honey bee pests, highlights possible factors that might influence their levels and provides vital information for the development of management options against these pests. These findings thus contribute significantly to the existing knowledge on the ecology of honey bee pests across Africa.

5.2 Introduction

There have been numerous global reports on the decline of managed honey bee colonies and wild insect pollinators (Le Conte *et al*., 2010; Moritz *et al*., 2010; Potts *et al*., 2010). This decline has been linked to several factors (Vanbergen and Insect Pollinators Initiative, 2013; Goulson *et al*., 2015) with pests and parasites among the plausible drivers of annual colony losses (Dainat *et al*., 2012a; Smith *et al*., 2013). This is due to their ability to cause both direct and indirect damage (Arbogast *et al*., 2009; Chen *et al*., 2004; Fombong *et al*., 2012; Le Conte *et al*., 2010; Rosenkranz *et al*., 2010; Suazo *et al*., 2003). *Varroa* mites for example have the ability to weaken the honey bee immune system, induce morphological deformities, and reduce longevity in honey bees by feeding on haemolymph as well as transmit and reactivate viruses (Bowen-Walker *et al*., 1999; Chen *et al*., 2004; Dainat *et al*., 2012a; Le Conte *et al*., 2010; Rosenkranz *et al*., 2010). The small hive beetle (SHB) *A. tumida* has also been implicated as a potential vector of honey bee viruses (Eyer *et al*., 2009). These two pests are widely distributed globally and are generally recognised as key pests of *A. mellifera* due to their damage to colonies (Dietemann *et al*., 2012; Neumann *et al*., 2016). They and other pests such as *A. woodi* and *Tropilaelaps* mites have been listed as notifiable infections by OIE, thus making them hazards in international trade (OIE, 2016).

Although the SHB was recorded to occur in Cameroon close to a decade ago (Neumann *et al*., 2016), there is no scientific information on the occurrence of other honey bee pests including the invasive mite *V. destructor* (Pirk *et al*., 2016). The effect of this mite and other honey bee pests has been shown to cause significant damage to honey bee colonies across Africa (Rasolofoarivao *et al*., 2013; Fombong *et al*., 2012; Neumann *et al*., 2016). Studies on the ecology and impact of these pests on Cameroon's honey bee population are therefore a priority. This is of great significance especially now that the Cameroon government is working towards boosting the commercialization of beekeeping (Awono *et al*., 2013).

Knowledge on the ecology of honey bee pests is vital for monitoring and management. The seasonal dynamics and spatial distribution of *Varroa* mite has been investigated in a few African countries (Akinwande *et al*., 2012; Muli *et al*., 2014; Mumbi *et al*., 2014). Similarly few studies in Africa have investigated the distribution and dynamics of the SHB (Neumann *et al*., 2016; Torto *et al*., 2010a). Given that Africa is so diverse in terms of ecological zones,

findings from different countries are bound to vary. Localized studies are therefore required to provide vital information for pest management within limited geographic boundaries and to contribute to the broader picture on the overall infestation levels and dynamics of honey bee pests across the continent. In addition, the dynamics of several pests occurring in colonies simultaneously have not been broadly considered. Such information will likely provide efficient information for pest management. The aim of this study was therefore to determine the occurrence and investigate the abundance of honey bee pests in colonies across selected agro-ecological zones and seasons in Cameroon.

5.3 Materials and Methods

5.3.1 Study sites

The assessment of honey bee colonies for pests was conducted in three major ecological zones that represent major honey producing areas of Cameroon. These include the highland Sudan savannah of the North West, the lowland dense evergreen forest zone of the South West and the degraded evergreen forest zone of the East. To determine the distribution of key pest species, colonies were also sampled in the Guinean savannah zone of the North and forest zone of the South and Central. A full description of the study sites is provided in section 3.1 above.

5.3.2 Sampling of honey bee pests and other arthropod hive invaders

Honey bee colonies were assessed for the small hive beetle, *Varroa* mite, bee louse, large hive beetles, moths, phorid flies, pseudoscorpions, and other arthropod hive invaders such as ants.

5.3.2.1 *Varroa* mite and bee louse

The presence and levels of *Varroa* mites in honey bee colonies was determined using the sugar roll assay (Ellis and Macedo, 2001). In this assay, a cup scoop of about 300 bees were collected from the brood comb in to a jar with a mesh lid. Sugar dust was added to the jar which was shaken on a white surface. Dislodge mites and lice were collected. Infestation levels were established by counting the recovered number of mites from the sugar dust. This was repeated thrice and the average number of mites and lice recorded. The assessment of honey bee colonies in the Central, South and Guinean savannah zone was done using the

alcohol wash method (Shimanuki and Knox 2000). Here approximately 300 bees were collected and preserved in 95 % ethanol. The bees were   later examined for mite and louse infestation in the laboratory.

5.3.2.2 Small hive beetle

The methods by Torto *et al*. (2010b) and Neumann *et al*. (2013) were adopted for sampling SHB from colonies housed in modern hives (e.g. KTB). This involved carefully checking and collecting beetles from top boards or covers, bottom of hives, frames and inside walls of hives with an aspirator. Sampling of SHBs from frameless hives was carried out by gently opening and checking the inner surfaces thoroughly for the presence of hive beetles as described in section 3.3 above. Escaping SHB around the edges and entrances of the hives were also counted and collected.

5.3.2.3 Large hive beetles and moths

Large hive beetles were sampled using a hive tool and/or hand after visual inspections of the colony as described in Torto *et al*. (2010b). Adult or larvae of wax moths or death's head hawkmoths were carefully sampled after visual inspection. Colonies were also checked for the presence of symptoms such as silken cocoons (Ellis *et al*., 2013).

5.3.2.4 Phorid flies

Colonies were examined for the presence of phorids by randomly sampling 30 worker honey bees from within the inner walls of the hives in 50 ml centrifuge tubes and from hive entrances using an aspirator. The bees were held at room temperature (25 ± 2.0 °C) within the same tubes. Each tube was capped with 0.25 mm fine mesh netting that excluded phorid flies and allowed sufficient air exchange. Emergent fly larvae were allowed to pupate and their development followed until adult emergence. Adult flies were collected and preserved in 95 % ethanol for subsequent molecular identification.

5.3.2.5 Ants

Various ant samples were collected inside honey bee colonies and at hive entrances using a bee brush and hive tool and placed in 50 ml centrifuge tubes containing 70 % ethanol for

subsequent identification. Damage caused by ants to honey bee colonies was recorded and photographed.

5.3.3 Identification of honey bee pests and other arthropod hive invaders

Collected pest samples were identified directly in the field using morphological features. Additional confirmatory studies were carried out in the laboratory using microscopy and diagnostic protocols (Ellis *et al*., 2013; Dietemann *et al*., 2013; Neumann *et al*., 2013). Samples whose identity could not be ascertained were forwarded to the Biosystematics unit of the International Centre for Insect Physiology and Ecology (ICIPE) (e.g. beetles and moths samples) and the National Museums of Kenya (NMK) (e.g. beetles, ants, and moth samples) for identification. Mite samples ($n = 230$) from across the different ecological zones were further processed and mounted on microscope slides and the length and width of the dorsal shield measured to determine their identities and morphological variability (Dietemann *et al*., 2013). The size of the mite may also vary as a result of variation in the size of its host, the honey bee or selective pressures of acaricide (Maggi *et al*., 2009). Measurement was done using the ZEISS microscope and ZEN (blue edition) Service pack2 software (Carl Zeiss Microscope GmbH Jena, Germany).

Molecular techniques were used to further provide detailed information on the identity and diversity of the main honey bee pests as determined by the survey data and also to confirm the occurrence of pests whose identity could not be ascertained using the available diagnostic protocols or by taxonomic experts. These included *Varroa* mite, small hive beetle and phorid fly specimens.

For *Varroa* mite, genomic DNA was extracted from 31 individual whole specimens from across all study sites (1 - 2 mites/locality) and from an additional sample from Kenya using the DNeasy 96 Blood and Tissue Kit (Qiagen, Germany) according to the manufacturer's instruction. A 454 base-pair (bp) region of the mitochondrial *CO1* gene of the extracted DNA was amplified in a T100™ thermal cycler (Bio Rad) using primer pairs COXF and COXR (Table 5.1). Polymerase chain reaction (PCR) was performed in a 25 µl final reaction volume containing 5 µl DNA template, 3 µl of each primer (20 µM each), 1.5 µl RNase free water, and 12.5 µl of Mytaq HS Mix 2x using the Mytaq HS Mix 2x kit (Bioline, UK). The thermal profile consisted of an initial denaturation step of 95 °C for 1 min, followed by 35 cycles of

95 °C for 20 sec, 45 °C for 20 sec, 72 °C for 15 sec and a final extension step of 72 °C for 7 min. The PCR products were verified by electrophoresis using a 100 bp Hyperladder (Bioline, UK) on a 2 % agarose gel stained with ethidium bromide in a 1X TAE buffer and visualized under UV in a GelDoc-It[TS2] imager.

In the case of the SHB and phorid flies, genomic DNA was extracted from the leg tissues and whole individuals of 18 and 6 specimens respectively using the ISOLATE II Genomic DNA kit (Bioline, UK) adhering to the manufacturer's instructions. The extracted DNAs were amplified using the Mytaq HS Mix, 2x kit (Bioline, UK) following the manufacturing instructions. For the SHB DNA, a ~ 1080 bp region containing part of the mitochondrial *CO1* gene was amplified using primer pairs AT1904S and AT2953A (Table 5.1). Thermocycling conditions were similar to those used for the mite samples with annealing at 54 °C for 20 sec. Polymerase chain reaction was carried out using the Mytaq HS Mix, 2x kit (Bioline, UK). The reaction was performed in a 25 µl final volume containing 1.5 µl DNA template, 0.5 µl of each primer, 10 µl RNase free water and 12.5 µl Mytaq HS Mix 2x. For the phorid DNA, the barcode primers LCO1490 and HCO2198 (Table 5.1) were used to amplify a ~710 bp region of the *CO1* gene. Amplification of the *CO1* gene of the phorid DNA was carried out in a 25 µl final volume constituting 1 µl of DNA template, 1 µl of each primer, 9.5 µl of RNase free water and 12.5 µl of Mytaq HS Mix 2x. The thermal profile was similar to that used to amplify the SHB and *Varroa* mite mitochondrial *CO1* gene with annealing at 45 °C for 20 s. The SHB and phorid PCR products sizes were verified on a 2 and 1 % agarose gel respectively stained with ethidium bromide against a 1 kb ladder (Fisher Scientific, UK). All amplicons were purified using the ExoSAP purification kit (Thermo Fisher Scientific, USA) adhering to the manufacturer's protocol and send for bidirectional sequencing at Inqaba Biotech (Pretoria, South Africa).

Sequences were edited using MEGA version 6.0 (Tamura *et al*., 2013) and Geneious version 8.1.8 (Biomatters, 2015). The edited nucleotide sequences were initially investigated through the Basic Local Alignment Search Tool (BLASTN) search within MEGA. Multiple alignment of the consensus ingroup sequences together with close taxonomic representatives and suitable outgroup of each of the pests extracted from GenBank was performed using the MUSCLE method with 8 interactions in MEGA. Alignment was done by codon since all the nucleotide sequences were from coding genes. Aligned sequences were trimmed to 458bp, 921bp and 658bp for the *Varroa* mite, SHB and phorid fly sequences respectively before

analysis. Model selection tests were used to find the best nucleotide substitution models for each of the sequence data sets of each pest using the Bayesian Information Criterion (BIC). The Hasegawa-Kishino-Yano (HKY) model fitted the *Varroa* mite nucleotide sequence sets while the Tamura 3-parameter (T92) model with discrete Gamma distribution (+*G*) was the best substitution model for the SHB and phorid fly nucleotide sequences. The phylogenetic relationship of each pest was inferred using the Maximum Likelihood (ML) method employing the best DNA models above, with 1000 bootstrap replication. Evolutionary divergence over sequence pairs within and between groups was estimated using the above models associated with each pest's nucleotide sequence set. Positions with gaps and missing data were excluded while the 1st, 2nd, 3rd and noncoding codon positions were included.

Table 5.1 Primer pairs used for amplification of the *CO1* gene of the extracted genomic DNA of honey bee pests

Primer name	Primer sequence	Primer sequence (5 -3)	Source
COXR	Reverse	GGWGACCTGTWAATAGCAAATAC	Anderson and
COXF	Forward	GGRGGWGAYATTYAWTATCAAC	Fuchs, 1998
AT1904S	Reverse	GGTGGATCTTCAGTTGATTTAGC	Evans *et al.*,
AT2953A	Forward	TCAGCTGGGGGATAAAATTG	2000
LCO1490	Reverse	GGTCAACAAATCATAAAGATATTGG	Folmer *et al.*,
HCO2198	Forward	TAAACTTCAGGGTGACCAAAAAATCA	1994

5.3.4 Data analysis

The *Varroa* mite measurement data (length and wide of dorsal shield) were analysed using ANOVA to test whether the mite populations across the different ecological zones differed in the measured characters.

The proportion of infested honey bee colonies was estimated for each pest by dividing the number of infested colonies by the total number of colonies and multiplying the results by 100 %. The negative binomial model was used to determine whether *Varroa* and SHB counts fluctuated with seasons and differed among agro-ecological zones. The model selection was based on the AIC (Akaike Information Criterion) and justified using Chi probability. The overall effect of the factors (season and ecological zone) on the model was assessed for each

pest using Wald test. Regression analysis was used to further test the association between *Varroa* mite and SHB numbers with altitude. Colonies within an apiary occurred at similar altitudes therefore, the mean *Varroa* and SHB numbers per apiary within each season, and cumulative averages over the entire study period were used (to eliminate temporal variability due to change in seasons). Both *Varroa* mite and SHB numbers were converted to logarithmic scale using the equation, $\log_{10}$ (*Varroa*/SHB numbers + 1) before the regression analysis. The presence and absence data of wax moth, bee louse and ants across ecological zones and seasons was analyzed using Pearson's chi-squared test for independence. Means were compared using pairwise comparisons with Bonferroni corrections of the *P* values. The occurrence of pests such as Dynastid beetle and hawkmoth were very rare and thus excluded from this analysis. In all these comparisons across ecological zones, only data from colonies housed in KTB hives were used.

The relationship between *Varroa* mite and SHB levels with beehive types was determined using Kruskal-Wallis test with multiple comparisons of mean ranks using Dunn's test. In this analysis, only data from the North West (NW) during the dry season were used (Table 3.1). Three different hive types (Kenya Top Bar (KTB), Cylindrical Indian Bamboo (CIB), and Cylindrical Log (CL)) were present during this period.

In order to determine the possible relationship between honey bee pest levels and colony losses, the levels of these pests were evaluated in four categories of colonies. These include: (1) colonies that collapsed (death) in the following season (drastic decline in bee population unable to sustain the colony), (2) absconded colonies in the following season (absence of bees, presence of food resources and young brood), (3) colonies about to collapse (decreased bee population and presence of wax moth and/or silken cocoon) and (4) healthy colonies that survived (colonies with enough bees and food reserves that did not collapse or abscond). The bee population in these groups of colonies was estimated by counting the number of frames occupied by bees. In this analysis only colonies (n = 75) that occurred in apiaries that were assessed in all the three sampling periods were included. All statistical analyses were performed using the R statistical software version 3.2.2 (R Core Team, 2015).

5.4 Results

5.4.1 Occurrence and diversity of honey bee pests

The following pests were recorded in honey bee colonies; *Varroa destructor*, the small hive beetle *Aethina tumida*, Dynastid (rhinoceros) beetle, wax moths *Galleria mellonella* and *Achroia grisella*, death's head hawkmoth *Acherontia* sp, bee louse *Braula* sp, and phorid *Megaselia scalaris*. Other arthropods recorded include various ant species. Genetic and/or morphological studies were further carried out to assess the diversity of *V. destructor*, *A. tumida*, phorids, and ants.

5.4.1.1 *Varroa destructor*

Morphological analysis carried out on the mites revealed no significant variation ($P > 0.05$) in body size in samples across the ecological zones with average dorsal length and width ranged of 1173.24 ± 17.35 µm and 1691.7 ± 28.97 µm respectively (Table 5.2). It also revealed the morphological similarity of the mite populations with that from previous reports in other parts of the world (Table 5.2).

