UNIVERSITY OF KWAZULU-NATAL

Identification of arthropods of forensic importance during cold and warm seasons in KwaZulu-Natal Province of South Africa

By

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PREFACE

The research contained in this thesis was completed by the candidate while based in the Discipline of Genetics, School of Life Sciences of the College of Agriculture, Engineering and Science of the University of KwaZulu-Natal, Westville campus, South Africa. The research was financially supported by Professor Samson Mukaratirwa from the University Research Productivity Funds awarded to him as well as from the National Research Foundation (NRF) of South Africa.

The contents of this work have not been submitted in any form to another university and, except where the work of others is acknowledged in the text, the results reported are due to investigations by the candidate.

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DECLARATION 1: PLAGIARISM

I, Danisile Tembe, declare that:

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DECLARATION 2: PUBLICATIONS

My role in each paper and presentation is indicated. The * indicates corresponding author.

1. Tembe D* and Mukaratirwa S. 2020. Forensic entomology research and application in southern Africa. A scoping review. South African Journal of Science, 116: 1-8. doi.org/10.17159/sajs.2020/6065

2. Tembe D*, Malatji MP, Mukaratirwa S. 2021. An exploratory study of beetles and flies of forensic importance on sheep carrion in KwaZulu-Natal province of South Africa. African Entomology, 29: 1-11. doi.org/10.4001/003.029.0000

3. Tembe D.* and Mukaratirwa S. 2021. Insect succession and decomposition pattern on pig (*Sus scrofa domesticus*) carrion during warm and cold seasons in Kwazulu-Natal Province of South Africa. Journal of Medical Entomology, XX: 1-11. doi.org/10.1093/jme/tjab099

4. Tembe D*, Malatji MP, Mukaratirwa S. 2020. Molecular identification and diversity of adult arthropod carrion community collected from pig (*Sus scrofa domesticus*) and sheep (*Ovis aries*) carcasses within the same locality during different stages of decomposition in the KwaZulu-Natal province of South Africa (In press –PeerJ)

ABSTRACT

Forensic entomology is the study and use of insects and other arthropods in forensic investigations associated with death, abuse and neglect of both humans and animals. Although there has been an increased interest in forensic entomology and its application in predicting post-mortem interval (PMI) amongst other issues in many developed countries, the results cannot be extrapolated beyond the countries/regions of study since the arthropods species spectra may vary with region and geographical conditions. The present study aimed to determine the arthropod species of forensic importance found during different stages of decomposition of sheep (*Ovis aries*) and pig (*Sus scrofa domesticus*) carrion during the warm and cold season in KwaZulu-Natal province of South Africa.

A scoping review was conducted to determine the state of knowledge of forensic entomology research and application in southern Africa. To determine the arthropod species associated with sheep and pig carcass during different stages of decomposition, two medium sized Large-White pigs and two medium sized Merino sheep were humanely killed and used for the cold and warm season. Adult arthropods found on and around the carcasses during different stages of decomposition were collected and identified using combined morphological identification keys and molecular technique based on the mitochondrial gene.

The review showed that arthropod species that were found on a decomposing carcass could be useful in the estimation of PMI and provided clues in cases of criminal investigations. The review also confirmed the scarcity of forensic entomology research, and its application in southern Africa. Experimental results from this study showed that dipteran flies from the families Calliphoridae, Muscidae and Sarcophagidae were the first to colonize the sheep and pig carcasses during both warm and cold seasons. These include species of *Chrysomya marginalis*, *Ch. putoria*, *Ch. albiceps*, *Ch. chloropyga*, *Lucilia cuprina*, *Musca domestica* and *Sarcophaga calcifera*.

On the sheep carcasses, *Ch. marginalis*, *Ch. albiceps* and *M. domestica* were the most dominant fly species, contributing 63.2 % of the collected flies in the warm season, and 68.9 % in the cold season. Colonization by coleopterans during the warm season started as early as the fresh stage with *Dermestes maculatus*, *Thanatophilus micans* and *Onthophagus crassicollis*. In the cold season these same beetle species were collected from the bloated stage of the sheep carcass.

On the pig carcasses, *Ch. marginalis* (n = 111), *Ch. albiceps* (n = 99) and *M. domestica* (n = 131) were the most abundant species during the warm season. The same species were the most abundant species in the cold season (n = 55), (n = 34) and (n = 81) respectively, although in lower numbers than the warm season. Among the collected Coleoptera species, *D. maculatus* (n = 112) and *N. rufipes* (n=

62) were the most abundant species found on the carcass during the warm season and the same species were the most abundant species in the cold season *(*n = 66) and (n = 48) respectively. In the warm season *Dermestes maculatus* was recorded on the pig carcass during the fresh stage and persisted on the carcass until the last of decomposition. However, in the cold season *Dermestes maculatus* was first recorded on the carcass during the active stage of decomposition.

Molecular analyses confirmed the identification of twelve (12) arthropod taxa collected from both sheep and pig carcasses during the cold season. Results showed that 11/12 arthropod species were common in both sheep and pig carcasses, with exception to *Onthophagus sp.* and *Atherigona soccata* species which were unique to sheep and pig carcasses respectively. However, during the warm season, the sheep carcass attracted more (n=13) taxa as compared to the pig carcass. The variation in the arthropod was due to the presence of *Onthophagus sp.* which was also unique to the sheep carcass during this season. Furthermore, there was an addition of a beetle species *Hycleus lunatus*, which was collected from both sheep and pig carcasses but unique to the warm season.

This study generated important information on the endemic arthropod species that are of forensic importance KwaZulu-Natal province. The arrival time and association of arthropods species with different stages of decomposition during the warm and cold season highlighted their value in estimating the PMI in forensic investigations in the locality of KwaZulu-Natal province. The studied arthropods can potentially be useful in the estimation of PMI and other cases of criminal investigations. The seasonal variations in abundance of both Diptera and Coleoptera in the two seasons seemed to indicate influence of seasons which subsequently influenced temperature.

It is recommended that similar studies be conducted at other geographical locations of South Africa with a different ecological system to build a database of dipteran and coleopteran species of forensic importance which are endemic in these areas.

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OUTLINE OF THESIS

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Chapter 2: Scoping review on forensic entomology research and application in southern Africa.

Chapter 3: Application of veterinary forensic entomology to domestic and game ruminant animals: an exploratory study using sheep carcass in KwaZulu-Natal province of South Africa.

Chapter 4: Insect succession and decomposition pattern on pig (*Sus scrofa domesticus*) carrion during warm and cold seasons in Kwazulu-Natal Province of South Africa.