Table 5.2 Mean body size (length and width) of female *Varroa* mites from different geographical locations in Cameroon

Mite source (Country and region/province)		Sample size	Length of dorsal shield (µm) (Mean ± SD)	Width of dorsal shield (µm) (Mean ± SD)
Cameroon	North West	100	1172.99 ± 18.73	1690.87 ± 27.94
	South West	60	1173.74 ± 15.18	1689.24 ± 25.92
	East	60	1174.8 ± 14.95	1693.4 ± 31.79
	Adamawa	10	1163.35 ± 26.38	1706.79 ± 37.68
Nigeria	*Varroa destructor**	20	1179.0 ± 8.96	1718.25 ± 1.51
Japan, Thailand and Vietnam	*Varroa destructor***	533	1167.3 ± 26.8	1708.9 ± 41.2

*Akinwande *et al*. 2013; **Anderson and Trueman, 2000; SD = Standard deviation

Phylogenetic inferences of *V. destructor* DNA sequences obtained from samples across all sites and ecological zones further confirmed the high degree of relatedness among samples of this pest across Cameroon. Sequences were 100 % identical to each other, to the Kenya

samples and to the Korean haplotype of *V. destructor* (Figure 5.1). The sequences have been deposited in GenBank with accession number KX255668.
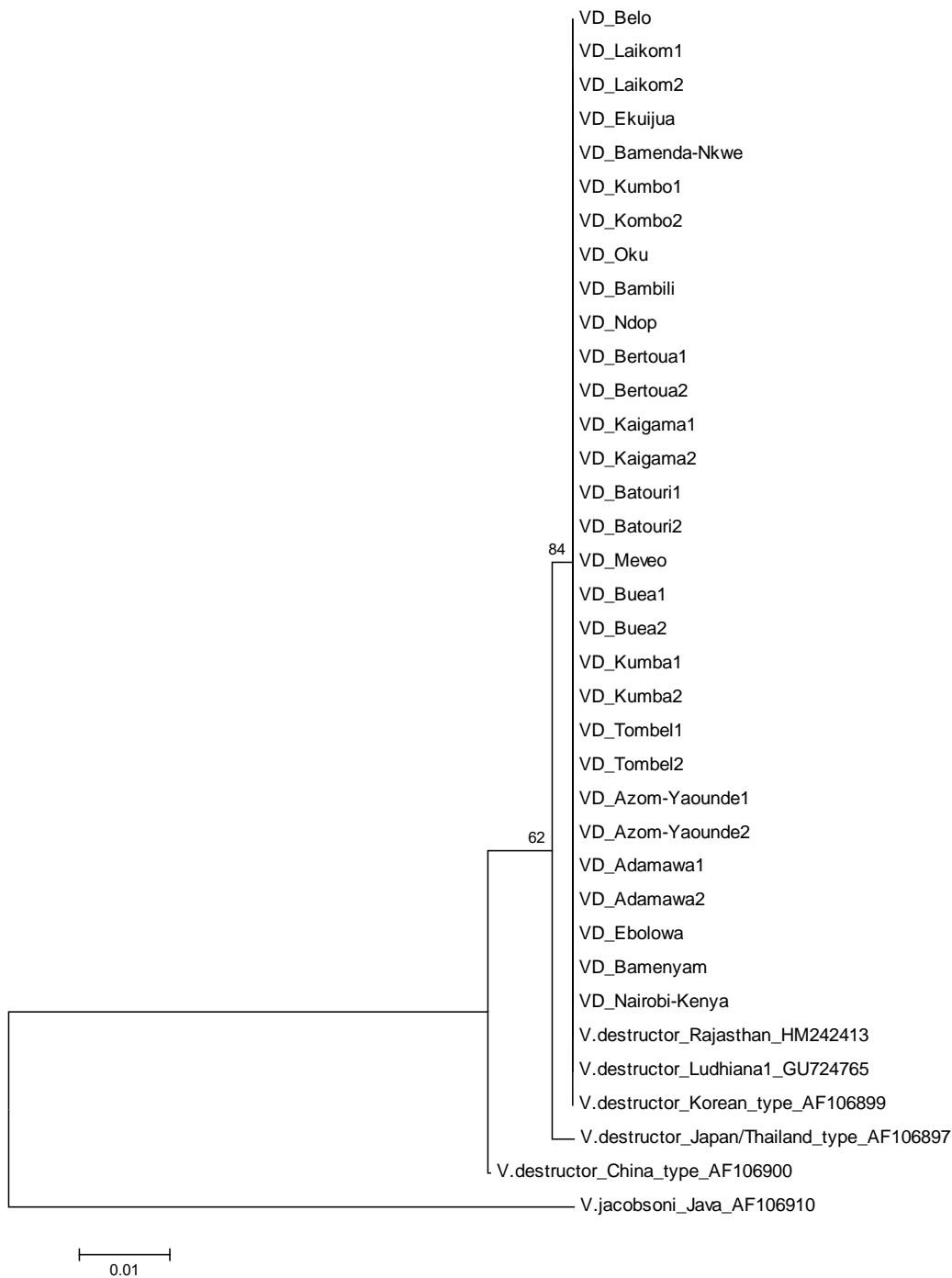
Figure 5.1 Maximum Likelihood phylogenetic tree of *Varroa destructor CO1* sequences from Cameroon and Kenya. VD stands for *Varroa destructor* while the associated initial represents the apiary sites. Sequences retrieved from GenBank are associated with their GenBank accession numbers. Boostrap support values are indicated above each branch.

5.4.1.2 *Aethina tumida*

The small hive beetle *A. tumida* (SHB), was recorded to be present across all the agro-ecological zones in this study. Phylogenetic analysis of 18 nucleotide sequences of the SHB produced two highly diverse haplotype groups represented as Cameroon 1 (CMR1) and Cameroon 2 (CMR2) (Figure 5.2). The average evolutionary divergence over sequence pairs between the two haplotypes was large (3.75 %) compared to the low average intra-haplotype diversity (0.74 % for CMR1 and 0.05 % for CMR2). Phylogenetic inferences further revealed that sequences belonging to haplotype CMR2 were closely related to SHB sequences from South Africa, Burkina Faso, and Zimbabwe (0.4 – 0.5 % divergence), and to SHB from North America (NA) (0.5 - 0.7 % divergence with NA1; and 0.1 – 0.2 % with NA2). Unlike CMR2, there was high sequence divergence between CMR1 and SHB sequences from South Africa, Burkina Faso, Zimbabwe and NA (3.1 – 4.2 % divergence) (Appendix 1). Beetle samples (*n* = 2) collected from Kenya were closely related to CMR2 with maximum percentage divergence of 0.1 (Appendix 1).

The geographic distribution of the two SHB haplotypes between apiaries across agro-ecological zones was irregular**.** The SHB haplotypes in the forest zone of the East were more biased towards CMR1 (80% (*n* = 5/6)) as opposed to the NW and SW with almost equal frequencies of the two haplotypes. Both haplotypes in most cases did not occur in the same or nearby apiaries with the exception of some localities in the SW (BU and TOB) (Figure 5.2, Appendix 1).

Figure 5.2 Phylogenetic relationships of *Aethina tumida CO1* haplotype sequences from Cameroon and those from other parts of the world. The tree topology was inferred using the Maximum Likelihood method. Bootstrap support values are presented at each branch. Asterisk (*) represents sequences retrieved from GenBank. The tree is rooted with *Stelidoda sp*. Sites names follow those in Table 3.1, while the abbreviations NA = North America; ZIM = Zimbabwe; SA = South Africa; AUST = Australia; BKF = Burkina Faso; CMR stands for the haplotype from Cameroon.

5.4.1.3 *Megaselia scalaris*

Phorid flies were recovered from incubated honey bee samples from three sites one each in the NW, SW and East (Figure 5.3A). DNA barcoding confirmed that all the phorid specimens recovered from dead honey bees in this study belonged to the species *Megaselia scalaris*. BLASTN search produced homologs that were 99 % similar to *M. scalaris* with 100 % query coverage and an E value of zero. Phylogenetic inferences of the DNA sequences using the ML method linked the ingroup sequences to *M. scalaris* sequences from China and Korea (Figure 5.3B). Maximum genetic distance between the ingroup sequences and the Korean/China *M. scalaris* was 1.9 %. The generated phorid sequences have been deposited in GenBank under accession numbers KX266966 (for *M. scalaris* from BE1 in the NW), KX266963 - KX266965 (for *M. scalaris* from KBA in the SW), and KX266967 - KX266968 (for *M. scalaris* from BT in the East).
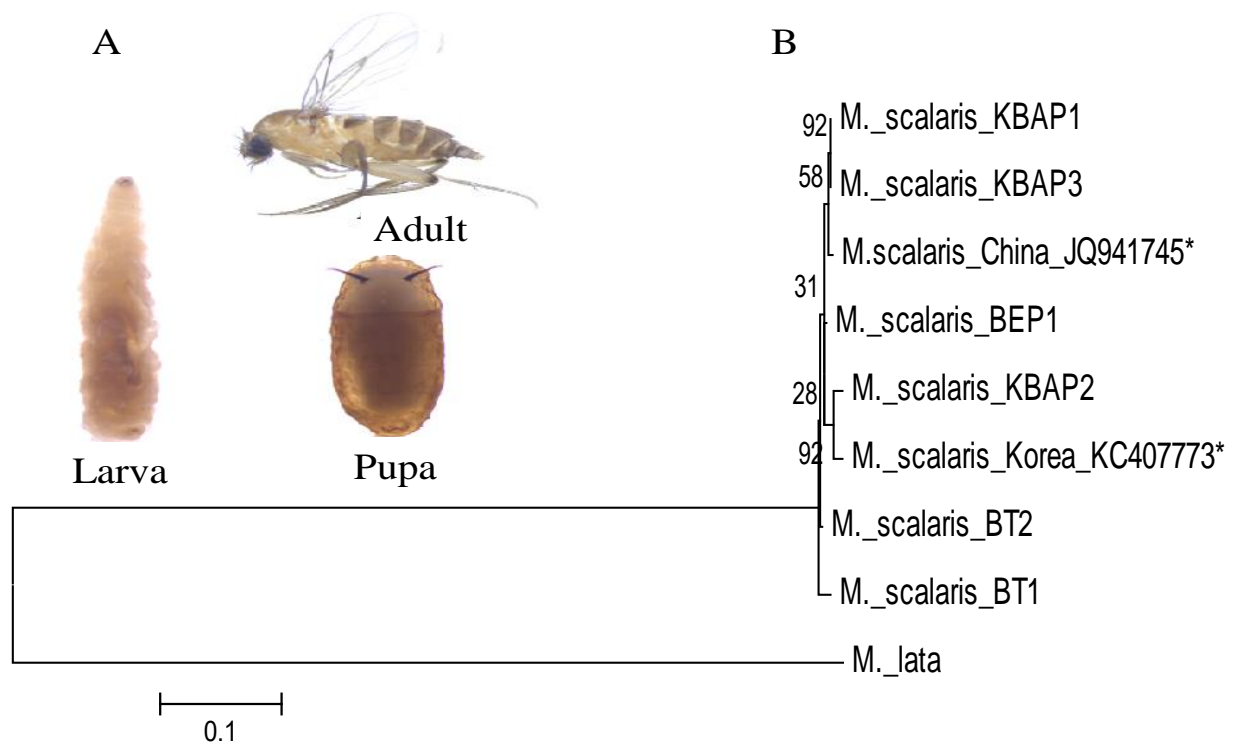
Figure 5.3 Phylogenetic relationships, immature and mature stages of *Megaselia scalaris*. (A) Different developmental stages of *M. scalaris* after the egg stage, (B) Maximum Likelihood phylogenetic tree of *M. scalaris CO1* sequences from Cameroon and reference sequences from GenBank (*). KBAP1-KBAP3 represents specimens from the South West, BT1-BT2 specimens from the East, and BEP1 specimen from the North West.

5.4.1.4 Ants

Taxonomic identification revealed the occurrence of 8 ant species infesting honey bee colonies (Figure 5.4). Of these, only three, *Monomorium cryptobium*, *Oecophylla longinoda* and *Dorylus molestus* were of economic importance as they were observed to cause damage to honey bee colonies (Figure 5.5). *Monomorium cryptobium* infestation resulted in the death of worker bees whose carcasses were left in place as the ants went after the honey reserves. Unlike *M. cryptobium*, workers of *O. longinoda* worked collectively to attack and pull away intact worker bees, while raiding parties of *D. molestus* carried away worker bees and their brood from bee colonies.
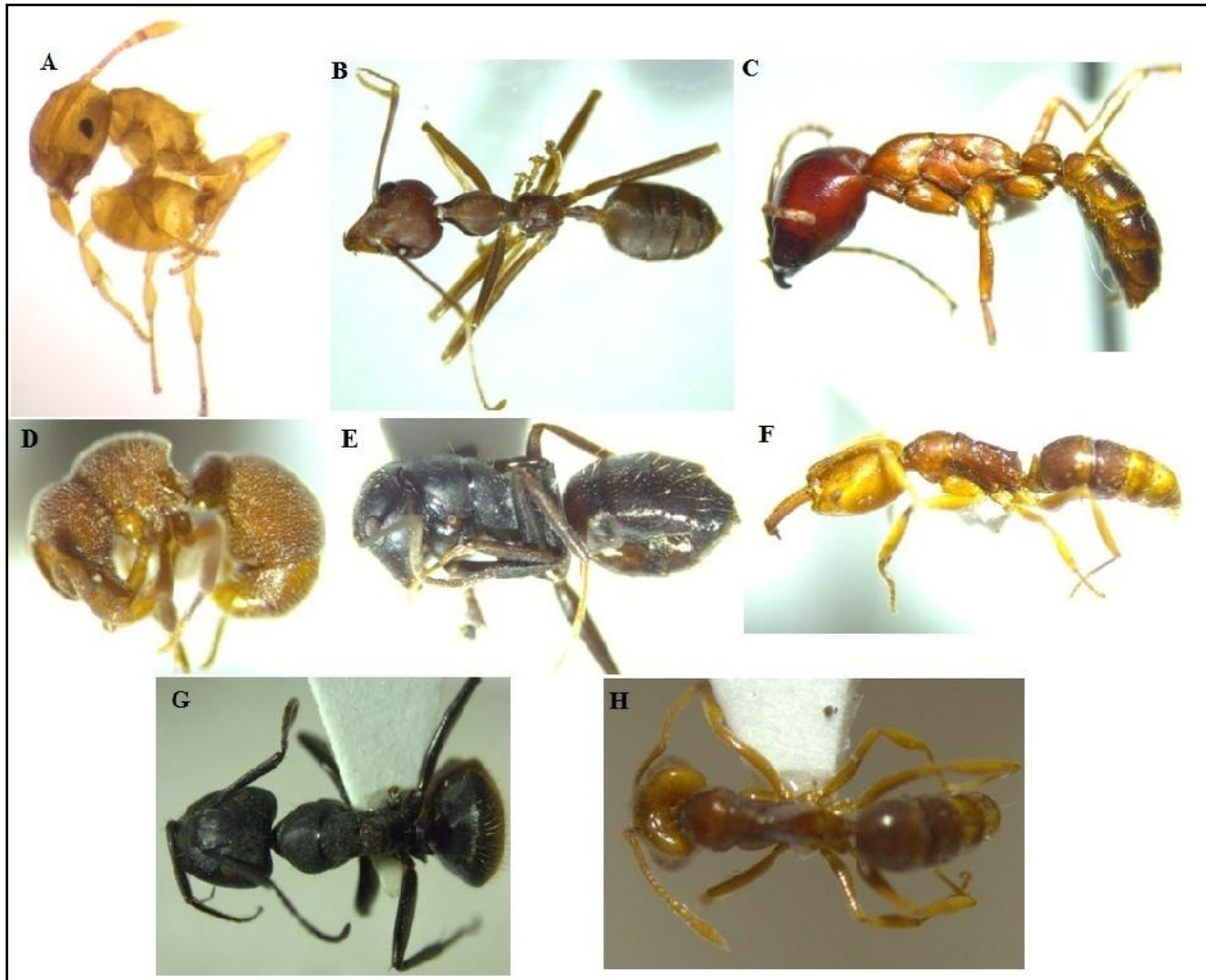
Figure 5.4 Ant species recorded in honey bee colonies. A = *Monomorium cryptobium*, B = *Oecophylla longinoda*; C = *Dorylus molestus*; D = *Discothyrea mixta*; E = *Crymatogaster depressa*; F = *Anochetus sp*; G = *Camponotus chrysurus*; H = *Tetramorium sp.*
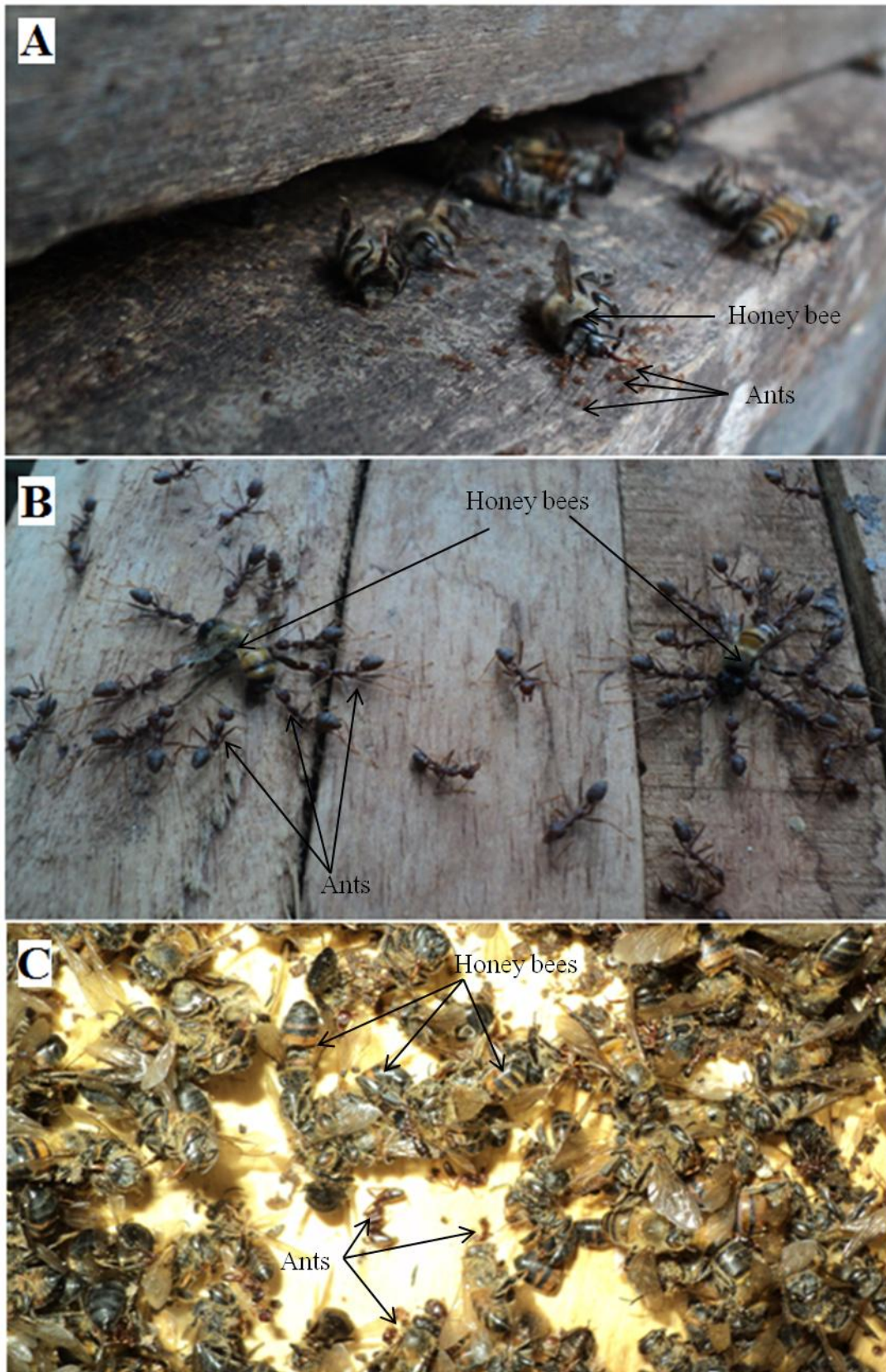
Figure 5.5 Damage by the ant species (A) *Monomorium cryptobium* (B) *Oecophylla longinoda* and (C) *Dorylus molestus* to honey bees.