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LIST OF ABBREVIATIONS

µL - Microlitre

µM – Micromolar

bp – Base pairs

cm – Centimetre

DNA – Deoxyribonucleic acid

EDTA - Ethylenediaminetetraacetic acid

m – Metre

mm – Millimetre

NCBI – National centre of biotechnology information

PMI – Post-mortem interval

PCR – Polymerase chain reaction

TBE buffer – Tris borate EDTA buffer

CHAPTER 1: Introduction

1.1 Background

There are over one million arthropods species currently described up to date, with insects dominating the metazoan class (Amendt *et al.*, 2004; Hamilton *et al.*, 2010; Aly *et al.*, 2013; Kokdener, 2016). These species colonize almost all habitats in large numbers, surviving on vertebrate carrion as source of food (Amendt *et al.*, 2004; Aly *et al.*, 2013). According to Pechal *et al.* (2014), access of these insects to carcasses accelerates the rate in which a carcass decomposes (Amendt *et al.*, 2004; Pechal *et al.*, 2014; Charabidze *et al.*, 2017).

Decomposition of a carcass proceeds through a sequence of 5 decomposition stages, namely fresh, bloated, active decay, advanced decay and dry stages (Pechal *et al.*, 2014; Sharma *et al.*, 2015). This occurs over a period of time (Sharma *et al.*, 2015; Charabidze *et al.*, 2017). Each stage of decomposition attracts different insect species (Dantas *et al.*, 2016; Charabidze *et al.*, 2017). For example, approximately, 400 insect species have been reported on a pig cadaver during its different stages of decomposition (Amendt *et al.*, 2004). A succession of insects follows a predictable pattern from the first phase to the last phase of decomposition, with first colonization occurring within few minutes of death (Vasconcelos *et al.*, 2012; Sharma *et al.*, 2015; Dantas *et al.*, 2016; Charabidze *et al.*, 2017). However, oviposition may not always occur (Wolff *et al.*, 2001; Amendt *et al.*, 2004).

Diptera (flies) and Coleoptera (beetles) are the main two major groups of insects associated with carcasses decomposition process (Carvalho and Linhares, 2001; Kokdener *et al.*, 2016). Diptera species are recognised as the first colonizers of the carcass (Kokdener, 2016; Charabidze *et al.*, 2017) and the most common species are from the families Calliphoridae (blow flies), Muscidae (house flies) and Sarcophagidae (flesh flies) (Kokdener, 2016). These species mainly indicate post-mortem interval (PMI), especially in cases where human or animal remains are discovered days, weeks, or even longer after death (Amendt *et al.*, 2004). Coleopterans are normally associated with the carcass at the later stage of decomposition (Kokdener, 2016). As a result, both diptenan and coleopteran species serve as a reliable indicator in forensic investigations (Goff, 1993; Wolff *et al.*, 2001; Amendt *et al.*, 2004; Charabidze *et al.*, 2017). Consequently, the use or study of insects and other arthropods in forensic investigations has gained interest in recent years (Harvey *et al.*, 2003; Amendt *et al.*, 2004; Chen *et al.*, 2004).

Forensic entomology is the study of insects and other arthropods in forensics investigation (Amendt *et al.*, 2007; Padonou *et al.*, 2017). It has been used as a significant source of information

since the the l3th century (Benecke, 2001; Kelly *et al.*, 2008). It was successfully used in the case of famous Buck Ruxton in 1935 in UK. From then, the importance of forensic entomology has increased significantly (Sharma *et al.*, 2015) and it has recently been successfully applied to various case studies of unnatural death (Anderson, 1995; Anderson, 1997; Benecke, 1998; Kelly *et al.*, 2008). In many developed countries forensic entomology is now recognized as one of the most precise and important method for estimating the elapsed time since death in forensic investigations, especially when 3 days or more have elapsed (Sharma *et al.*, 2015). Furthermore, recent research has showed that arthropods can be used to detect drugs or toxins in a decomposed body (Campobaso, 2005; Brundage and Byrd, 2016) and create a link between a victim, suspect and a crime scene (Brundage and Byrd, 2016). These applications have made forensic entomology a valuable tool in cases of murder or suicide investigations.

According to Kokdener (2016) accurate species identification is a crucial and first step and essential necessity in forensic entomology (Chen *et al.*, 2004). If a species identification is incorrect, the estimated PMI results and other interpretations will consequently be incorrect. Different insect species vary in their developmental rates and maturation (Kokdener, 2016). Identification of insect species has been mainly done using morphological techniques (Kokdener, 2016; Rolo *et al.*, 2013). Identification of different arthropods species using morphological techniques requires the knowledge and understanding of taxonomy and the ability to identify and distinguish arthropods species using identification keys (Zehner *et al.*, 2004; Amendt *et al.*, 2004). Identification of insect larvae and adult using morphological characteristics has its challenges (Amendt *et al.*, 2004; Zehner *et al.*, 2004; Boehme *et al.*, 2012; Kokdener, 2016). For instance, insect samples collected from carcass at the crime scene are often undeveloped (i.e. larval stage) and often undifferentiated (Zehner *et al.*, 2004; Velasquez *et al.*, 2010; Boehme *et al.*, 2012). As a result, rearing of the insect larva to adult stage under proper rearing conditions is an option (Amendt *et al.*, 2004; Chen *et al.*, 2004) of which is time consuming, and may delay the criminal investigation or cause difficulties when rearing fails (Amendt *et al.*, 2004; Velasquez *et al.*, 2010). Due to these mentioned circumstances or limitations, species identification based on genetic examination is more accurate as a method for identifying different insect species to complement morphological identification (Harvey *et al.*, 2003; Amendt *et al.*, 2004; Zehner *et al.*, 2004; Wells and Williams, 2007; Boehme *et al.*, 2012; Kokdener, 2016).

DNA-based methods for identification of arthropods have advanced rapidly since 1994 (Park *et al.*, 2013) and have been used successfully to differentiate insects of forensic value (Park *et al.*, 2013), and as a result, and the methods have been applied in forensic investigations (Park *et al.*, 2013; Charabidze *et al.*, 2017). DNA-based methods are reliable, precise, and rapid and can accurately

identify different arthropod species at all developmental stages (Oliveira *et al.*, 2011). As a result, identifying important forensic insects such as, blowflies and flesh flies, using DNA-based techniques provides accurate data for reliable estimation of the post-mortem interval (PMI) (Park *et al.*, 2013). Among widely used DNA based techniques is the PCR which mainly focuses on amplification of specific regions of the insect genome (Amendt *et al.*, 2004; Wells and Williams, 2007).

Although there has been an increased interest in the identification of arthropods species and application of forensic entomology in predicting the PMI in many developed countries, including South Africa, their results cannot be extrapolated beyond the countries/regions of study since the arthropods species spectrum vary with region and geographical conditions. Hence this study contributed new knowledge on the spectra of arthropods species and their succession pattern associated with sheep and pig carrion during different stages of decomposition during warm and cold season in KwaZulu-Natal Province of South Africa.

1.2 Aim

The aim of this study are:
To explore history of forensic science research in southern Africa and determine the arthropod species associated with carrion at different stages of decomposition during warm and cold seasons in KwaZulu-Natal province of South Africa.