5.4.2 Abundance of honey bee pests across ecological zones and seasons

5.4.2.1 *Varroa* mite

Results of *Varroa* mite colony infestation across ecological zones revealed that the percentage of infested colonies was consistently high between 96.7 % and 100 % throughout the year in the NW and East (Table 5.3). In the SW the percentage of infested colonies was slightly lower ranging from 76.7 % during the long rainy season to 96.7 % in the short rainy season (Table 5.3). Colony infestation levels (number of mites/300 bees) was largely variable. Comparison of mite infestation levels between seasons within each ecological zone showed that most colonies had higher mite levels in the dry season compared to the rainy seasons within each of the ecological zones (Appendix 2). Across ecological zones, most colonies in the NW had higher mite levels than the East and SW (Appendix 2). In general, a majority of the colonies (33 %, n = 89) had between 0 – 5 mites, 28.2 % (n = 76) had 6 – 10 mites, 16.3 % (n = 44) had 11 – 15 mites, 6.3 % (n = 17) colonies had 16 – 20 mite, 8.9 % (n = 24) had 21 – 30 mites, while the rest 7.4 % (n = 20) had more than 30 mites.

The abundance of *Varroa* mite differed across ecological zones and seasons. Across ecological zones mite abundance was significantly higher in the highland savannah zone of the NW than in the forest zones of the East (Wald test = 17.7, df = 1, P = 0.001) and SW (Wald test = 26.5, df = 1, P = 0.001). It was also significantly more abundant in the East than the SW (Wald test = 5.1, df = 1, P = 0.03) (Figure 5.6). Comparison of the abundance of *Varroa* between seasons within each individual ecological zone showed that the mite was significantly more abundant in the dry season than in the long rainy season (Wald test, P > 0.05) irrespective of ecological zone (Figure 5.6). *Varroa* mite was also more abundant in the short rainy season than in the long rainy season. This pattern of seasonal variability was consistent within each of the individual agro-ecological zones. Mite abundance was also largely variable across sites (Appendix 3). Results from the regression analysis of cumulative mean apiary *Varroa* mite numbers over the entire study period with altitude revealed a significant positive relationship with increase in mite numbers with elevation gradient (r (15) = 0.5, P = 0.05) (Figure 5.7). This positive association was confirmed by regression analysis using data within each season independently, during the dry season (r (15) = 0.36, P = 0.02), short rainy season (r (15) = 0.4, P = 0.15) and long rainy season (r (15) = 0.1, P = 0.84).
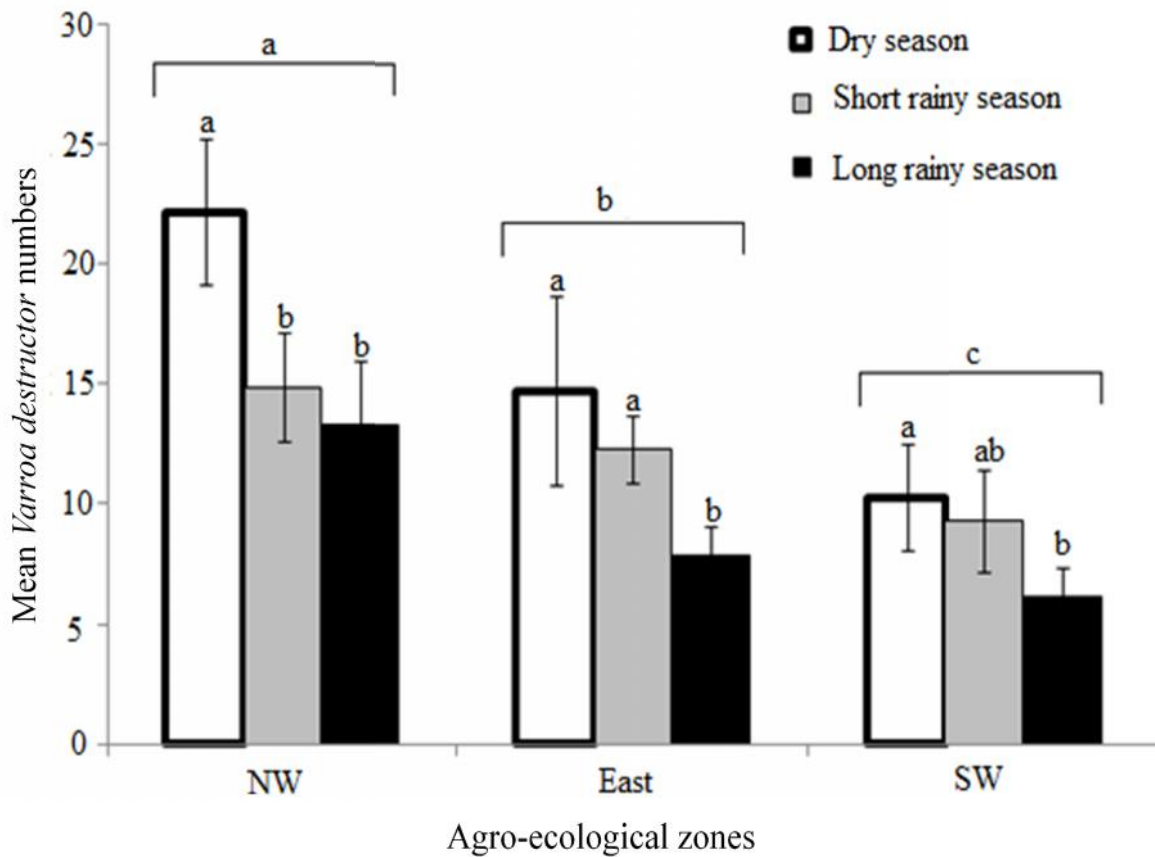
Figure 5.6 Mean (± SE) *Varroa destructor* numbers between seasons and across ecological zones. NW = highland Sudan savannah of the North West, East = degraded evergreen forest of the East, SW = evergreen forest of the South West. Different letters (e.g. a, b) represent significant differences in pest numbers between seasons and across ecological zones (= 0.05).
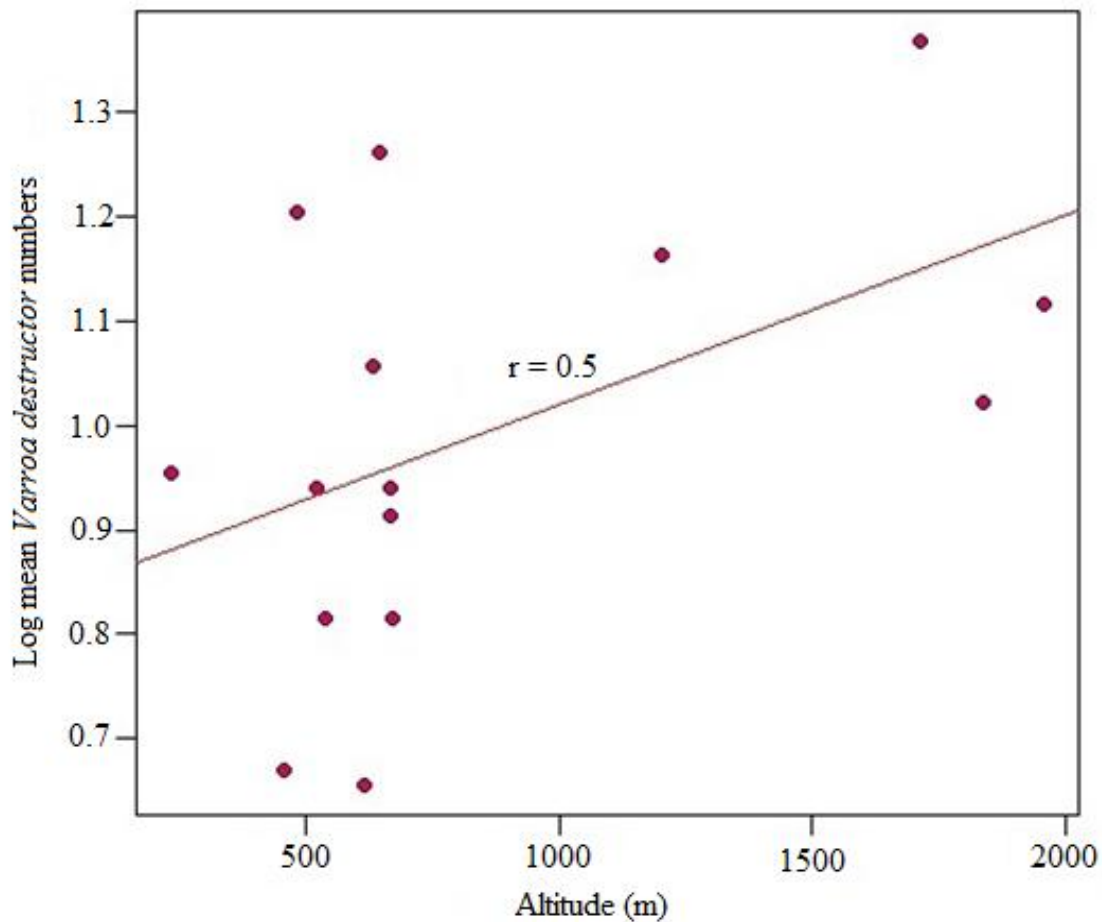
Figure 5.7 Natural log of mean *Varroa destructor* numbers with altitude. The plot shows a positive relationship between *V. destructor* numbers with increasing altitude in the cumulative mean apiary data over the entire study period.

5.4.2.2 *Aethina tumida*

The SHB was present in all (100 %) of the apiaries and in 93.3 % (n = 252) of the overall colonies assessed. The percentage of colonies infested was slightly higher in the forest zones of the SW and East (90 – 100 %) compared with the savannah zone of the NW (80 – 96.7 %) irrespective of seasons (Table 5.3). Infestation levels (SHB/colony) varied between seasons and across ecological zones. SHB infestation levels between seasons within each individual ecological zone revealed higher levels in the rainy seasons compared to the dry season (Appendix 4). Comparison between ecological zones revealed higher SHB infestation levels in colonies in the forest zones of the East and SW than in the NW (Appendix 4). The infestation levels in general ranged between 0 – 250. Most colonies (61.1 %, n = 165) had

between 0 – 20 and 21 – 50 (21.5 %, n = 58) SHB while the rest (17.4 %, n = 47) had more than 50 SHB.

The negative binomial analysis showed that rainfall led to significant increase in the SHB recovery from honey bee colonies. The SHB was significantly more abundant (2 folds) during the long rainy season compared with the dry season (Wald test = 24.3, df = 1, P = 0.001) or short rainy season (Wald test = 6.3, df = 1, P = 0.012). It was also significantly more abundant in the long than in the short rainy season (Wald test = 5.9, P = 0.015) (Figure 5.8). This pattern of SHB seasonal variation was consistent within each of the three ecological zones. In terms of ecological zones, SHB was significantly higher in the forest zone of the East compared with the savannah zone of the NW (Wald test = 27.5, df = 1, P = 0.001) and SW (Wald test = 11.5, df = 1, P = 0.001) (Figure 5.8). It was also more abundant in the SW than in the NW although there was no significant difference between these two zones (Wald test = 3.4, df = 1, P = 0.064) (Figure 5.8). The SHB abundant also varied between sites within each of the ecological zones and seasons (Appendix 3). Regression analysis of cumulative mean apiary SHB numbers throughout the entire study with altitude produced a significant negative correlation of SHB numbers with increasing altitude (r (15) = - 0.53, P = 0.04) (Figure 5.9). Higher numbers of SHB were therefore recorded at lowland located apiaries. Regression analysis carried out using data within each season independently further supported the negative relationship during the dry season (r (15) = - 0.39, P = 0.15); short rainy season (r (15) = - 0.67, P = 0.01) and long rainy season (r (15) = - 0.3, P = 0.37).
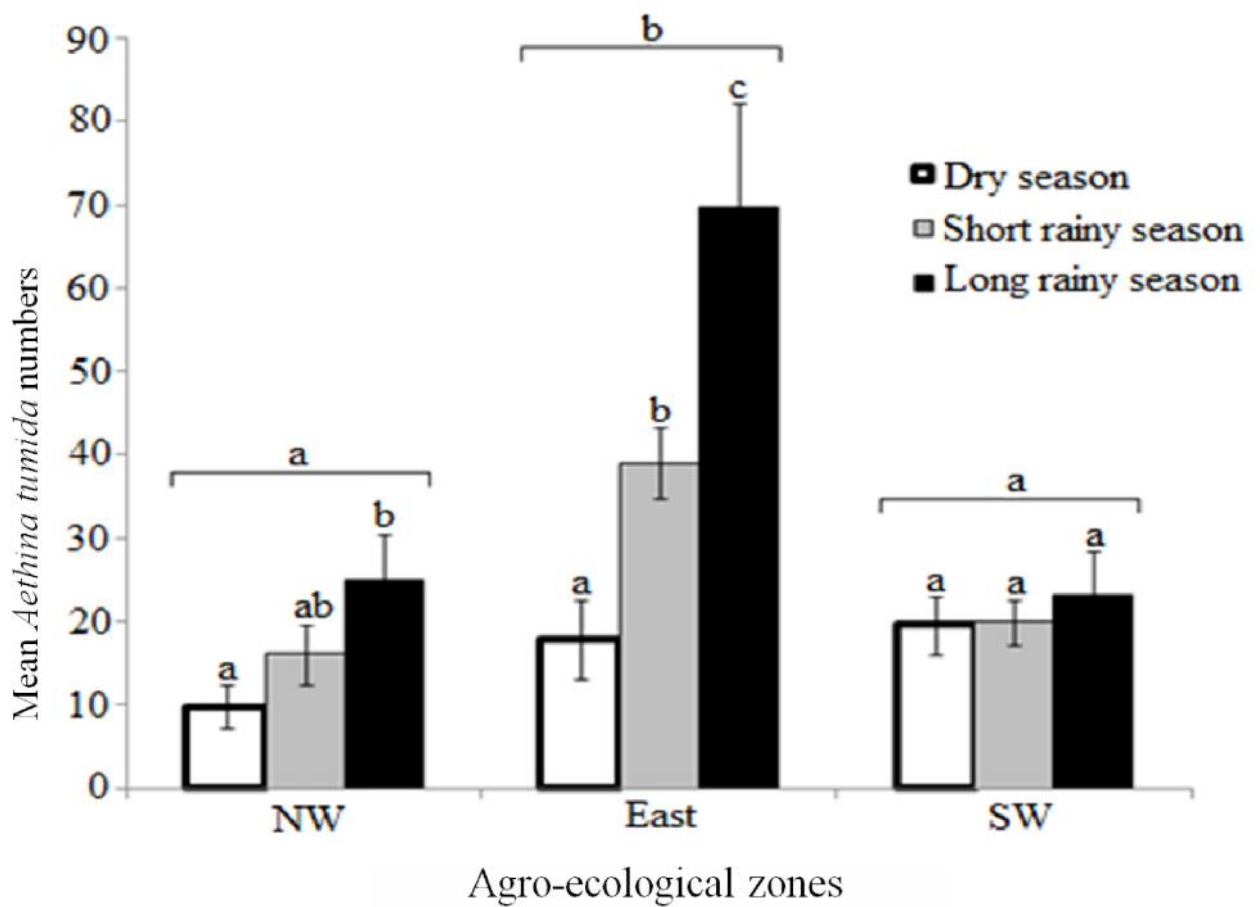
Figure 5.8 Mean (± SE) *Aethina tumida* numbers between seasons and across ecological zones. NW = highland Sudan savannah of the North West, East = degraded evergreen forest of the East, SW = evergreen forest of the South West. Different letters (e.g. a, b) represent significant differences in pest numbers between seasons and across ecological zones (= 0.05).
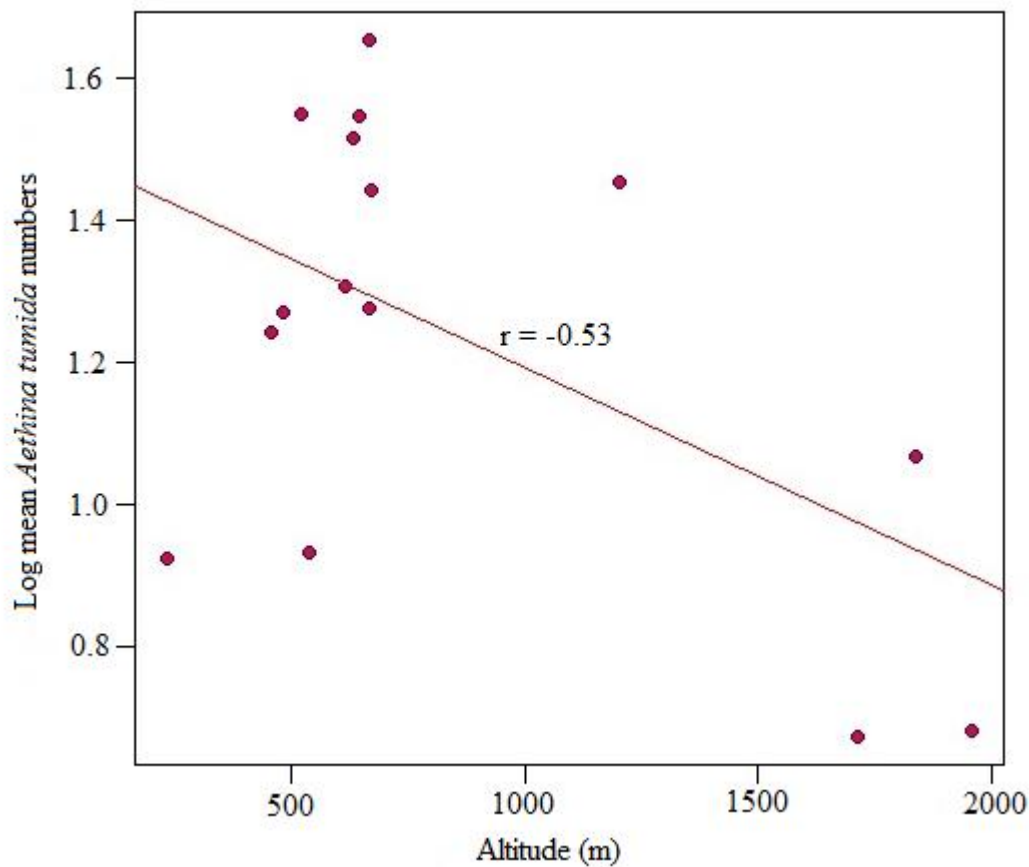
Figure 5.9 Natural log of mean *Aethina tumida* numbers with altitude. The plot shows a negative relationship between *A. tumida* numbers with increasing altitude in the cumulative mean apiary data over the entire study period.

5.4.2.3 Incidental arthropod pests

Pests such as wax moths *G. mellonella* and *A. grisella*, hawkmoths *Acherontia* sp, dynastid beetle and bee louse *Braula sp* were only occasionally recorded in honey bee colonies. The percentage of wax moth infested colonies was low across all the ecological zones throughout the year (0 – 30 %) (Table 5.3). The only significant result was between the highland savannah zone of the NW and lowland forest zone of the SW where wax moth incidence was higher for colonies in the SW compared with the NW (^{2} = 6.19, df = 1, *P* = 0.013) (Table 5.4). Hawkmoths were very rare and occurred exclusively in East and SW with maximum incidence of 10 % during the long rainy season (Table 5.3). Dynastid beetles were recorded only in the East in 6.7 % of the colonies sampled during the short and long rainy seasons. *Braula* sp was recorded in between 10 – 30 % colonies across ecological zones and seasons

(Table 4.3). *Braula* colony infestation however, did not differ across seasons ($^2 = 0.24$, df = 2, $P = 0.89$) or between ecological zones ($^2 = 4.34$, df = 2, $P = 0.11$) (Table 5.4).

Table 5.3 Percentage of pest infested honey bee colonies between seasons and across ecological zones

Zone	Season	n	Varroa mite	Small hive beetle	Bee louse	Wax moths	Hawk moth	Dynastid beetle
NW	Dry	30	96.7	96.7	23.3	20	0	0
	Short rainy	30	100	83.3	26.7	0	0	0
	Long rainy	30	96.7	80	30	6.7	0	0
East	Dry	30	96.7	100	16.7	30	3.3	0
	Short rainy	30	100	96.7	10	6.7	3.3	6.7
	Long rainy	30	96.7	100	23.3	10	10	6.7
SW	Dry	30	93.3	90	16.7	20	6.7	0
	Short rainy	30	96.7	100	23.3	23.3	0	0
	Long rainy	30	76.7	93.3	10	26.7	6.7	0

The header "% Pest infestation of honeybee colonies per ecological zone" spans the pest columns.

NW, Sudan savannah grassland of the North West; SW, dense evergreen forest of the South West; East, degraded evergreen forest of the East; n, number of colonies inspected in Kenya Top Bar hives in the NW, SW and East.

Table 5.4 Effect of season and location on wax moth and *Braula* sp incidence

Pest	Factor	Comparison	x^2	DF	*P* value
Wax moth	Season	D vs mW vs MW	5.761	2	0.056
		D vs mW	4.267	1	0.039
		D vs MW	1.942	1	0.164
		mW vs MW	0.271	1	0.603
	Location	East vs NW vs SW	7.351	2	0.025
		East vs NW	1.287	1	0.257
		East vs SW	1.431	1	0.232
		NW vs SW	6.193	1	0.013*
Braula sp.	Season	D vs mW vs MW	0.238	2	0.888
		D vs mW	0.020	1	0.886
		D vs MW	0.087	1	0.768
		mW vs EW	0.000	1	1
	Location	D vs mW vs MW	4.337	2	0.114
		East vs NW	2.572	1	0.109
		East vs SW	0.000	1	1
		NW vs SW	2.341	1	0.126

D, dry season; W, short rainy season; EW, long rainy season; NW**,** Sudan savannah grassland of the North West; SW, dense evergreen forest of the South West (SW); East, degraded evergreen forest of the East. *P value < Bonferroni-adjusted P value of 0.017

5.4.2.4 *Megaselia scalaris*

Parasitism by the phorid *M. scalaris* was generally low across all the study zones (NW, SW, and East). Of the total number of colonies (n = 120) from which honey bee specimens were collected for incubation, only 11 (9.2 %) were infested by *M. scalaris*. Of these, 6 (54.6 %) were located in the forest zone of the East, 4 (36.4 %) in the forest zone of the SW, and 1 (9 %) in the savannah zone of the NW.

5.4.2.5 Ants

The recorded ant species were widely distributed in honey bee colonies across the ecological zones of the North West, South West and East throughout the year (Table 5.5). The incidence of ants in honey bee colonies in general was significantly different across locations (x^2 = 18.32, df = 2, $P < 0.001$). Ants infestation was higher for colonies in the forest zone of the East compared to the savannah zone of the NW (x^2 = 17.10, df = 1, $P < 0.001$). There was no significant difference between the two forest zones or between the SW and NW. In particular, some species such as *M. cryptobium* were found to occur only in the forest zone of the East

(Table 5.5). Ant incidence did not differ significantly across seasons (x^2 = 6.02, df = 2, P = 0.049).

Table 5.5 Diversity of ant species infesting honey bee colonies in three ecological zones during the dry and rainy seasons in Cameroon

Ant species	Season	Ant presence (+) and absence (-)			% infested colonies		
		NW	SW	East	NW	SW	East
Dorylus molestus	D	+	+	+	3	10	3
	W	+	+	+	6	3	3
	EW	+	+	+	6	13	6
Anochetus sp	D	+	-	-	3	0	0
	W	+	-	-	3	0	0
	EW	+	+	+	3	3	3
Camponotus chrysurus	D	+	+	+	16	7	3
	W	+	+	+	6	13	13
	EW	+	+	+	6	9	16
Crematogaster depressa	D	+	+	+	16	3	3
	W	+	+	+	12	10	10
	EW	+	+	+	3	6	3
Discothyrea mixta	D	-	-	-	0	0	0
	W	-	-	+	0	0	3
	EW	-	+	+	0	3	3
Tetramorium sp	D	-	+	+	0	3	3
	W	-	+	+	0	17	3
	EW	-	+	+	0	16	6
Oecophylla longinoda	D	-	-	+	0	0	6
	W	-	+	-	0	10	7
	EW	-	-	+	0	0	9
Monomorium cryptobium	D	-	-	+	0	0	16
	W	-	-	+	0	0	27
	EW	-	-	+	0	0	25

NW = Highland savannah of the North West; SW = Dense evergreen forest of the South West; East = degraded evergreen forest of the East; D = dry season, W = short rainy season, EW = Long rainy season.

5.4.3 *Varroa* mite and *Aethina tumida* levels in different hive types

Hive type was found to have an influence on *A. tumida* and not on *Varroa* mite. The CIB hive was found to harbour significantly less *A. tumida* compared with the CL and KTB hives (Kruskal-Wallis test, H = 26.64, $P < 0.001$). *Varroa* mite infestation levels on the other hand, did not differ with hive types during the dry season (Kruskal-Wallis test, H (2) = 3.52, p < 0.172, Figure 5.10).
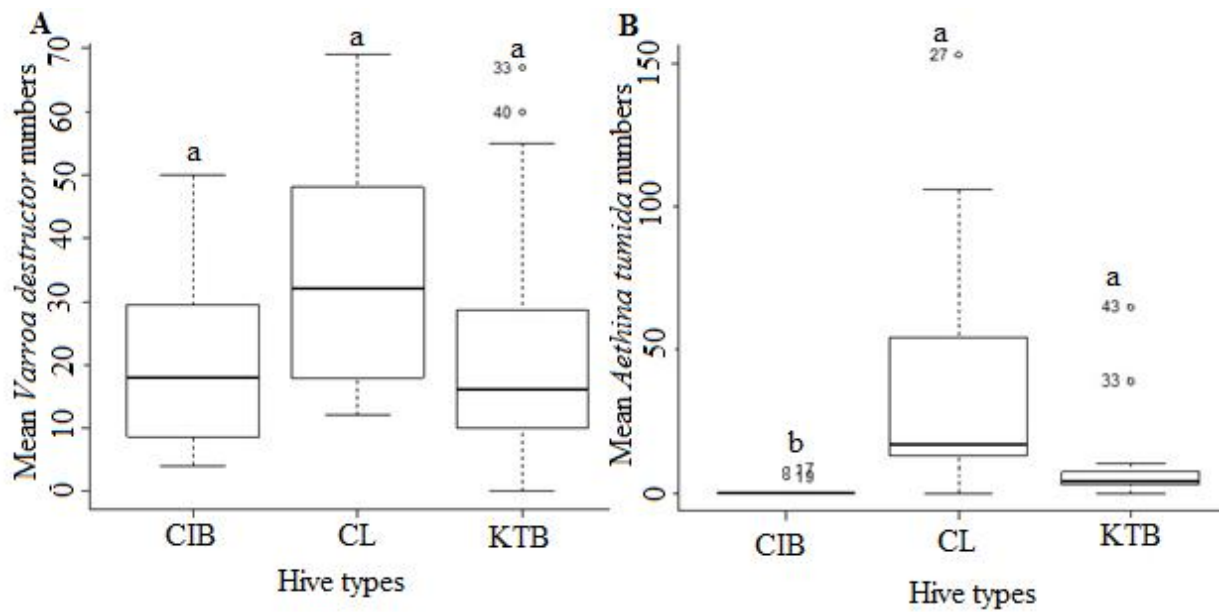
Figure 5.10 Abundance of *Varroa destructor* (A) and *Aethina tumida* (B) in different hive types. CIB, Cylindrical Indian Bamboo; CL, Cylindrical Log; KTB, Kenyan Top Bar. Different letters (e.g. a, b) represent significant differences in pest abundance between hive types ($P < 0.05$, Kruskal-Wallis test with multiple comparisons of mean ranks). Dots represent data points.

5.4.4 Overall colony losses and pest infestation levels

Of the 75 colonies considered in this study, a total of 17 (22.7 %) were lost (absconded or collapsed) while an additional 6 (8 %) colonies were at the verge of collapse. Nine of the colonies that were lost, absconded/migrated while 8 collapsed. Majority of the colonies lost were in the forest zone of the East (64.7 %, n = 11) and SW (29.4 %, n = 5), while only 1 (5.9 %) was from the savannah zone of the NW. Most of the colonies were lost during the rainy season (64.7 %, n =11) compared with the dry season (35.3 %, n = 6) (Table 5.6). Average *Varroa* numbers in the collapsed colonies (CC) was between 4 to 12 times higher than that in absconded colonies (AC) and approximately twice that in the survived colonies (RC). This was contrary to mean SHB numbers which were lowest in the CC compared with the high numbers that occurred in the RC and AC respectively. The incidence of moths was generally high in collapsed and absconded colonies than in the remaining colonies. All colonies at the verge of collapse were infested with wax moth (Table 5.6). This was in contrast to the

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incidence of the bee louse and dynastid beetles (Table 5.7). Bee population was higher in the RCs than in the collapsed colonies.