1.3 Objectives

The objectives of this study are:

a. To review the status of forensic entomology research in southern Africa.
b. To determine the species of arthropods associated with sheep and pig carrion at different stages of decomposition during the warm and cold seasons.
c. To determine the genetic diversity of arthropods carrion communities of sheep and pig carcasses collected within the same locality during different stages of decomposition.

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CHAPTER 2: Forensic entomology research and application in southern Africa: A scoping review.

2.1 Abstract

The use of forensic entomology is well established in the northern hemisphere, but is still emerging in the southern hemisphere, where most of the current research is not explicitly undertaken in the context of forensics. In this review, we provide an update on the status of forensic entomology research and its application in relation to estimation of post-mortem interval in various criminal investigations ranging from murder cases, cases of human neglect and the poaching of wildlife in southern Africa, among other issues. A literature search was conducted using Google Scholar, PubMed, Scopus and EBSCOhost databases. The studies reviewed were focused on arthropod diversity during different stages of carcass decomposition, effect of seasons on the abundance and diversity of carrion feeding arthropod species during carcass decomposition, and diurnal and nocturnal oviposition of forensically important insect species during carcass decomposition. It was further observed that arthropod species that established on a decomposing carcass are potentially useful in the estimation of post-mortem interval and determining clues in cases of criminal investigations. The review confirmed the paucity of research in forensic entomology, and its application in southern Africa. Future studies on the research and application of forensic entomology in various criminal investigation scenarios – such as murder cases, human neglect, and wildlife poaching in southern Africa – are therefore needed.

Key words: Forensic science, Arthropods, Post-mortem interval, Stages of decomposition, Insect diversity

2.2 Background

Forensic entomology has been applied in forensic investigations for decades (Harvey *et al.*, 2003; Amendt *et al.*, 2007; Amendt *et al.*, 2011) and is now recognised as an important investigative tool (Greenberg, 1991; Harvey *et al.*, 2003). Forensic entomology can be classified into urban, stored-product and medico-legal divisions (Goff, 1993; Villet *et al.*, 2006). According to Goff (1993), urban forensic entomology involves civil actions regarding insect activity associated with construction as in cases of termite damage. Stored-product forensic entomology deals with cases involving commercial property that is infested or damaged by insects. Medico-legal forensic entomology deals with insect evidence collected at a crime scene (Goff, 1993; Anderson, 2001; Villet *et al.*, 2006). Such evidence is commonly used to estimate the time of death or post-mortem interval (PMI) of the decomposing remains of animals or humans (Goff, 1991; Goff, 1993). This field has been gaining more recognition than have urban and stored-product forensic entomology worldwide (Goff 1993; Sukontason, 2007).

In this review, we focus on medico-legal forensic entomology in this review.

Insects have been used mainly for estimating PMI (Greenberg, 1990; Greenberg, 1991; Catts, 1992; Badenhorst, 2018) drug verification (Catts, 1992; Sukontason *et al*, 2007; Dupont *et al.*, 2011), determination of ante-mortem trauma, and confirmation of the relocation of carcasses (Prichard *et al.*, 1986; Smith, 1986; Sukontason *et al.*, 2007). This is achieved by analysing the carrion-feeding insect communities recovered from crime scenes and on the carcasses to produce evidence in forensic investigation cases such as human neglect, suicide, homicide, animal poaching and accidental death (Louw and Van der Linde, 1993; Kelly *et al.*, 2009; Tomberlin *et al.*, 2011; Villet, 2011; Brundage and Byrd, 2016).

The stage of decomposition as well as other processes that lead to complete decomposition that are likely to affect the remains of a person or animal, all need to be considered for the accurate estimation of PMI (Megyesi *et al.*, 2005; Simmons *et al.*, 2010; Brundage and Byrd, 2016). The application of forensic entomology to estimate the PMI requires accuracy and consideration of several factors, such as the ability to correctly identify the insect species colonising the carcass (i.e. insect community) (Harvey *et al.*, 2003;Villet, 2011; Badenhorst and Villet, 2018); the understanding of the role of different insect species and their colonisation processes throughout carcass decomposition (Denno and Cothran, 1975; Villet, 2011); the effect of temperature, seasons and climatic zones; and the presence of toxins (Catts, 1992; Williams, 2003).

Forensically significant insects and other arthropods vary among regions due to varying geographical conditions. Data obtained from the northern hemisphere cannot be applied to the southern hemisphere (Villet, 2011; Brundage and Byrd, 2016). The application of forensic entomology has been successfully explored mainly in developed countries such as the USA, Britain and Australia (Byrd and Castner, 2010; Villet, 2011; Mabika *et al.*, 2014) and in some other European countries. Only a few studies have been conducted in some African countries, including South Africa, Cameroon, Egypt, Ghana and Nigeria (Okiwelu *et al.*, 2008; Mabika *et al.*, 2014).

Although Villet (2011) reported that the southern hemisphere is recognised as home to many forensically important insect species not found in the northern hemisphere, there is a paucity of information on the geographical distribution and abundance of these forensically important insect species (Harvey *et al.*, 2003; Villet, 2011). These species have not been fully studied and exploited to determine their importance and role in forensic investigations (Harvey *et al.*, 2003; Williams and Villet, 2006); the majority of research on carrion insects conducted in southern Africa was not undertaken in the context of forensic investigation (Williams and Villet, 2006). Consequently, lack of information

on the importance of these insects in forensics limits the application of entomology in forensic investigation.

According to Villet (2011), African scientists have been aware of the potential application of entomology in forensic investigations for several years. For example, in South Africa and Zimbabwe, there have been cases in which entomological evidence was used in solving criminal cases (Harvey *et al.*, 2003; Williams and Villet, 2006; Brundage and Byrd, 2016). To date, southern African forensic entomology research has been carried out on animals (i.e. pigs) as models for solving human cases (Goff, 1993). In a previous study, Smith (1986), carcasses of several vertebrates were used as models to study different insect communities on a decaying human body but there have been no applications in cases of animal poaching or neglect (Goff, 1993; Villet, 2011; Brundage and Byrd, 2016). The application of forensic entomology in cases that involve animal remains are still needed, given the high rate of wildlife poaching taking place in southern Africa (Brundage and Byrd, 2016).

Research conducted in southern Africa to date has generated useful results that can be used as evidence in forensic investigations if assessed carefully. Consequently, research in forensic entomology in southern Africa has great potential as a complementary investigative technique in criminal investigations taking place in countries in southern Africa. According to Villet *et al.*, (2006), southern African research has focused more on species that are useful for urban or stored-product forensic entomology cases than on insects that are important in medicolegal cases (Villet *et al.*, 2006) This calls for more research on insects of medico-legal importance to assist in solving criminal cases.