Table 5.6 Mean (±SD) *Varroa destructor*, *Aethina tumida* and bee population in collapsed, absconded and survived colonies across seasons

Season	Colony category	No of colonies	*V. destructor*	*A. tumida*	Bee population
Dry	CC	3	24.67 ± 31.39	6 ± 7.94	3.33 ± 3.62
	RC	69	14.48 ± 17.02	16.45 ± 21.92	10.83 ± 5.02
	AC	3	2.33 ± 1.53	8.67 ± 6.03	5 ± 3.12
Short rains	CC	5	26.2 ± 11.54	31.2 ± 19.47	6.3 ± 2.97
	RC	58	10.67 ± 9.2	29.55 ± 21.9	9.53 ± 3.09
	AC	6	6.83 ± 5.0	32.5 ± 31.07	9.17 ± 3.30
Long rains	AtC	6	26.17 ± 24.39	15.17 ± 13.96	3.58 ± 1.53
	RC	52	8.37 ± 7.07	52.12 ± 60.91	6.92 ± 2.93

CC, colonies that collapsed in the following season; AC, absconded colonies in the following season; AtC, colonies about to collapse; RC, survived colonies

Table 5.7 Percentage of infested colonies by incidental honey bee pests in collapsed, absconded and survived colonies across seasons

Season	Colony category	No of colonies	Wax moth	Bee louse	Hawkmoth	Dynastid beetle
Dry	CC	3	1 (33%)	3 (100%)	1 (33%)	0
	RC	69	15 (21.7%)	12 (17.4%)	2 (2.9%)	0
	AC	3	1 (33%)	0	0	0
Short rains	CC	5	3 (60%)	1 (20%)	0	0
	RC	58	3 (5%)	13 (22.4%)	0	2 (3.5%)
	AC	6	1 (16.7%)	0	1 (16.7%)	0
Long rains	AtC	6	6 (100%)	1 (16.7%)	0	0
	RC	52	2 (3.9%)	9 (17.3%)	5 (9.6%)	2 (3.9%)

CC, colonies that collapsed in the following season; AC, absconded colonies in the following season; AtC, colonies about to collapse; RC, survived colonies

5.5 Discussion

Varroa destructor

These findings represent the first report of the occurrence of *V. destructor* in Cameroon. It adds to increasing literature on the mite's occurrence in Africa (Pirk *et al*., 2016). The observed morphological and genetic similarities in the studied samples support the mite's identity as a member of the virulent Korean haplotype and agree with previous studies (Akinwande *et al*., 2012; Anderson and Trueman, 2000). This high level of phenotypic and genetic similarity may be due to the inbreeding reproductive life style of the mite (where siblings mate with one another) (Dietemann *et al*., 2013). Additionally, it suggests that its host appears to exert minimal selection pressure that may trigger genetic and associated phenotypic changes (Maggi *et al*., 2009). This is supported by recent reports of mites from other parts of Africa belonging to this haplotype (Pirk *et al*., 2016). Although, now known as a well-established pest in Cameroon its origin is not known. It is therefore suspected that it

might have invaded Cameroon either through the North from where it first invaded Africa (Allsopp *et al*., 1997), or from East or West Africa (Nigeria) where it has been reported long before now (Fazier *et al*., 2009; Akinwande *et al*., 2012).

This study also showed that *Varroa* levels in Cameroon are highly variable. This may have relative impact on colonies across the different ecological zones and seasons. The distribution and abundance of this mite was also found to vary across ecological zones. The higher mite numbers in the savannah zone of the NW compared with the forest zones of the East and SW might be the result of differences in environmental conditions and/or honey bee subspecies haplotypes. *Varroa* for example has been reported to occur in higher numbers in cooler climatic locations in Brazil (Moretto *et al*., 1991) while grooming (defence behaviour against *Varroa* mite) has been shown to vary with honey bee subspecies (Invernizzi *et al*., 2015). Mites also showed variability in levels across the seasons. The high abundance of *Varroa* mites during the dry season might be related to the availability of increased foraging resources in the previous season. Blooming of flowers towards the end of the rainy season (November – December) (Pamo, 2008) might have boosted brood rearing and thus led to an increase in mite population later in the year (during the dry season). Brood availability has been shown to be closely related to *Varroa* abundance (Martin, 1998). Our findings on the high abundance of *Varroa* mite at highland located apiaries might be the result of variation in several factors including but not limited to honey bee subspecies, brood availability, and environmental conditions as mentioned above. This finding supports previous reports by Muli *et al*. (2014) and Mumbi *et al*. (2014). Further surveillance studies of *Varroa* mite in areas not covered in this study such as the North and Extreme North regions of Cameroon may add more information on the extent of spread and scale of its infestation.

Small hive beetle, *Aethina tumida*

The small hive beetle (SHB) sequences showed divergence within the ingroup and outgroup sequences from GenBank. The large average SHB inter-haplotype diversity (3.75 %) between CMR1 and CMR2 compared to the low intra-haplotype divergences signals the possible existence of two separate groups of the SHBs in Cameroon. This is well supported by the fact that SHB sequences from other African countries and those from North America and Australia (Evans *et al*., 2000, Lounsberry *et al*., 2010) all fell between the two SHB haplotypes from Cameroon. The similarity between haplotype CMR2 and those from the

other African countries, USA and Australia is an indication that CMR2 and these sequences might belong to the same haplogroup. The low boostrap support further support this view. CMR1 was quite unique from all the other haplotypes. However, large intraspecific distances have been reported in beetles CO1 sequences (Pentinsaari *et al*., 2014). The two SHB haplotypes (CMR1 and CMR2) were distributed across all ecological zones. This wide geographical distribution is possibly facilitated by humans through inter-regional trade of hive products (honey and wax) (Awono *et al*., 2013). The two haplotypes mostly occur in separate apiaries. However, the occurrence of the two haplotypes in some apiaries in the SW may be due to movement of colonies between apiaries by beekeepers since swarm catching from areas far off from the apiary is common in the SW. Movement of SHBs between apiaries of less than 10 km apart is also possible (Neumann *et al*., 2016). Biasness of the SHB haplotypes towards CMR1 in the East compared with the almost equal frequencies of both haplotypes in the NW and SW, is likely due to limited trade in hive products between these zones and the East and the high trade that exist between the two neighbouring regions of the NW and SW (Ingram, 2014; Awono *et al*., 2013). The implication of the two SHB haplotypes in colony health needs further investigation.

Ecological studies revealed differences in SHB abundance with ecological zones, and seasons. The high SHB numbers recorded in the forest zones of the East and SW compared with the low numbers in the savannah zone of the NW might be linked to differences in vegetation, climate, soil or topology. Higher SHB infestations have been reported in apiaries located in forest areas (Spiewok *et al*., 2008; Torto *et al*., 2010a), while soil type and temperature variation have also been shown to affect pupation and fecundity of the SHB (Ellis *et al*., 2004; de Guzman and Frake, 2007; de Guzman *et al*., 2009; Meikle and Patt, 2011) which might affect their abundance. SHB abundance also varied with seasons. Moist and humid conditions have been shown to favour the pupation success of the SHB (Ellis *et al*., 2004). The high levels of SHB observed during the long rainy season support this finding and that by Torto *et al*. (2010b) on the seasonal abundance of SHB in Kenya. However, unlike in Kenya where SHB was observed in low densities (< 15 mean SHB/hive) all year round, the infestation level in this study was higher with infestation as high as 17 and 66 mean SHB/hive during the dry and long rainy seasons respectively. Infestation levels were also higher compared to that reported in Uganda (Kugonza *et al*., 2009). This study also reports changes in SHB abundance with altitude. The strong negative correlation between SHB with increasing altitude may be the influence of several factors including soil type,

moisture or vegetation as indicated above. This study therefore highlights the relative importance of this pest across different ecological zones and seasons.

Incidental honey bee pests

The very low colony infestation by other pests such as dynastid beetles, wax moths, hawkmoths, and bee louse, in addition to the restricted occurrence of some (e.g. dynastic beetle and hawkmoths) in apiaries located in the forest zones suggest their opportunistic infestation of honey bee colonies. Most of these pests such as dynastid beetle and hawkmoths were reported for the first time in honey bee colonies in Cameroon. The incidental pests in general have been reported to attack mostly weak colonies (Hepburn and Radloff, 1998).

Phorid fly, *Megaselia scalaris*

This study represents the first report of *M. scalaris* as a pest of honey bees in Cameroon. Although these observations are not surprising as there have been previous reports of the fly's infestation on the continent (Disney, 2008), it adds to the limited reports of the fly's occurrence in honey bee colonies in Africa (Pirk *et al*., 2016). The results from the DNA barcode confirmed the identities of our samples as *M. scalaris* and corroborated their identity to those from Asia. The low numbers observed and their widespread nature are suggestive of the opportunistic infestations, probably facilitated by the weakening of colonies and worker bees caused by a number of conditions well known to adversely affect honey bees (Goulson *et al.*, 2015). *Megaselia scalaris* is a well-known parasitoid that targets slow moving, injured and dying host as well as scavenges on dead remains of various insects (Disney, 2008; Robinson, 2005). It has also recently been reported to infest healthy honey bees in Italy (Ricchiuti *et al*., 2016). The highest record of phorid in the East and South West is likely linked to the high number of worker bees injured by ants which occurred around these apiaries. Thus, predators such as ants which either disable or kill worker bees around the apiary tend to increase the risk of *M. scalaris* infestation. The control of ants infestation and proper hygiene of hive surroundings are therefore necessary for *M. scalaris* management.

Ants

This study revealed for the first time the identities of 8 ant species infesting honey bee colonies in Cameroon. Of these, 3 species namely; *M. cryptobium*, *O. longinoda* and *D.*

molestus seemed to cause the most damage compared to the others. The observation of these species in honey bee colonies supports previous reports of members of some of these genera being predators of honey bees. For instance, *Dorylus* sp is considered a widespread predator of honey bees in Africa (Hepburn and Radloff, 1998; Schöning *et al*., 2006) and *M. minimum* is considered a serious pest of honey bees in Uganda (Kugonza *et al*., 2009). Aside from *A. m. adansonii,* the major honey bee subspecies in Cameroon, *Monomorium* spp and *Oecophylla* sp also infest *A. cerana* in Asia (Ritter and Akratanakul, 2006).

As most ants are generalists, the availability of alternative food sources within these agro-ecological zones may in part explain their somewhat consistent occurrence across seasons. The high occurrence of deleterious ant species in the forest zone of the East might also be related to the higher availability of foraging resources in these zones. The observed damage patterns of the different ant species suggest the employment of different strategies to seize and capture prey which range from solitary to group hunting that may or may not involve the use of chemical weapons such as repellent secretions and paralyzing venoms (Cerdá and Dejean, 2011). Ants species have also been reported to trigger colony absconding and to cause nuisance to beekeepers (Ritter and Akratanakul, 2006). It is therefore recommended to prioritize the control of deleterious ant species to prevent absconding and improve the health of the honey bee colonies.

Effect of hive types on *Varroa* mite and *Aethina tumida* levels

Hive types significantly affected SHB levels but had very little influence on *Varroa* mite levels. However, as shown above, levels of *Varroa* mite and *A. tumida* can vary with space and time. Therefore, since the different hive types were located in different apiaries, a well design experiment with all the different hive types place in the same apiary is needed to ascertain the actual impact of hive types on pest levels.

Influence of pests on colony losses

This study also revealed that honey bee colony losses were minimal. Losses were however high during the rainy season and in the forest zones. Main colony losses in temperate regions have been reported to occur during winter (period following high *Varroa* density) (Amdam *et al*., 2004), with *Varroa* mite as one of the major contributing factors (Dainat *et al*., 2012a;

van Dooremalen *et al*., 2012; Francis *et al*., 2013). This was similar in our study where *Varroa* numbers were generally high during the dry season and thus probably also influencing colony losses during the rainy season. This is supported by the higher *Varroa* level (4 to 12 times) in colonies that collapsed in the following season (rainy season) compared to that in the absconded or survived colonies. Colony mortality was however lower compared to that reported in Madagascar (Rasolofoarivao *et al*., 2013). The high colony losses recorded in the East is probably due to contribution from other factors and pest as well. Moths have also been reported to speed up colony absconding or collapse (Hepburn and Radloff, 1998). Moths as well as the SHB were recorded in high numbers in the forest zone of the East. Also there was high incidence of deleterious ant species in the forest zones particularly in the East. The high colony losses in the East and SW compared with the NW is an indication that these other pests may be contributing factors to the observed higher losses. *Varroa* associated viruses have also been reported as contributing factors to colony death (Dainat *et al*., 2012b). Further research on these viruses is needed to provide more answers to the variation in colony death recorded in this study. Although majority of the incidental pests reported here (e.g. moths) attack mostly weak colonies (Hepburn and Radloff, 1998) they can also be used as indicators of colony heath status, since their high numbers in the forest zone was correlated to high colony losses (death and absconding). The distribution of *G. mellonella* for example has been reported to be limited to low altitudinal gradients where it causes the most damage (Ben, 1999).

5.6 Conclusion

Overall, this study has shown that honey bee populations of Cameroon are infested by a number of diverse arthropods which may be pests, parasites or predators. It also provided a number of new records on honey bee arthropod hive invaders thus adding on to the existing literature on arthropods associated with honey bee colonies. It further highlighted the impact of these arthropods to honey bee health and showed that *V. destructor* and *A. tumida* are the main pests of honey bees in Cameroon. Although findings on the high abundance of *V. destructor* at highland located apiaries and high abundance of *A. tumida* during the rainy season support previous studies, this study further showed that the abundance of these pests across ecological zones, seasons and along altitudinal gradients were in contrast with each other. In addition, the occurrence of minor pests (e.g. wax moths) although low, is shown to serve as an indicator of colony health status. This study therefore contributes to knowledge

on the ecology of honey bee pests across the African continent which is vital for pest management.

CHAPTER SIX

IMPACT OF BEEHIVE TYPE ON HONEY BEE COLONY ESTABLISHMENT AND PEST INFESTATION

6.1 Summary

Understanding the factors affecting the health of honey bees is crucial in developing sustainable solutions for their conservation. One of these factors includes management practices such as the use of different hive types. Yet there is dearth of knowledge on the impact of beehive types on bee health, especially in Africa where it is common for beekeepers to use traditional hives in keeping their bee colonies. Moreover, results from a preliminary honey bee pest survey conducted in Cameroon in 2014 suggested that hive types may have an influence on pest levels. The finding however, could not be concluded since the hive types assessed occurred in different apiaries across different geographic locations, implying that other factors (e.g. climate or honey bee subspecies) might be contributing to the differences in pest infestation levels recorded. To resolve this uncertainty, three hive types, Cylindrical Indian Bamboo (CIB), Kenya Top Bar (KTB) and Langstroth (L) were constructed and all three hive types installed in the same apiaries in three different locations. These locations include Bamenda and Oku in the savannah zone of the North West, and Buea in the forest zone of the South West. The hives were monitored for colonization and pest infestation. Results revealed that apiary location and hive type may have an influence on colonization. Colonization was higher for hives in the North West (100 % in Bamenda; 28.6 % in Oku) compared to the South West (23.8 %) and faster in the CIB (4 – 39 weeks) and KTB hives (3 – 35 weeks) compared with the L hives (30 – 47 weeks) in the North West. Hive types was also found to have an influence on the infestation of pest such as wax moth *Galleria mellonella* with high infestation of *G. mellonella* in the L hives (28.6 %) compared to CIB and KTB (0 %). However, the infestation and levels of main honey bee pests such as *Varroa destructor* and *Aethina tumida* did not differ with hive types. Thus the differences in levels of these pests recorded in the preliminary study may probably be the consequence of the location of the apiary sites and the absence of movement of honey bee stocks between apiaries or across locations. This study therefore shows that although hive type may have an influence on colonization and infestation of pests such as *G. mellonella*, it seems not to have an impact on the infestation and levels of *V. destructor* and *A. tumida*. Long term studies across all seasons and apiary sites are, however, needed to confirm these findings. This study

also highlights the significance of restricted colony movement between apiaries in minimizing the spread of honey bee pests.

6.2 Introduction

The recent global decline in honey bee colonies has led to increase in global research in an attempt to understand the possible causes for this decline and to mitigate colony losses. This has led to reports on the influence of a number factors (Goulson *et al*., 2015; Vanbergen and Insect Pollinators Initiative, 2013) including management practices (Goulson *et al*., 2015). In Africa where beekeeping is largely unprofessional, traditional practices may contribute to decline in bee population and number of colonies. For example, honey harvesting using traditional methods by unskilled beekeepers often results in low quantity and quality of hive products and in the destruction of bee brood (Ingram, 2014; King, 2014). Proper education on good management practices may therefore help to improve the quality of the hive products and curb the loss of honey bee populations.

The use of different beehive types both traditional (e.g. CL, CIB, grass, and pods hives) and modern (e.g. KTB and L hives) (Ingram, 2014; King, 2014) in beekeeping is common across Africa. However, there is little or no knowledge on the impact of these hives on honey bee health. These hive types differ in their construction materials, shapes, sizes, and volume (Ingram, 2014; King, 2014). These differences often lead to variation in hive type microenvironments which is likely to influence the hive acceptance by bees and the infestation and establishment of honey bee pests. Variation in the shape and depth of containers for example has been shown to influence the pupation success of the small hive beetle (Meikle and Diaz, 2012). It is therefore important to establish the impact of these hives on bee health to aid the adoption of the most suitable hive type with minimal effect on the bee population within different geographic locations.