Molecular research in species identification in forensic entomology is established (Florin and Gyllenstrand, 2002; Tomberlin *et al.*, 2011; Boehme *et al.*, 2012) but an understanding of the role of quantitative genetics in the development and behaviour of arthropods found at crime scenes has been less appreciated in forensic entomology (Tomberlin *et al.*, 2011). Quantitative genetics is used to identify and analyse differences in phenotypes (Wayne and Ostrander, 2007; Tomberlin *et al.*, 2011), which reduces error in estimating the PMI with insects as evidence because each insect species has its own unique phenotype and developmental profile (Williams and Villet, 2006; Wells and Stevens, 2008; Velasquez *et al.*, 2010). Hence, it is essential to accurately identify the insect species collected as evidence in solving criminal cases (Velasquez *et al.*, 2010; Tomberlin *et al.*, 2011).

The present review aims to provide an update on the status of forensic entomology research and its application in relation to estimation of PMI in various criminal investigations such as murder cases, human neglect, and poaching of animals in the southern Africa region.

2.3 Methods

2.3.1 Scoping review

The results of this scoping review address the question: What is known from the existing literature about forensic entomology research and its application in relation to estimation of PMI in various criminal investigations in southern Africa? Peer-reviewed research articles from southern Africa that explicitly report on forensic entomology research in a country or countries within southern Africa were collected through a comprehensive approach to answer this question. The procedure followed was consistent with a scoping review approach, which is to synthesise what is known about a particular matter across various literature forms to achieve clarity about the state of knowledge and evidence that exists (Johnston *et al.*, 2010). The scoping review approach outlined by Arksey and O' Malley (2005) was followed: (1) identify the research question; (2) identify relevant literature; (3) select the literature; (4) chart the data; and (5) collate, summate and report the results.

2.3.2 Search strategy and selection of the literature

A literature search was conducted on four databases – Google Scholar, PubMed, Scopus and EBSCOhost – and the search was executed using the Boolean operators AND, OR and the following search terms: forensic entomology, post-mortem interval and/or index, forensic entomology research in southern Africa (Angola, Botswana, Madagascar, Mozambique, Namibia, South Africa, Zambia andZimbabwe), identification of forensically important insects and southern Africa, and application and limitations of forensic entomology in southern Africa. Selected search terms were relevant to the scoping question and were developed in consultation with a librarian. Articles that were identified were then screened by reading through their titles and abstracts. Consistent with the scoping review protocol, post-hoc inclusion criteria were developed (Arksey and O'Malley, 2005). The exclusion criteria for this review were developed based on the three inclusion criteria which were peer-reviewed research articles from southern Africa explicitly reporting on forensic entomology research in a country or countries from southern Africa between 1932 and 2017 that included: (1) colonisation and succession pattern of arthropods during different stages of decomposition; (2) variation spectrum of carrion-feeding insects; and (3) diversity and/or abundance of arthropods colonising a carcass during different seasons. Any study that may have been referenced under forensic entomology but did not report on one of the above-mentioned inclusion criteria, was not included. Furthermore, any study that had no information to contribute to answering the scoping questions was also excluded.

Once the titles and abstracts had been reviewed, articles meeting the criteria were reviewed in full. Some articles were screened for any additional relevant information to be included in the

review by manually scanning the reference lists (Johnston *et al.*, 2010). The selection process and search flow are shown in Figure 2.1.

2.3.3 Charting, collating and summarising the data

A spreadsheet was created to chart the data extracted from the articles which contributed to answering the research question. Details regarding publication information, aims and objectives of the study, the country in which the study took place, outcomes of the study and data pertinent to the scoping question were recorded in this spreadsheet. This process was carried out by (D.T.). The information extracted was discussed with (S.M.) in order to work towards an overall perspective on the factors emerging from the literature reviewed. The final step was to work together to identify key knowledge or research gaps resulting from the reviewed articles that have direct relevance to the scoping review question. The verification of the data set used in the final analysis was done by S.M.

2.4 Results

2.4.1 Search flow

As shown in Figure 2.1, the literature search yielded a total of 282 hits from the databases searched, of which 277 were excluded because they were either duplicates or not focused on forensic entomology research. At the end of the selection process, only five peer-reviewed articles fulfilled the inclusion criteria (Figure 2.1); these are shown in Tables 2.1-2.3. The review included literature from 1934 to 2017.

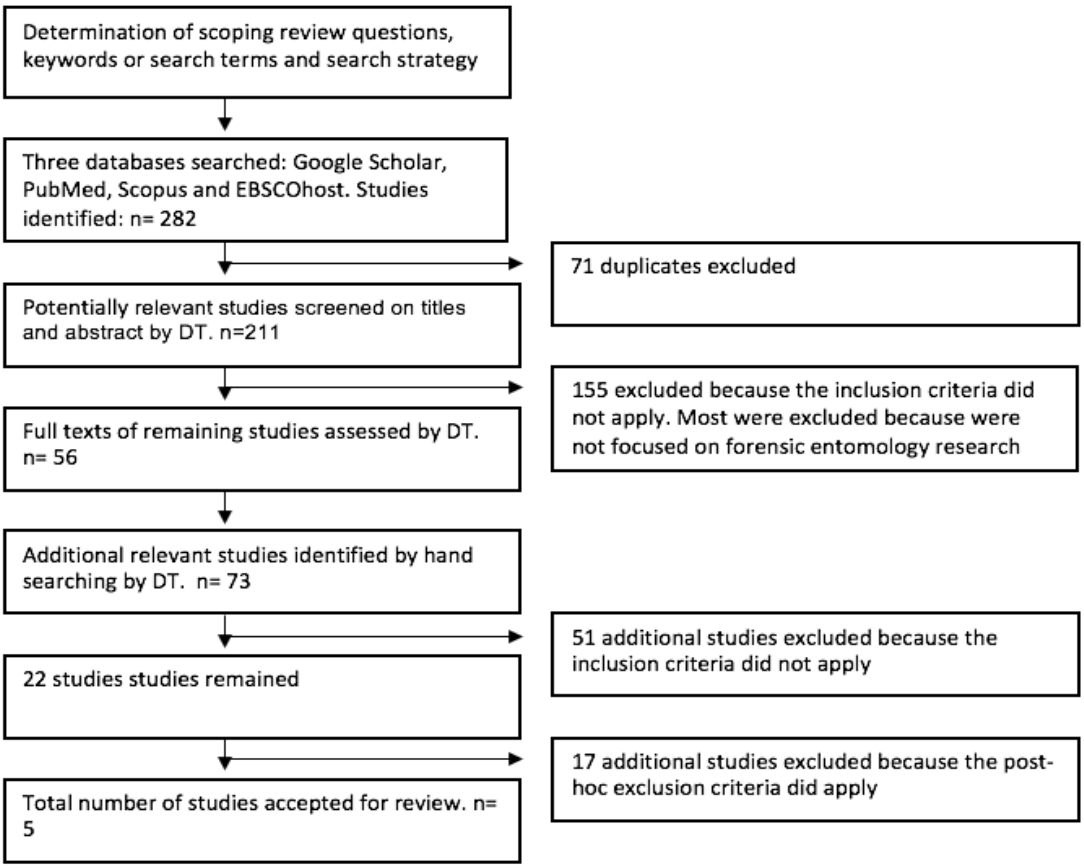

Figure 2.1: Selection process and search flow

2.4.2 Arthropod diversity during different stages of carcass decomposition

Only two articles reported on the diversity of arthropods colonizing decomposing carcasses from the southern African region (Kelly *et al.*, 2011; Mabika *et al.*, 2014) (Table 2.1). Kelly *et al.* (2011) observed that during the fresh stage of a pig carcass, only *Musca spp*. were found, and they persisted during the bloated stage, where seven new species (*Calliphora vicina*, *Chrysomya chloropyga*, *Chrysomya marginalis*, *Chrysomya albiceps*, *Lucilia spp*., Sarcophagidae and *Hydrotaea capensis*) visited the carcass, but did not persist past this stage. Instead, four new species (*Dermestes maculatus, Necrobia rufipes, Thanatophilus micans* species and one unidentified species) visited the carcass during the decay stage and only *D. maculatus* and *Necrobia rufipes* persisted on a carcass (Table 2.1). Mabika *et al.* (2014) compared the pattern of arthropod colonisation between a rabbit carcass left to decompose in the sun and one left in the shade. During the fresh stage of a carcass exposed to the sun, *Lucilia cuprina*, *C. albiceps* and *Musca domestica* visited the carcass; they persisted during the bloated and decay stages and only disappeared during the dry stage. *Saprinus spp*. and *Dermestes spp*. were recorded only during decay and dry stages of the carcass exposed to the sun, whereas *Pheidole spp*. persisted throughout the four stages of decomposition on the carcass exposed to the sun. Similarly, *L.*

cuprina, *C. albiceps* and *M. domestica* species visited the carcass in the shade during the fresh stage and persisted during the bloated and decay stages, but then disappeared during the dry stage. *Saprinus spp*. and *Dermestes spp*. visited the carcass in the shade only during the decay and dry stages, whereas Pheidole spp. visited the carcass in the shade throughout the four stages of decomposition. It was further observed that *N. rufipes* only visited the carcass exposed to the sun during the dry stage but persisted during both decay and dry stages on the carcass in the shade. Similarly, *Hydrotaea spp*. appeared on the carcass exposed to the sun during the dry stage only, but during both bloated and decay stages of the carcass in the shade. However, Sarcophangidae spp. and *Drosophilia* spp. persisted during the decay stage only on the carcass exposed to the sun. In the study of Kelly *et al.* (2011), *L. cuprina* and *C. albiceps* visited the carcass only during the bloat stage, whereas in Mabika *et al.* (2014) study, *L. cuprina* and *C. albiceps* persisted during the fresh, bloated and decay stages. Furthermore, in both studies, *Dermestes spp*. visited the carcass during decay and dry stages only.

Table 2.1: Summary of studies (1934-2017) reporting on different arthropods colonising carcasses during different stages of decomposition in southern Africa

Study	**Country of study**	**Location of study**	**Objectives of study**	**Host animal**	**Outcome of study**						
					Arthropods			**Insect species collected at different stages of decomposition**			
					Order	**Family**	**Genus/species**	**Fresh**	**Bloated**	**Decay**	**Dry**
Kelly *et al.*, 2011	South Africa	Bloemfontein	Determine the influence of clothing and wrapping on carcass decomposition and arthropod succession to provide data to enable estimated post-mortem interval in homicide investigations	Pig	Diptera	Muscidae	*Musca* spp.	*	*		
					Diptera	Calliphoridae	*Calliphora vicina*		*		
					Diptera	Calliphoridae	*Chrysomya chloropyga*		*		
					Diptera	Calliphoridae	*Chrysomya marginalis*		*		
					Diptera	Calliphoridae	*Chrysomya albiceps*		*		
					Diptera	Calliphoridae	*Lucilia* spp.		*		
					Diptera	Sarcophagidae	Sarcophagidae		*		
					Diptera	Muscidae	*Hydrotea capensis*		*		
					Coleoptera	Piophilidae	Unidentified			*	
					Coleoptera	Dermestidae	*Dermestes maculatus*			*	*
					Coleoptera	Cleridae	*Necrobia rufipes*			*	*
					Coleoptera	Silphidae	*Thanatophilus micans*			*	
Mabika *et al.*, 2014	Zimbabwe	Harare	Investigate insects visiting sun exposed and shaded decomposing rabbit carcasses and establish the relationship between insects and carcasses	Rabbit	Diptera	Calliphoridae	*Lucilia cuprina* (S)	4	2	6	0
					Diptera	Calliphoridae	*C. albiceps* (S)	1	4	8	0
					Diptera	Calliphoridae	Unidentified (S)	1	1	5	0
					Diptera	Calliphoridae	Unidentified (S)	0	0	0	37
					Diptera	Muscidae	*Musca domestica* (S)	4	55	47	0
					Diptera	Muscidae	*Hydrotaea* sp. (S)	0	0	0	8
					Diptera	Phoridae	Unidentified (S)	0	0	1	0
					Diptera	Sarcophagidae	Sarcophagidae sp. (S)	0	0	1	0
					Diptera	Drosophilidae	*Drosophilia* sp. (S)	0	0	1	0
					Coleoptera	Histeridae	*Saprinus* sp. (S)	0	0	9	8
					Coleoptera	Cleridae	*N. rufipes* (S)	0	0	0	9
					Coleoptera	Dermestidae	*Dermestes* sp. (S)	0	0	18	65

			which may be of forensic importance		Hymenoptera	Formicidae	*Pheidole* sp. (S)	22	29	19	34
					Diptera	Calliphoridae	*L. cuprina* (s)	1	11	1	0
					Diptera	Calliphoridae	*C. albiceps* (s)	2	12	1	0
					Diptera	Calliphoridae	Unidentified (s)	1	27	0	0
					Diptera	Calliphoridae	Unidentified (s)	0	0	0	17
					Diptera	Muscidae	*M. domestica* (s)	2	276	40	0
					Diptera	Muscidae	*Hydrotaea* sp. (s)	0	1	1	0
					Diptera	Phoridae	Unidentified (s)	0	0	1	0
					Diptera	Anthomyiidae	Unidentified (s)	0	0	1	0
					Coleoptera	Histeridae	*Saprinus* sp. (s)	0	0	2	4
					Coleoptera	Cleridae	*N. rufipes* (s)	0	0	3	8
					Coleoptera	Dermestidae	*Dermestes* sp. (s)	0	0	31	141
					Hymenoptera	Formicidae	*Pheidole* sp. (s)	30	14	18	30

2.4.3 Seasonal abundance and diversity of carrion-associated arthropods

There was an observed difference in the abundance and diversity of arthropods colonising impala carcasses during different seasons (Table 2.2). More arthropod species were identified during the rainy season (Braack, 1986) than the dry season (Ellison, 1990). *Necrobia rufipes* was found on the carcass during the dry season by Ellison (1990); however, Braack (1986) found and identified the same species during the warm season. In both studies, *Lucilia spp.* were found on decomposing carcasses during the dry season only.