In Cameroon for example, beekeepers especially in the major honey producing areas of the savannah zones use a variety of different hive types to keep their bee colonies (Ingram, 2014). The impact of these hives types on pest infestation and establishment has not been investigated. During honey bee pest survey carried out between January 2014 to December 2014 (see Chapter 5), colonies were assessed in both traditional and modern hives in the savannah zone of the NW. The results obtained suggested that hive types may have an

influence on pest levels. For example, during the dry season where three hive types (KTB, CIB and CL) were assessed, the CIB was found to harbour significantly less SHB compared to CL and KTB hives, while there was no different in *Varroa* numbers. SHBs were absent in colonies situated in apiaries sampled in Oku. Although these preliminary findings, especially on the impact of hive type on SHB levels, they are not conclusive and may be the result of variation in environmental conditions. This is because all the different hive types that were sampled occurred in different apiaries with probably different climatic conditions. Differences in climatic conditions have been reported to have differential effect on the performance of different honey bee subspecies (Abou-Shaara *et al*., 2013). Furthermore, different soil types and temperature variation have been shown to have differential impact on the pupation success and fecundity of the SHB (deGuzman *et al*., 2009; deGuzman and Frake, 2007; Ellis *et al*., 2004). It is therefore necessary to carry out proper investigation to ascertain the actual impact of hive types on honey bee pest levels in Cameroon by installing the different hive types on the same apiaries across different locations. The objective of this study was therefore to investigate the impact of beehive types on honey bee colony establishment and pest infestation.

6.3 Materials and methods

6.3.1 Selection and installation of beehive types across apiary sites

Three hive types, L, KTB, and CIB were selected for use in this experiment. The KTB hive is the most widely use hive type in Cameroon, followed by the CIB hive. The L hive is a modern hive type used by beekeepers in many areas in Europe and America. It is also increasingly being adopted in many African countries due to the ease of management (King, 2014). A total of 21 hives of each of these three hive types were constructed and baited with the same type of beeswax. Seven of each hive type was installed in three different apiary sites. These apiary sites include Buea, in the forest zone of the SW, Bamenda and Oku in the savannah zone of the NW. Detailed description of these ecological zones is provided in section 3.1 above.

In each apiary site, the hives were randomly placed on hive stands approximately 90 cm above the ground. The hives where arranged with their entrances facing the same direction. Hive installation took place during the third week of January 2015 in all the apiary sites.

6.3.2 Monitoring beehives for colonization and pest infestation

The occupation of the hives by bees commenced immediately after installation of the hives in all the three apiary sites. Colonization was monitored weekly throughout the study period from the third week of January 2015 to the last week of April 2016. This covers a period of approximately 56 weeks.

Assessment of the hives for pest infestation was carried out after successful colonization of 100 % of the installed hive types in at least one of the three apiary sites. Established colonies were assessed for pests such as *Varroa* mite, *Braula* sp, *A. tumida*, large hive beetles, wax moths, hawkmoths and other arthropod hive invaders following the procedures described in section 5.3.2 above**.**

6.3.3 Colony performance parameter estimation

The established colonies were evaluated for difference in performance by estimating strength parameters such as number of combs with bees, honey, and brood. This estimate was done by dividing the frames in to four parts and estimating the total area occupied by bees, honey, or brood. The methods used are similar to those described in Delaplane *et al*. (2013) and Chemurot *et al*. (2016) for African honey bees.

6.3.4 Data analysis

The time taken for the colonization of the different beehives (rate of colonization) was analyzed using Kruskal-Wallis test with multiple comparisons of mean ranks using Dunn's test. The percentage of arthropod infested colonies in each hive type was estimated for each pest by dividing the number of infested colonies by the total number of colonies and multiplying the results by 100 %. To determine the influence of beehive type on *V. destructor* and *A. tumida* abundance, counts of these pests were analyzed using the non-parametric Kruskal-Wallis test. The same statistical tests were used to analyze data for the strength parameters between hive types. All analyses were carried out in the R statistical software (R Core Team, 2015).

6.4 Results

6.4.1 Honey bee colonization of hive types across apiary sites

Occupation of the hive types by bees varied across apiary sites. Of the total number of hives (n = 21) installed in each of the three apiary sites, 100 % of the hives (n = 21) were occupied in Bamenda; 28.6 % (n = 6) in Oku; and 23.8 % (n = 5) in Buea (Table 6.1). Besides Bamenda and Oku where equal number of the three hive types were occupied, colonization was biased towards the KTB hives in Buea. All the 5 colonies in Buea where housed in KTB hives. However, 2 of the L and CIB hives were temporary occupied but the colonies absconded shortly after colonization.

Table 6.1 Percentage of hive colonization by honey bees in three different apiary sites in Cameroon

Apiary site	Number of colonized hives (% of total hives)			Total colonized hives (% of total hives)
	L	KTB	CIB	
Bamenda	7 (100)	7 (100)	7 (100)	21 (100)
Oku	2 (28.6)	2 (28.6)	2 (28.6)	6 (28.6)
Buea	0 (0)	5 (71.4)	0 (0)	5 (23.8)

L = Langstroth hive, KTB = Kenya Top Bar hive, CIB = Cylindrical Indian Bamboo hive

6.4.2 Rate of colonization of the different hive types

The rate of colonization was estimated only in Bamenda where all hive types were occupied by bees. The colonization rate ranged between 3 to 47 weeks. The KTB hives were colonized between 3 and 35 weeks; the CIB hives between 4 and 39 weeks and the L hives between 30 and 47 weeks (Figure 6.1). The mean colonization rate differed significantly with hive types (Kruskal–Wallis test, H (2) = 9.76, P = 0.008). It was significantly lower in the KTB than L hives and in CIB than in the L hives. However, colonization rate did not differ between the KTB and CIB hives (Figure 6.1). The honey bee populations around Bamenda in the savannah zone of the North West therefore seem to have a higher preference for the CIB and KTB hives over the L hives.
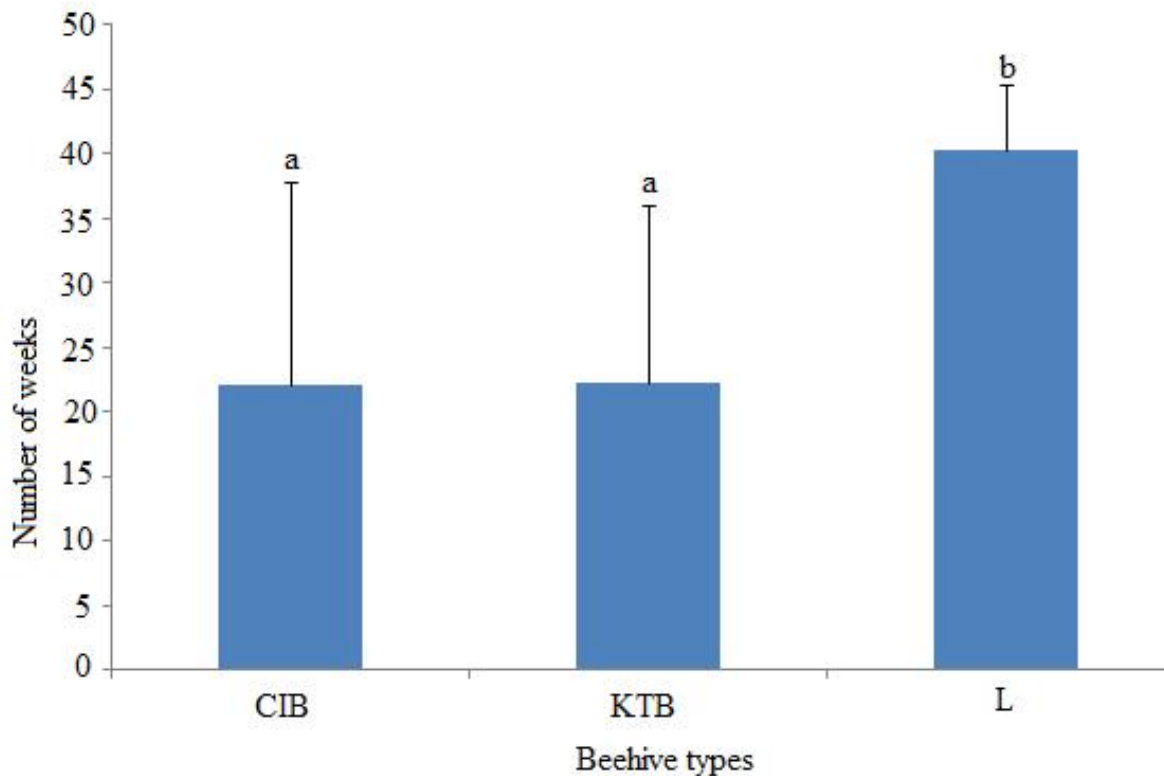
Figure 6.1 Mean (± SD) number of weeks prior to colonization of the different beehive types in Bamenda. CIB = Cylindrical Indian Bamboo, KTB = Kenya Top Bar, L = Langstroth. Significant differences (Kruskal-Wallis test, = 0.05) are indicated by the different letters (a, b).

6.4.3 Arthropods infesting honey bee colonies in different beehive types

The assessment of honey bee colonies in the KTB, CIB and L hives for pests in Bamenda, North West revealed the presence of 6 arthropod pest species, 1 species of pseudoscorpion and predators. Pests such as *V. destructor* and *A. tumida* were common in all the 3 hive types. The pseudoscorpion *Ellingsenius ugandanus,* and predators such as ants were also recorded in at least one of each of the three hive types. The wax moth *Galleria mellonella* was recorded only in the L hives, hawkmoth *Acherontia sp* in KTB and CIB, *Braula* sp in CIB only (Table 6.2).

The number of pest infested colonies in each hive type varied with pest species. Pests such as *V. destructor* was recorded in 100% of the assessed colonies in all the beehive types while *A.*

tumida was recorded in about 71% of the KTB hives and in 85% of the L and CIB hives (Table 6.2). Other pests such as *G. mellonella*, *Acherontia sp*, and *Braula sp*, did not occur in all the beehive types. The wax moth *G. mellonella* for example was recorded only in the L hives and *Braula* sp only in CIB hives. The percentage of infested colonies housed in each of the beehive types by these pests was less than 30% (Table 6.2). Predators such as ants were recorded more in the L and KTB hives (71 % colony infestation) than in the CIB hives (28% colony infestation). Other arthropods such as pseudoscorpions were commonly encountered in the L hives (42%) than in the KTB (28%) or CIB hives (14%) (Table 6.2).

Table 6.2 Arthropod pests and predators infesting honey bee colonies housed in different hive types

Arthropod pests and predators	% infested colonies		
Pests	L	KTB	CIB
Varroa destructor	100	100	100
Aethina tumida	85.7	71.4	85.7
Galleria mellonella	28.6	0	0
Acherontia sp	0	14.3	14.3
Braula sp	0	0	28.6
Ellingsenius ugandanus	42.8	28.6	14.3
Predators			
Ants	71.4	71.4	28.6

CIB, Cylindrical Indian Bamboo hive; KTB, Kenya Top Bar hive; L, Langstroth hive

6.4.4 Influence of hive types on *Varroa destructor* and *Aethina tumida* levels

The infestation levels of *V. destructor* ranged from 10 to 32 mites/300 bees for the CIB hives; 7 to 63 for KTB and 9 to 30 for the L hives. Mean *Varroa* mite numbers however, did not differ significantly between the three beehive types ($H = 0.036$, $P = 0.982$) (Figure 6.2).
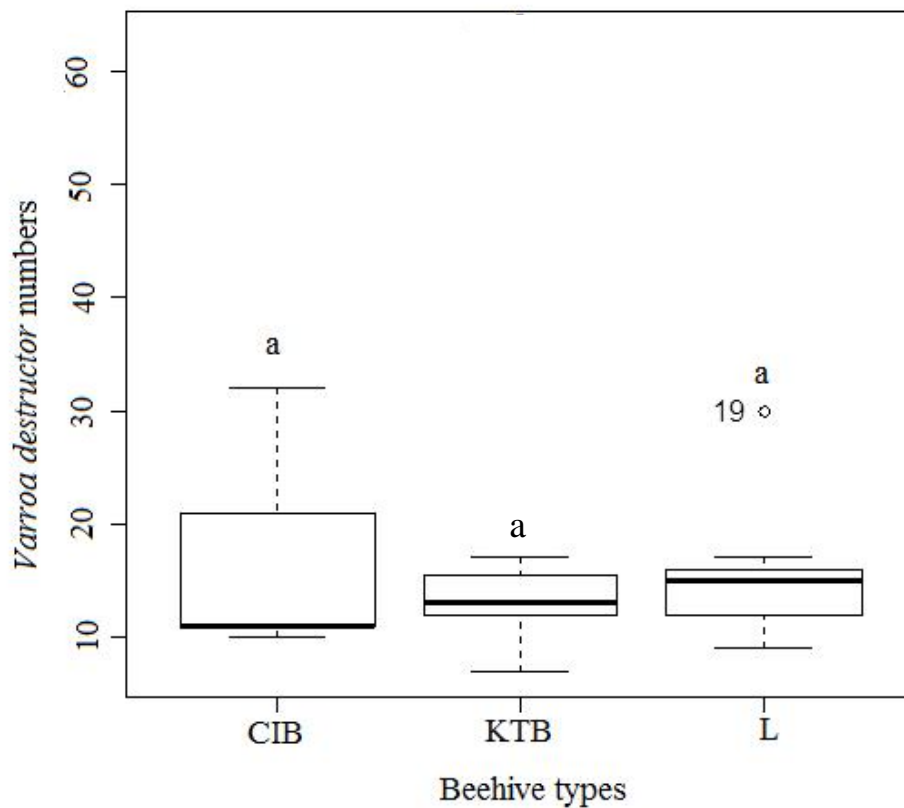
Figure 6.2 Mean *Varroa destructor* numbers between the Cylindrical Indian Bamboo (CIB), Kenya Top Bar (KTB) and Langstroth (L) beehive types.

The infestation level of *A. tumida* in the different beehive types was also variable. The number of beetles/hive range from 0 to 215 for CIB hives; 0 to 350 for the KTB hives and 0 to 300 for the L hives. Like *V. destructor*, the *A. tumida* mean numbers did not differ significantly between the three hive types ($H = 0.058$, $P = 0.972$) (Figure 6.3).
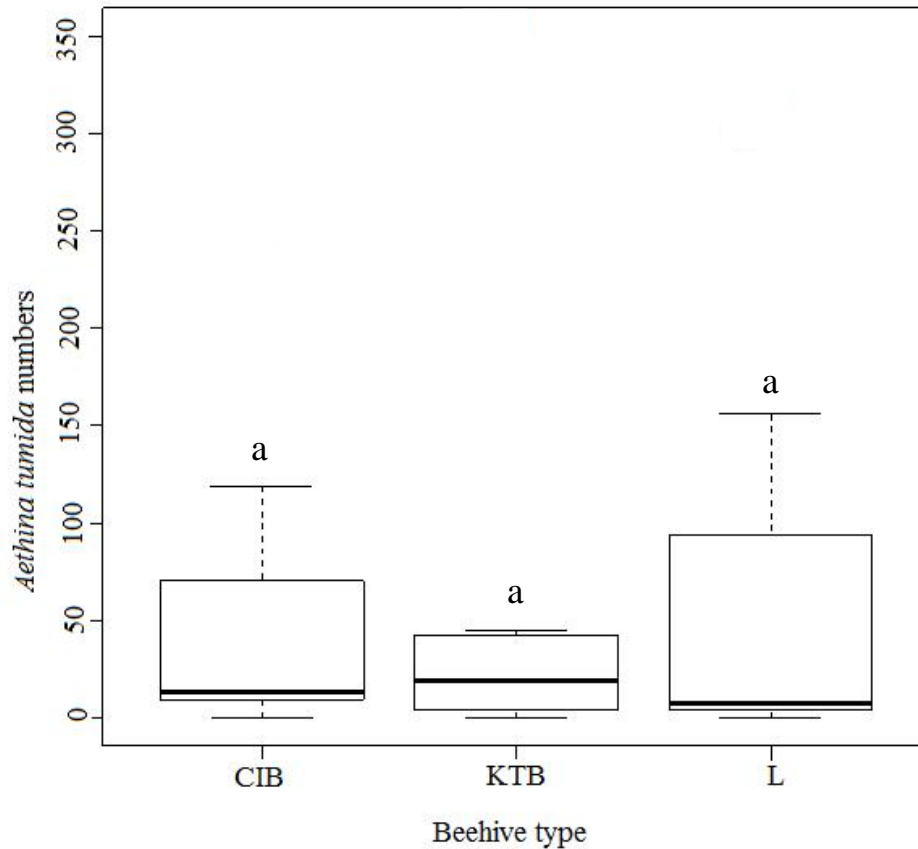
Figure 6.3 Mean *Aethina tumida* numbers between the Cylindrical Indian Bamboo (CIB), Kenya Top Bar (KTB) and Langstroth (L) beehive types.