Table 2.2: Summary of studies (1934–2017) on the diversity and abundance of arthropods collected during different seasons in southern Africa

Study	Country of study	Location of study	Objectives of study	Host animal	Outcome of study		
					Order/family/species	Average number of carrion-associated arthropods	
						Dry season	Rainy season
Braack, 1986	South Africa	Kruger National Park	To collect and identify the species found on the large mammal carcasses during both summer and winter	Impala	*Anisolabis* sp.	–	<10
					Bormansia meridionalis Burr	–	<10
					Euborellia annulipes (Lucas)	–	<10
					Fusius rubricosus (Stal)	–	<10
					Lisarda rhodesiensis Miller	–	<10
					Rhinocoris albopunctatus (Stal)	–	<10
					R. violentus (Germar)	–	<10
					Xylocoris (Proxylocoris) afer Reuter	–	±60
					Solenostethium liligerum	–	<10
					Metagonum sp.	–	<10
					Platymetopus curtulus (Peringuey)	–	<10
					Xenodochus melanarius (Boheman)	–	<10
					Thanatophilus (Chalcosilpha) micans (Fabricius)	–	265
					Staphylinidae	–	625
					Trogidae	–	422
					Allogymnopleurus thalassinus (Klug)	–	<30
					Anachalcos convexus (Boheman)	–	164
					Aphodius sp.	–	<100
					Caccobius convexifrons (Roth)	–	<30
					C. nigritulus (Klug)	–	<30
					Catharsius philus (Kolbe)	–	<30
					Copris amyntor (Klug)	–	<30
					C. elphenor (Klug)	–	<30
					C. evanidus (Klug)	–	<30
					C. mesacanthus (Harold)	–	<30
					Garreta nitens (Olivier)	–	<30
					Gymnopleurus virens (Erichson)	–	<30
					Metacatharsius opacus (Waterhouse)	–	<30
					Milichus sp. *probably apicalis* (Fahraeus)	–	<30
					Onitis fulgidus (Klug)	–	<30

					O. granulisetosus (Ferreira)	–	<30
					O. inversidens (van Lansberge)	–	<30
					O. obenbergeri (Balthasar)	–	<30
					O. picticollis (Fabricius)	–	<30
					Onthophagus (Proagoderus) dives (Klug)	–	670
					Pedaria sp.	–	<30
					Phaeochrous madagascariensis (Westwood)	–	486
					Phalops ardea (Klug)	–	<30
					Sarophorus costatus (Fahraeus)	–	304
					Scarabaeus ebenus (Klug)	–	<30
					Sisyphus calcaratus (Klug)	–	<30
					S. goryi (Harold)	–	<30
					S. impressipennis (van Lansberge)	–	<30
					S. injuscatus (Klug)	–	<30
					S. seminulum (Gerstaecker)	–	<30
					Sybax distortus (Schaum)	–	<30
					Tiniocellus spinipes (Peringuey)	–	<30
					Dermestes maculatus (De Geer)	191	–
					Necrobia rufipes (De Geer)	–	–
					Phloeocopus sp.	–	572
					Carpophilus nr. *quadrisignatus* Er.	–	1
					Carpophilus sp.	–	<10
					Bactria sp.	–	<10
					Euscelidia rapax (Westwood)	–	<10
					Hoplistomerus nobilis Loew	–	<10
					Neolophonotus (Lophopeltis) sp.	–	<10
					Ommatius sp (Loew).	–	<10
					Stichopogon caffer (Hermann)	–	<10
					S. punctus (Loew)	–	<10
					Hypocerides spinulicosta (Beyer)	–	<10
					Megaselia curtineura	–	<10
					Megaselia sp. n. *pauculitincta*	–	<10
					Plethysmochaeta sp.	–	<10
					Australosepsis niveipennis (Becker)	–	<10
					Paratoxopoda depilis (Walker)	–	<50
					Xenosepsis sp.	–	97

					Cestrotus n. sp.	–	<50
					Homoneura (Keisomyia) n. sp.	–	<10
					Curtonotum cuthbertsoni (Duda)	–	<10
					Sphaeroceridae	–	<10
					Chlorichaeta albipennis (Loew.)	–	<10
					Discomyza eritrea (Cresson)	–	223
					Mosillus beckeri (Cresson)	–	<10
					Apotropina n. sp.	–	<10
					Chloropsina sp.	–	<10
					Conioscinella sp.	–	<40
					Oscinella sp.	–	<40
					Siphunculina ornatifrons (Loew)	–	<40
					S. punctifrons (Sabrosky)	–	<40
					Siphunculina sp.	–	250
					Desmometopa m-nigrum (Zetterstedt)	–	<40
					Leptometopa latipes (Meigen)	–	<40
					Leptometopa n. *sp.*	–	<40
					Meoneura n. sp.	–	<40
					Milichiella lacteipennis (Loew)	–	<40
					*Atherigona aberrans (Mallocch), A. naqvil (*Steykal) *A. steeleae* (Emden), *Atherigona* spp. indet.	–	574 289
					Fannia leucosticta (Meigen)	–	1
					Graphomya leucomelas (Wiedemann)	–	1
					Gymnodia mervinia (Walker)	–	5
					Gymnodia tonitrui (Wiedemann)	–	3
					Haematobosca latifrons (Malloch)	–	1
					H. spinigera (Malloch)	–	6
					H. thirouxi ssp. *potans* (Bezzi)	–	7
					Morellia nilotica (Loew)	–	3
					Lucilia sp.	47	–
					Nasonia vitripennis	–	<40
					Trichopria lewisi (Nixon)	–	>35
					Lardoglyphus sp.	–	<100
					Macrocheles muscaedomesticae	–	<100
					Pygmephorus sp.	–	<100

Ellison, 1990	South Africa	Klaserie Private Nature Reserve	The effect of scavenger mutilation on the subsequent rate of decomposition and insect colonisation of such carcasses	Impala	*Saprinus* spp.	1.3	–
					Necrobia rufipes	6.6	–
					Dermestes maculatus	9.2	–
					Aleochara spp.	<1	–
					Thanatophilus spp.	<1	–
					Mycetophagidae spp.	<1	–
					Onthophagus spp.	<1	–
					Piophila spp.	36.5	–
					Ophyra capensis	3.4	–
					Musca spp.	10.9	–
					Chrysomya albiceps	3.4	–
					Chrysomya chloropyga	<1	–
					Chrysomya marginalis	4	–
					Chrysomya putoria	<1	–
					Tricyclea spp.	9.7	–
					Lucilia spp.	11	–
					Sarcophaga spp.	0.75	–
					Auchmeromyia luteola	0.25	–
					Ceratophaga vastella	<1	–
					Brachynieria spp.	<1	–
					Acrididae spp.	<1	–

2.4.4 Diurnal and nocturnal oviposition of forensically important insect species

While attempting to determine the nocturnal oviposition behaviour of blowflies in the southern hemisphere, Williams *et al.* (2017) found that *Lucilia spp*., *Chrysomya putoria* and *C. chloropyga* laid eggs during the day and night (Table 3). However, *Chrysomya megacephala* laid eggs only during the day and at a lower rate than the above-mentioned species. For all species, oviposition rate was generally higher during the day than at night.

Table 2.3: Summary of the diurnal and nocturnal oviposition by forensically important arthropods on pig carcasses in southern Africa

Study	**Country of study**	**Location of study**	**Objective of study**	**Host animal**	**Outcome of study**		
					Species identified	**Day**	**Night**
Williams *et al.*, 2017	South Africa	Grahamstown	To determine the nocturnal oviposition behaviour of blowflies in the southern hemisphere	Pig	*Chrysomya megacephala* *Lucilia sericata* *Chrysomya putoria* *Chrysomya chloropyga*	1 8 7 2	0 1 1 1

2.5 Discussion

To date, there has been limited research published on forensic entomology in southern Africa – a finding supported by the review by Villet *et al.* (2006) on the history of forensic entomology. Available studies are limited to identification of insect taxa found on carcasses during different stages of decomposition, and presumably this information can then be used in determining PMI (Mabika *et al.*, 2014). Several factors affect the rate and pattern of decomposition, and thereby influence the abundance and diversity of arthropod species found colonising the carcass (Braack, 1981), which in turn affects the accuracy of PMI and consequently any legal investigation (Catts, 1992). These factors include season, temperature, geographical distribution, and vertebrate class (category) studied (Mabika *et al.*, 2014, Padonou *et al*, 2017).

2.5.1 Arthropod diversity during different stages of carcass decomposition

Different stages of decomposition of a carcass attract different arthropod species. Kelly *et al.* (2009) observed and described these stages as follows:

a. Fresh stage – the stage commencing directly after the animal is killed, characterised by a soft torso and flexible limbs. This stage is very short and associated with no odour.
b. Bloat/bloated stage – this stage is when the torso begins to harden, and the abdomen becomes inflated because of a build-up of gases. The carcass appears like a balloon, and the body colour changes. Oviposition by arthropods takes place during this stage.
c. Decay stage – at the beginning of this stage, the carcass is deflated because of maggots feeding on the carcass tissue which consequently allows gases to be released. Limbs collapse into the resting position, and the skin begins to peel, allowing maggots to feed underneath. At the end of this stage, little tissue remains on the carcass and thus the bones of the skull, ribs and legs are often visible.
d. Dry stage – this stage is characterised by little to no moisture. The gut contents are dried out, with only hair and small patches of skin remaining.

Mabika *et al.* (2014) observed that *L. cuprina*, *C. albiceps* and *M. domestica*, from the families Calliphoridae and Muscidae, were the first to colonise rabbit carrion during the first three stages of decomposition (fresh, bloat and decay stages). However, these species were found only during the bloated stage of a pig carcass (Kelly *et al.*, 2011). Results were consistent with a report by several authors that species from the Calliphoridae and Muscidae families are the first to colonise any carcass as the tissue is still soft (Carvalho and Linhares, 2001; Picard and Wells, 2009; Abuje *et al.*, 2014; Padonou *et al.*, 2017), and the arthropod species from these two families can potentially be useful in the estimation of PMI and determining clues in cases of criminal investigations (Padonou *et al.*, 2017).

The difference in arrival pattern and colonization time of the same arthropod species on different carcasses of different animal species as observed by Kelly *et al.* (2011) and Mabika *et al.* (2014) could be associated with the difference in body size (Simmons *et al.*, 2010). According to Sutherland *et al.* (2013), smaller animals decompose faster than larger animals and this faster decomposition leads to earlier attraction of arthropods. This in turn influences the sequence of arthropod colonisation, hence, *L. cuprina*, *C. albiceps* and *M. domestica* were found during the fresh, bloat and decay stages of a rabbit (Louw and Van der Linde, 1993) but only at the bloating stage of a pig carcass (Kelly *et al.*, 2011).

The environmental or physical conditions (sun, shade, buried, housed) under which a carcass is disposed of also influence the type of arthropods arriving and colonising the carcass (Braack, 1981). For instance, Mabika *et al.* (2014) observed that *N. rufipes* colonised a carcass exposed to the sun during the dry stage but were found during both decay and dry stages of a carcass in the shade. *Hydrotaea spp*. were also found on the carcass exposed to the sun during the dry stage but were found during both bloated and decay stages of the carcass in the shade. This variation in insect arrival and colonisation pattern may be because of the difference in relative temperature and humidity – higher temperature and lower humidity lead to chemical reactions that often result in faster decomposition of the carcass (Padonou *et al.*, 2017). *Pheidole* spp. (Family: Formicidae) were found throughout the decomposition stages (Mabika *et al.*, 2014). Although Morreti *et al.* (2013) showed that these species feed on both carcasses and maggots, they do not affect the decomposition process (Mabika *et al.*, 2014).

2.5.2 Seasonal abundance and diversity of carrion-associated arthropods

The abundance and diversity of arthropod species seem to vary with seasons (Braack, 1986). Braack (1987) collected and identified more arthropod species during the rainy (summer) season than during the dry (winter) season. This observation is congruent with that of Kelly *et al.* (2011) and Parry *et al.* (2016), who observed more arthropod species colonising carcasses during summer as compared to winter. The authors also observed that there were no other factors influencing the difference in abundance and the diversity of these arthropod species other than the change in season, which subsequently influenced temperature. For instance, the dry season is characterised by low temperatures, which consequently result in reduced arthropod activity, subsequently leading to a gradual decrease in the number of arthropods colonising carcasses (Gruner *et al.*, 2007). Although PMI can still be estimated during the winter (dry) seasons, the reduction in the number of arthropod species present in this season often leads to difficulties in estimating the PMI accurately (Kelly *et al.*, 2011).

The absence of certain species during a particular season is expected, as many species are specific to a season and geographical area or locality (Villet, 2011). Arthropod species colonising an impala carcass, as observed by Braack (Braack, 1986) and Ellison (1990), varied from season to season, with few exceptions. Ellison (1990) surprisingly found *N. rufipes* colonising an impala carcass during the dry season, which was previously found by Braack (1986) on an impala carcass during the warm (wet) season. Kelly *et al.* (2011) found this species during both seasons. It can be suggested that the presence of this species during both seasons might be because it occurs throughout the year, as was observed and reported by Bensaada *et al.* (2014) in Turkey. Furthermore, Ellison (1990) and Kelly *et al.* (2011) found *Dermestes maculatus DeGeer* and *Lucilia spp*. on decomposing carcasses during the dry season only. This observation contradicts that of Villet (2011) who stated that although other *Dermestes* species such *D. peruvianus* and *D. haemorrhoidalis* are common in winter, *D. maculatus* and *Lucilia spp*. are typically common and more active in summer, and rare in winter. Therefore, knowledge of the seasonal occurrence of arthropod species is important as it provides useful information about which insect to expect during a given season and is thus essential in determining PMI in forensic investigations (Williams, 2003).