6.4.5 Variation in colony strength parameters with beehive types

Bee population was significantly different among the beehive types ($H = 11.117$, $P = 0.004$). The CIB had significantly higher bee population compared to the KTB hives or the L hives (Figure 6.4A). There was however, no significant difference in bee population between the KTB and L hives (Figure 6.4A). There was significant difference in the number of combs with honey between hive types ($H = 7.92$, $P = 0.019$), with the CIB containing higher number of combs with honey compared to the KTB and L hive types (Figure 6.4B). The number of combs occupied by brood did not differ between hive types ($H = 4.57$, $P = 0.102$) (Figure 6.4C).
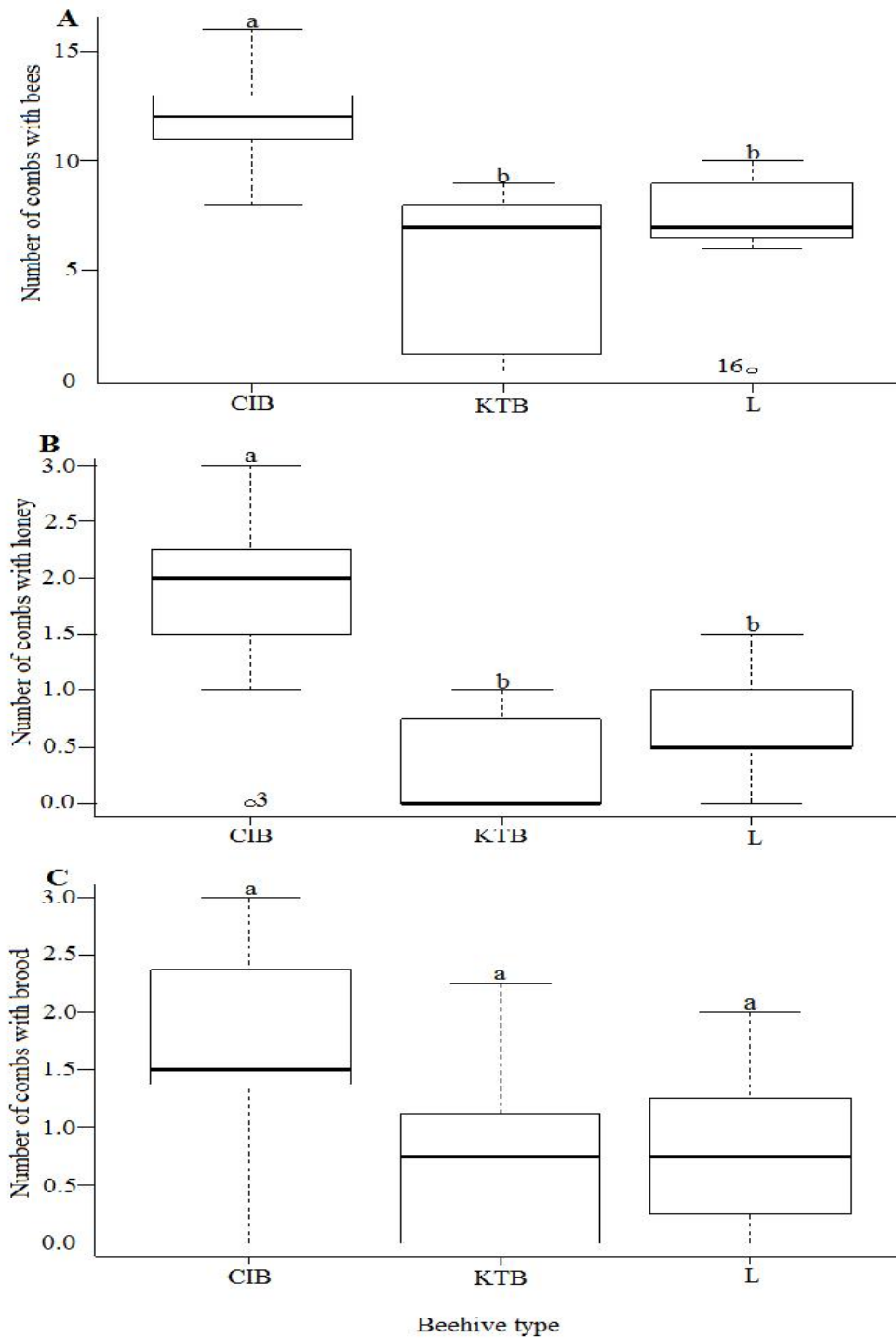
Figure 6.4 Influence of beehive type on colony strength parameters. Influence of hive types on (A) bee population, (B) honey, (C) brood. The hive types include; Cylindrical Indian Bamboo (CIB), Kenya Top Bar (KTB), and Langstroth (L) hives. Significant differences ($P < 0.05$, Kruskal-Wallis test with multiple comparisons of mean ranks) are indicated using different letters.

6.5 Discussion

This study represents the first attempt to evaluate the impact of hive types on honey bee colony establishment and pest infestation levels in the CIB, KTB and L hive types. The differences in colonization of hive types across locations recorded in this study may be due to differences in a number of environmental and anthropogenic factors. The higher colonization of hives in Bamenda in the savannah zone of the NW compared to Buea in the forest zone of the SW for example, may be due to differences in vegetation cover. Also, the availability of large monoculture plantations which are often being sprayed with chemical pesticides might also be a contributing factor to the low colonization in Buea. Pesticides (especially neonicotinoid insecticides) have been linked to the decline of wild bee populations (Woodcock *et al*., 2016). However, further studies on the effect of the commonly used pesticides around Buea on bee health would be required to ascertain this point. The high availability of wild colonies in Bamenda compared to Oku both in the savannah zone is probably due to the low beekeeping activities and less number of beekeepers around Bamenda compared to Oku a predominant beekeeping area. Increase in the number of beekeepers has been linked to decline in the number of feral colonies (Goulson *et al*., 2015). Other factors including the lack and diversity of foraging resources, pests and diseases (Goulson *et al*., 2015), may also influence the availability of feral colonies across locations.

Besides differences in colonization across locations, the rate of colonization was found to differ with hive types. In particular, bees were found to have a high preference for the CIB and KTB hives over the L hives. This may be due to either familiarity of specific hive types to the local bee populations or differences in the hive designs. In the North West for example, the CIB and KTB hives are the two commonly used hive types while in Buea, in the South West, the KTB is the only hive type used by beekeepers. In addition, the L hive type used in this study is generally new to Cameroon. The designs of the CIB hives with outer covering wrapped with grass and the KTB hives with closed frames make these hives probably safer than L hives. This may also explain the variation in colonization of the hive type by bees. This finding supports previous study by Kugonza *et al*. (2009) where they reported faster colonization of traditional grass hives compared to KTB and L hives in Uganda and by Ande *et al*. (2008) where they showed that bees in Nigeria had a higher preference for Clay-pot and KTB over the L and other hive types. In common with these previous studies, this study also showed that traditional hives (although different in each study) and KTB hives tend to be

better in terms of colonization than the L hives. The use of these hives is therefore encouraged especially for swarm catching.

The differences in pest occurrence in the different hive types may be linked both directly and indirectly to the design of the hives. Directly, the design of the traditional hives with a single entrance makes it easier for worker bees to guard against pests. In some of the KTB and L hives most of the floor entrances were abandoned (probably due to rain splashing through) and an alternative entrance created close to the roof of the hive. The abandoned entrances can serve as a passage for pests or predators. This was unlike in CIB hives where the entrance (on the side of the hive) was never abandoned. However, in some instances the CIB hive entrances were reduced with propolis leaving only a tiny entrance small enough to prevent entry of intruders. This reduced disturbance of the bees and is likely to impact on the higher bee population in these CIB hive types compared to KTB or L hives. Indirectly, variation in colony strength (e.g. bee population) may also influence the ability of bees to defend themselves against pests or predators. The occurrence of wax moths in some L hives for example was probably not only as a result of abandoned entrances but also an indication of weakness of most of the bee colonies in the L hives. The mean number of frames occupied by bees in the L hives for example was lower compared to that in the CIB and KTB hives. Wax moths have been reported to attack mostly weak colonies (Hepburn and Radloff, 1998). This finding also support previous studies by Kugonza *et al*. (2009) where they recorded least number of pests in traditional hives compared to modern beehives (KTB and L) in Uganda. Unlike in L hive types the presence of bee louse in the CIB hive was not an indication of weakness but rather strength of the colonies since most of the CIB hives had high bee population with the presence of honey. Bee louse eggs are known to hatch and developed only when oviposited on honey cappings (Ellis and Nalen, 2010). Therefore colonies with high bee population and presence of honey are likely to harbor large bee louse population. Bee lice are also regarded as minor pests that are unlikely to cause any major damage to honey bees (Ellis and Nalen, 2010). All the hawkmoths recovered from the CIB hives were already dead and propolised. This is an indication that they probably gain access in to these hives before the reduction of the entrances and that the bees were able to defend themselves against this intruder.

The findings that hive types do not have an impact on the infestation levels of *V. destructor* and *A. tumida* contradicts the preliminary findings that prompted this study. Therefore the

absence of SHB in the CIB hives in Oku as shown by the preliminary study may have been due to either the unsuitability of the climatic conditions of Oku to the survival and development of the SHB, or limited dispersal of SHB from neighboring localities in to Oku. The former requires further study. However, it has been shown that under extreme environmental conditions, the microclimatic conditions of modified hive types can influence the performance of honey bee colonies and colony losses (Abou-Shaara *et al*., 2013; Erdogan *et al*., 2009). The latter reason for the absence of SHB in Oku as shown in the preliminary study may also be likely since there is limited or no movement of honey bee colonies from other localities in to Oku. In Oku, beekeepers practice migratory beekeeping where hives are installed in the lowland warmer savannah for colonization then transported to the forest for honey production still around Oku. Also, unlike *Varroa* mite that is dispersed by bee swarms, SHB is rarely dispersed by bee swarms (Ellis *et al*., 2003b). Although SHBs are active flyer and can detect stress colonies over distances of greater than 10 km (Neumann *et al*., 2016), their long distance dispersal has been shown to be restricted (Ellis *et al*., 2003b; Spiewok *et al*., 2008). Human aided dispersal has been suggested to be the main long distance dispersal mode of this pest (Hood, 2000; Spiewok *et al*., 2008). Thus the absence of SHB in Oku is probably aided by absence of the movement of honey bee stocks from other localities by beekeepers in to Oku. Therefore it is recommended that beekeepers limit movement of colonies between apiaries to avoid spread of this pest in to free zones.

6.6 Conclusion

Apiary location and beehive type had an influence on the colonization and establishment of honey bee colonies. In addition, hive types also influenced the infestation of pests such as *G. mellonella*. It did not, however, have an influence on the infestation and levels of pests such as *V. destructor* and *A. tumida*. The use of hives such as CIB and KTB are recommended since they performed better in terms of colonization and were less infested by wax moths. This study also highlighted the importance of limited colony movement between apiaries in minimizing the spread of *A. tumida*. Investigations on pest levels over time and in other apiary sites and seasons are needed to confirm these findings.

CHAPTER SEVEN

GENERAL DISCUSSION, CONCLUSIONS, AND RECOMMENDATIONS

7.1 General discussion

In this study, field assessments and laboratory studies encompassing morphological and molecular analysis were used to investigate the diversity of *A. mellifera* subspecies and their associated arthropod pests across selected geographic locations of Cameroon with focus on the conservation of this important pollinator species.

The study on honey bee subspecies diversity represents the first most comprehensive attempt to describe *A. mellifera* populations of Cameroon using a combination of both morphological and molecular markers. Morphological analysis of honey bee samples revealed the existence of three distinct morphotypes of *A. mellifera*. This finding agrees with previous reports by Hepburn and Radloff (1997) on *A. mellifera* morphotypes of Cameroon. It further shows that bee populations of the lowland forests zones of the South, East and Central that were not described in previous studies are morphologically similar to the bee populations of the South West. This study therefore expanded the range of the described *A. mellifera* populations across Cameroon.

Genetic analysis and phylogenetic studies showed that the *A. mellifera* morphotypes represented five mtDNA *A. mellifera* subspecies haplotypes. The recorded *A. m. scutellata* and *A. m. adansonii* haplotypes were similar to those reported in Kenya and Nigeria respectively (Arias and Sheppard, 1996). The other three ND2 subspecies haplotypes are new. Mountain bees of the North West were represented by two of these new ND2 subspecies haplotypes (Cam2 and Cam3). The haplotype Cam4 was morphologically similar to the *A. m. jementica* described previously in the northern part of Cameroon by Hepburn and Radloff (1997). Although this seems to suggest that Cam4 is likely *A. m. jementica*, this cannot be conclusive since genetic diversity studies revealed that beside the *A. m. scutellata* haplotypes, all the subspecies haplotypes were more closely related to the *A. m. adansonii* haplotype (the dominant haplotype) than to each other. This suggests that all the mountain bees probably descended from the same lowland *adansonii* population. It also supports the view that mountains bees should be considered differentiated populations of the subspecies surrounding the mountains (Hepburn *et al*., 2000).

Through morphology and DNA barcodes, this study also showed that the *A. mellifera* populations of Cameroon are infested by a number of different pest species, parasites and predators. Further phylogenetic studies using species specific primers, detected the virulent strain of *V. destructor*, the Korean haplotype and two haplotypes of *A. tumida*. The *V. destructor* was similar to that reported to cause damage to honey bees in Madagascar (Rasolofoarivao *et al*., 2013) or other parts of Africa (Pirk *et al*., 2016). One of the *A. tumida* haplotype CMR2 was genetically similar to that from other parts of the world (Evans *et al*. 2000, Lounsberry *et al*. 2010) while CMR1 was a unique *A. tumida* haplotype, not reported before. The implications of these haplotypes to bee health require further studies.

Honey bee pest survey results revealed *V. destructor* and *A. tumida* as the key pests of honey bees due to the high infestation levels. The contrasting pattern in levels of these two pests along altitudinal gradients, across ecological zones and seasons suggest the varying impact of these pests across seasons and locations. The variation in infestation levels by the mite can be due to differences in climatic conditions (Moretto *et al*., 1991) or honey bee subspecies (Invernizzi *et al*., 2015). Differences in temperature, soil types, and moisture are factors that can influence the populations of *A. tumida* (deGuzman and Frake, 2007; Ellis *et al*., 2004; Meikle and Patt, 2011).

The high colony losses recorded in the lowland forests zones may be the result of pests such as *V. destructor* and *A. tumida*. The high abundance of *V. destructor* in the collapsed colonies seems to support this claim. This mite alone or in combination with other pests, has been reported to play a central role in colony losses in the United States (Schäfer *et al.*, 2010). Besides *Varroa* mite and *A. tumida* other pests may act in synergy to contribute to honey bee colony losses. For example ants were found to cause injuries and death in honey bees especially in the East. This mortality and injury to bees by ants also probably contributed to the relatively high *M. scalaris* infestation of colonies in the East. Species of the genus *Megaselia* for example have been shown to infest slow moving or dying bees (Dutto and Ferrazzi, 2014) and contributes to poor honey bee health (Ricchiuti *et al*., 2016). Colony infestation by other minor pests such as wax moths *G. mellonella* and hawk moths *Acherontia sp* were also relatively high in forest zones. These minor pests have been reported to speed up colony absconding and collapse (Hepburn and Radloff, 1998). The combination of these pests is therefore likely to result in relatively high colony losses.

The differences in *A. mellifera* populations and vegetation (nutritional resources quality and quantity) across ecological zones may also be contributing factors to colony losses. It has been shown that differences in resistance between honey bee populations can explain the survival of honey bee colonies infested with *V. destructor* (Invernizzi *et al*., 2015; Strauss *et al*., 2016). In addition, genetic diversity has been reported to increase fitness and productivity in honey bees (Mattila and Seeley, 2007; Tarpy, 2003). Furthermore the quality and quantity of nutritional resources (e.g. pollen) can influence bee health (Di Pasquale *et al*., 2013). Thus the high *A. mellifera* genetic diversity in the highland savannah zones as well as differences in vegetation across Cameroon may also be contributing factors to the low colony losses recorded. Other factors such as diseases, pesticides and even management practices (Goulson *et al*., 2015) may also be contributing to the observed losses. However, this requires further studies.

Beehive types may also affect beekeeping and bee health. The high rate of colonization of the CIB hives by bees over the KTB and L hive types suggests that factors such as use of different hive types may influence beekeeping dynamics across localities. The variation in pest occurrence with hive types further suggests that the choice of the hive type may help reduce pest infestation. The non significant impact of hive types on the infestation levels of the main pests, *V. destructor* and *A. tumida*, may require further confirmatory studies involving colonies in the other agro-ecological zones and over long time periods. Of significance is the fact that this study revealed the importance of restricted movement of bee stocks in limiting the spread of honey bee pests (e.g. *A. tumida*).

7.2 Conclusions

This study has led to a number of conclusions with regards to honey bee diversity, arthropod pests of honey bees, and on the influence of beehive types on honey bee colony establishment and pest levels.

Morphological and molecular studies on *A. mellifera* populations of Cameroon have led to the conclusions that:

1. There exist at least three morphotypes of *A. mellifera* adapted to different ecological zones of Cameroon.

2. The *A. mellifera* morphotypes represent distinct subspecies haplotypes and that there are five *A. mellifera* subspecies haplotypes in Cameroon.

3. Three of the five *A. mellifera* haplotypes represent new ND2 mtDNA subspecies haplotypes.

4. *Apis mellifera adansonii* is the dominant subspecies haplotypes in Cameroon.

Identification of the sampled pests using diagnostic protocols, assistance from taxonomic experts and through DNA barcoding provided conclusive evidence that:

1. *Apis mellifera* populations in Cameroon are infested by at least eight known honey bee pests including *V. destructor*, *A. tumida*, *G. mellonella*, *A. grisella*, *Acherontia sp*, *Braula sp*, *M. scalaris* and dynastid beetles.

2. The Korean haplotypes is the only variant of *V. destructor* infesting honey bees in Cameroon

3. There are two *A. tumida* haplotypes infesting honey bees colonies with one of these (CMR1) being unique to Cameroon

4. *Megaselia scalaris* is the only recorded endoparasitic fly infesting honey bees populations. Predators such as ants increase the risk by *M. scalaris* due to their injuries and mortality to bees

5. The ant species, *M. cryptobium*, *O. longinoda* and *D. molestus* are the most damaging predators of honey bees in Cameroon.

Studies on the ecological and seasonal abundance of honey bee pests revealed that the occurrence and levels of honey bee pests, may be influenced by ecological zones, seasonality and elevation gradients. In particular it led to the conclusions that:

1. *Varroa destructor* and *A. tumida* are the two major pests infesting both the lowland and highland *A. mellifera* populations in Cameroon.

2. The abundance of *V. destructor* and *A. tumida* are in contrast with each other. While levels of *V. destructor* are high at highland located apiaries, in the savannah zones, and during the dry season, that of *A. tumida* tend to be high at lowland located apiaries, in the forest zones, and during the rainy seasons.

3. Although pests such as wax moths *Galleria mellonella* and *Achroia grisella,* hawkmoths *Acherontia* sp, phorid *M. scalaris,* and dynastic beetle are regarded as minor or opportunistic pests due to their low infestation levels, their synergistic effects can increase the risk of colony collapse or absconding.

4. The occurrence of minor pests such as wax moths and hawk moths in honey bee colonies may act as indicator of colony health status.

Overall colony losses in Cameroon are minimal. The losses can be attributed to a number of factors including pests such as *V. destructor*, *A. tumida*, and *G. mellonella*, and predators such as *M. cryptobium*, *O. longinoda* and *D. molestus*.

The study on the effect of hive types on honey bee colony establishment and pest infestation led to the conclusions that:
1. Honey bee colonization may be influence by apicultural practices such as hive types
2. Beehive type may influence the infestation by pests such as *G. mellonella* but not *V. destructor* or *A. tumida*.
3. Limited movement of bee stocks between localities, or apiaries may limit the spread of pests such as *A. tumida*.

In general, this study has provided new and expanded information on the diversity and geographic distribution of *A. mellifera* populations of Cameroon. It has also revealed new insights on the diverse pest species infesting these honey bee populations and provided baseline information that may be developed to aid the management of the recorded pests. This study therefore adds to the existing knowledge on the ecology of honey bees in Africa.

7.3 Recommendations

The findings of this study have led to a number of recommendations directed to researchers, policy makers and beekeepers. These include the following:
1. The movement of colonies between apiaries and across ecological zones should be limited so as to preserve the unique genetic identities of the *A. mellifera* subspecies haplotypes and morphotypes recorded in this study. Also, based on the findings that some honey bee pests such as *A. tumida* and some of its haplotypes where absent in some localities it is strongly recommended that beekeepers limit the movement of colonies between apiaries and localities to avoid or minimize the spread of this pest to new locations
2. Since the objective of this study was to determine the diversity of *A. mellifera* across ecological zones, it did not describe in detail the within zonal variation of the *A.*

mellifera populations. Detailed characterization of honey bee populations of the Far-North, Central, and other parts of Cameroon are therefore needed to determine the within zonal variations in bee populations.

3. Since only one molecular marker (mtDNA) was used in this study, it is recommended that more extensive studies using a combination of both mtDNA and nuclear markers be carried out to further discriminate between the *A. mellifera* haplotypes and to resolve the taxonomic uncertainty regarding the Northern *A. mellifera* populations.

4. Detailed studies on the occurrence and infestation levels of honey bee pests in areas not covered in this study such as the Extreme-North and South are needed to determine the extent of spread, levels and impact of these pests within Cameroon.

5. More research is needed to determine the implication of the two *A. tumida* haplotypes on honey bee health.

6. Long term studies are needed to establish the actual impact of the recorded pests on honey bee population decline.

7. Further research on the impact of factors such as honey bee associated viruses and bacterial diseases, as well as pesticides levels is needed to provide more answers to the variation in colony losses recorded.

8. The study on the colonization of hive types by honey bees, did not take into consideration the subspecies. Further study is therefore required to determine the influence of beehive types on colonization by different honey bee subspecies.

9. Since pest assessment in the different hive types (in the hive type experiment) took place within just one season and location, long term studies in other apiary sites are necessary to further ascertain the impact of these hive types on pest levels.

10. Further studies on colony production (e.g. honey yield) in different hive types are necessary to have a better understanding on the influence of hive types on colony productivity.

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APPENDICES

Appendix 1. Evolutionary divergence (%) over *Aethina tumida* sequence pairs within and between haplotypes from Cameroon, Kenya, and reference sequences from Genbank.

		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26
1	LKB2 CMR1																										
2	NJB1 CMR1	1.2																									
3	NJB2 CMR1	1.4	0.2																								
4	TOBB2 CMR1	1.1	0.9	0.9																							
5	BUB1 CMR1	1.4	0.5	0.5	0.8																						
6	KMAB1 CMR1	1.1	0.4	0.4	0.7	0.3																					
7	KMAB3 CMR1	0.9	0.7	0.7	0.4	0.8	0.4																				
8	BTRB1 CMR1	1.2	0.7	0.7	0.7	0.8	0.7	0.7																			
9	BTRB2 CMR1	1.1	0.8	0.8	0.5	0.9	0.8	0.5	0.1																		
10	BTB1 CMR2	4.1	3.7	3.7	4.0	4.3	4.2	3.7	3.5	3.4																	
11	BLB1 CMR2	4.1	3.7	3.7	4.0	4.3	4.2	3.7	3.5	3.4	0.0																
12	BUB2 CMR2	4.0	3.6	3.6	3.8	4.2	4.1	3.6	3.4	3.3	0.1	0.1															
13	MVB1 CMR2	4.0	3.6	3.6	3.8	4.2	4.1	3.6	3.4	3.3	0.1	0.1	0.0														
14	TOBB1 CMR2	4.0	3.6	3.6	3.8	4.2	4.1	3.6	3.4	3.3	0.1	0.1	0.0	0.0													
15	NDB1 CMR2	4.0	3.6	3.6	3.8	4.2	4.1	3.6	3.4	3.3	0.1	0.1	0.0	0.0	0.0												

		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26
16	NDB2 CMR2	4.0	3.6	3.6	3.8	4.2	4.1	3.6	3.4	3.3	0.1	0.1	0.0	0.0	0.0	0.0											
17	NBIB1 KE	4.0	3.6	3.6	3.8	4.2	4.1	3.6	3.4	3.3	0.1	0.1	0.0	0.0	0.0	0.0	0.0										
18	KEB1 KE	4.1	3.7	3.7	4.0	4.3	4.2	3.7	3.5	3.4	0.0	0.0	0.1	0.1	0.1	0.1	0.1	0.1									
19	AF227645 NA1*	4.0	3.6	3.6	3.8	4.2	4.1	3.6	3.4	3.3	0.7	0.7	0.5	0.5	0.5	0.5	0.5	0.5	0.7								
20	AF227646 NA2*	3.8	3.5	3.5	3.7	4.1	4.0	3.5	3.3	3.1	0.2	0.2	0.1	0.1	0.1	0.1	0.1	0.1	0.2	0.7							
21	AF227650 SA*	4.1	3.5	3.5	4.0	4.1	4.0	3.7	3.5	3.4	0.5	0.5	0.4	0.4	0.4	0.4	0.4	0.4	0.5	0.5	0.5						
22	AF227653 SA*	4.1	3.7	3.7	4.0	4.3	4.2	3.7	3.5	3.4	0.5	0.5	0.4	0.4	0.4	0.4	0.4	0.4	0.5	0.1	0.5	0.4					
23	AF522357 ZIM*	4.1	3.7	3.7	4.0	4.3	4.2	3.7	3.5	3.4	0.4	0.4	0.3	0.3	0.3	0.3	0.3	0.3	0.4	0.9	0.2	0.8	0.8				
24	HM056043BKF*	4.2	3.6	3.6	3.8	4.2	4.1	3.6	3.4	3.3	0.5	0.5	0.4	0.4	0.4	0.4	0.4	0.4	0.5	0.5	0.5	0.4	0.4	0.8			
25	HM056063AUST*	4.1	3.7	3.7	4.0	4.3	4.2	3.7	3.5	3.4	0.4	0.4	0.3	0.3	0.3	0.3	0.3	0.3	0.4	0.4	0.4	0.3	0.3	0.7	0.3		

CMR1 and CMR2 = Haplotypes sequences from Cameroon; KE = Kenya; * Reference sequences from Genbank. NA1 and NA2 = Sequences from North America; SA= South Africa; Zim = Zimbabwe; BKF = Burkina Faso; AUST = Australia. Site names follow those in Appendix 1.

Appendix 2. *Varroa destructor* infestation levels between seasons and across ecological zones in Cameroon

Zone	Season	n	Infestation levels (number of mites/300bees)					
			0 - 5	6 - 10	11 - 15	16 - 20	21 - 30	>30
NW	Dry	30	2(6.7%)	5(16.7%)	6(20%)	4(13.3%)	7(23.3%)	6(20%)
	Short rains	30	3(10%)	13(43.3%)	5(16.7%)	3(10%)	4(13.3%)	2(6.7%)
	Long rains	30	9(30%)	8(26.7%)	6(20%)	2(6.7%)	1(3.3%)	4(13.3%)
East	Dry	30	10(33.3%)	9(30%)	4(13.3%)	1(3.3%)	3(10%)	3(10%)
	Short rains	30	6(20%)	10(33.3%)	7(23.3%)	1(3.3%)	6(20%)	0
	Long rains	30	14(46.7%)	9(30%)	5(16.7)	1(3.3%)	0	1(3.3%)
SW	Dry	30	14(46.7%)	5(16.7%)	6(20%)	2(6.7%)	1(3.3%)	2(6.7%)
	Short rains	30	14(46.7%)	9(30%)	3(10%)	1(3.3%)	1(3.3%)	2(6.7%)
	Long rains	30	17(56.7%)	8(26.7%)	2(6.7%)	2(6.7%)	1(3.3%)	0

NW, Sudan savannah grassland of the North West; SW, dense evergreen forest of the South West; East, degraded evergreen forest of the East; *n*, number of colonies inspected

Appendix 3. Mean (± SE) numbers of *Aethina tumida* and *Varroa destructor* across study sites in the North West, South West, and East of Cameroon.

Pest	Ecological zones	Site	Season		
			Dry	**Short rainy**	**Long rainy**
Aethina tumida	**NW** (16.94 ± 2.46)	BE1	9.2 ± 6.22	41.11 ± 6.49	61.33 ± 7.07
		BN	NA	3.71 ± 1.96	NA
		EJ	15.33 ± 5.78	NA	28.2 ± 14.68
		KBO	14.5 ± 8.19	3.25 ± 2.14	2.25 ± 1.32
		LK	5.3 ± 0.92	7.3 ± 1.69	2.9 ± 1.06
		MN	NA	NA	8.5 ± 1.5
	East (42.17 ± 5.19)	BT1	10.0 ± 5.12	26.5 ± 15.35	25.5 ± 7.52
		BT2	9.5 ± 2.46	41.88 ± 6.33	53.86 ± 18.76
		BTR	26.9 ±13.23	39.36 ± 7.62	75.8 ± 27.57
		KMA	18.75 ± 5.65	42.57 ± 9.13	114.29 ± 25.53
	SW (20.87 ± 2.29)	BU1	21.0 ± 12.91	17.8 ± 6.73	17.5 ± 11.53
		BU2	11.0 ± 10.49	15.0 ± 1.87	3.86 ± 1.18
		KBA	13.86 ± 7.86	12.29 ± 4.35	3.5 ± 2.02
		MV	15.2 ± 1.98	12.2 ± 1.88	45.0 ± 10.49
		TOB1	23.33 ± 6.34	42.67 ± 6.65	5.33 ± 2.85
		TOB2	34.17 ± 6.09	32.0 ± 7.78	40.71 ± 14.85
Varroa destructor	**NW** (16.77 ± 1.58)	BE1	15.8 ± 5.42	8.78 ± 1.31	22.22 ± 6.79
		BN	NA	10.0 ± 1.45	NA
		EJ	21.0 ± 3.47	NA	5.6 ± 0.4
		KBO	36.25 ± 14.5	35.0 ± 10.24	10.0 ± 3.46
		LK	23.6 ± 3.67	15.7 ± 3.16	6.0 ± 1.73
		MN	NA	NA	35.5 ± 0.5

Appendix 3 Continue

Pest	Ecological zones	Site	Season		
Varroa destructor	**East** (11.63 ± 1.47)	BT1	12.5 ± 4.45	6.25 ± 1.6	7.0 ± 1.81
		BT2	7.0 ± 2.34	8.5 ± 1.4	4.71 ± 0.64
		BTR	28.6 ± 10.52	17.46 ± 2.68	9.9 ± 3.28
		KMA	6.25 ± 1.28	11.86 ± 2.44	9.0 ± 1.54
	SW (8.59 ± 1.1)	BU1	11.0 ± 4.8	17.0 ± 7.12	22.0 ± 7.02
		BU2	6.6 ± 2.94	12.4 ± 9.67	3.43 ± 2.52
		KBA	11.29 ± 4.19	10.0 ± 1.99	6.5 ± 1.45
		MV	4.8 ± 0.66	5.2 ± 2.08	3.71 ± 1.3
		TOB1	6.0 ± 0.58	2.33 ± 0.88	7.33 ± 3.85
		TOB2	18.5 ± 8.98	6.0 ± 1.51	6.0 ± 2.07

Site names follow that in Appendix 1.

Appendix 4. *Aethina tumida* infestation levels between the dry and rainy seasons and across ecological zones in Cameroon

Zone	Season	n	Infestation levels		
			0 - 20	**21 - 50**	**> 50**
	Dry	30	27(90%)	2(6.7%)	1(3.3%)
NW	Short rains	30	22(73.3%)	5(16.7%)	3(10%)
	Long rains	30	19(63.3)	2(6.7%)	9(30%)
	Dry	30	22(73.3%)	7(23.3%)	1(3.3%)
East	Short rains	30	8(26.7%)	13(43.3%)	9(30%)
	Long rains	30	9(30%)	8(26.7%)	13(43.3%)
	Dry	30	20(66.7%)	6(20%)	4(13.3%)
SW	Short rains	30	19(63.3%)	9(30%)	2(6.7%)
	Long rains	30	19(63.3%)	6(20%)	5(16.7%)

NW, Sudan savannah grassland of the North West; SW, dense evergreen forest of the South West; East, degraded evergreen forest of the East; *n*, number of colonies inspected.