2.5.3 Diurnal and nocturnal oviposition of forensically important insect species

Knowledge of the developmental stages of breeding arthropods on the carcass, estimating the date and time of egg or larva deposition, and taking into consideration the influence of environmental factors, can all assist in estimating the PMI of a carcass (Williams, 2003; Willliams *et al.*, 20172). Williams *et al.* (2017) observed that *Lucilia spp*., *C. putoria* and *C. chloropyga* species laid eggs during both the day and night, and *C. megacephala* only laid during the day. The authors observed that oviposition was higher during the day than night. This observation may have been because ambient temperatures were very low at night, and according to Digby (1958) and Nicholson (1934), temperature is one of the important factors influencing flying activity. Williams (2003) also observed that numerous blowfly species are unable to fly in ambient temperatures below 15 °C, and Richards *et al.* (2009) observed that *C. marginalis, C. albiceps* and *C. chloropyga* were unable to fly in temperatures below 20.8 °C, 21.7 °C and 23 °C, respectively. Therefore, Williams *et al.* (2017) concluded that the low number of species of arthropods colonising the carcass, and low oviposition at night, may have been due to lower temperatures and less light, which hindered the arthropod's ability to fly and lay eggs on the carcass. However, those species which were closer to the body were able to walk to the carcass, which explains why eggs from other arthropod species were found during the night.

In view of the above studies, arthropods can be an excellent source of evidence in forensic

investigations. For instance, if the stage of a decomposing body is not known, it can be easily estimated by observing the species of arthropods colonising the carcass (e.g. *Dermestes* species can only be found during decay and dry stages). Furthermore, knowledge of seasonal occurrence of certain arthropod species provides useful information in determining PMI because arthropod species vary with season.

Animal species also play a significant role in determining which arthropod species are attracted to them during different stages of decomposition. As such, there is need to document the variation of arthropod species attracted to different animal species in different geographical regions/locations. Lack of ideal tools for identification of arthropods to species level in southern African countries might have hampered the wide use of insects in forensic investigations. Although morphological tools have been widely used to identify important arthropod species for forensic studies, there are limitations. For example, morphological techniques require expertise in taxonomy and the ability to identify and differentiate arthropod species using identification keys which are lacking in many southern African countries. Furthermore, differentiation of some species at larval stage, using morphological approaches, is challenging. With the current advances in DNA technology, molecular tools are now available to facilitate species identification based on genetic examination. In view of the above, we can anticipate that estimates of PMI based on arthropod evidence will become more accurate and probably contribute to accurate interpretation and application of entomology data in medico-legal forensic investigation in southern Africa. Forensic entomology data or research have not been incorporated in cases of poaching, which are reported frequently in southern African countries including South Africa. Therefore, more studies need to be conducted and incorporated with available research so that research can be applied to solve cases of poaching of game animals. Additionally, occurrence of diurnal oviposition by carrion-feeding insects is well known, whereas there is still great debate about the occurrence of nocturnal oviposition, as most forensic entomologists assume that flies are nocturnally inactive. Therefore, future studies on nocturnal oviposition may be necessary, because a high number of deaths occur at night and nocturnal oviposition may be used in the determination of PMI.

2.6 Conclusion

Although forensic entomology is useful in criminal investigations, it is still an emerging field in southern Africa. Studies completed to date have been limited to identification of insect taxa found on carcasses during different stages of decomposition, and this information can subsequently be used to determine PMI. Some of the research conducted in southern African on carrion-feeding insects was not undertaken in a forensic context; however, it has generated useful results which can be used as

evidence in forensic investigations and improve the current status of forensic entomology in southern Africa. Nonetheless, future studies on the application of forensic entomology in various criminal investigation such as murder cases, human neglect, and the poaching of animals in southern Africa are recommended. Additionally, few studies have investigated nocturnal oviposition in southern Africa, despite many of deaths occurring at night and nocturnal oviposition therefore being applicable for the estimation of PMI.

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CHAPTER 3: Application of veterinary forensic entomology to domestic and game ruminant animals: an exploratory study using sheep carcass in KwaZulu-Natal province of South Africa

3.1 Abstract

There have been increasing cases of game and wildlife poaching in South Africa and this also includes illegal killing or neglect of domestic animals. This has resulted in an increased interest in developing an application of forensic entomology to solve legal cases of poaching and illegal killings of wildlife and domestic animals. This study made use of domestic sheep as an animal model for domestic and wildlife ruminants to gather and assess information on arthropods of forensic value in post-mortem interval (PMI) estimation. Two adult Merino sheep were humanely killed, (one in cold and the other in warm season) at the Ukulinga research and training farm of the University of KwaZulu-Natal, South Africa and the carcasses were used to assess the succession patterns of arthropod species colonization and to determine the different stages of decomposition. The following stages of decomposition and their duration were observed on both carcasses; fresh stage (0-1day), bloated stage (2-6 days), active decay (7-12 days), advanced decay (13-51 days) and dry stage (52-58 days). Dipteran flies from the families Calliphoridae, Muscidae and Sarcophagidae were the first to colonize the carcasses during both warm and cold seasons. These include *Chrysomya marginalis*, *Ch. putoria*, Ch. albiceps, *Ch. chloropyga*, *Lucilia cuprina*, *Musca domestica* and *Sarcophaga calcifera*. Colonization by beetle species during the warm season started as early as the fresh stage with *Dermestes maculatus*, *Thanatophilus micans* and *Onthophagus crassicollis*, whilst in the cold season the same beetle species were collected from bloated stage. *Chrysomya marginalis*, *M. domestica, D. maculatus*, *T. micans* and *O. crassicollis* persisted on the carcass throughout all five stages of decomposition during the warm season. In the cold season only *M. domestica* and *S. calcifera* persisted on the sheep carcass throughout all five stages of decomposition. In arthropod taxa *Ch. marginalis* (103), *Ch. albiceps* (110), *M. domestica* (110), *D. maculatus* (146) and *T. micans* (72) were the most abundant species colonizing the sheep carcass in the warm season. The same species were also abundant during the cold season. However, the number of adult arthropods collected during the warm season (n = 902) was significantly higher than during to the cold season (n = 371), and season had no statistical significant influence in the abundance of adult arthropods across all stages ($p \geq 0.05$). Results from this study generated important information on the endemic arthropod species that are attracted to sheep carcass during the warm and cold season in a locality of the KwaZulu-Natal province and their succession patterns can further be investigated as potential indicator species of PMI estimates in this area. This information may also be useful in legal cases of relocation or movement of a carcass after death. Results from this study also constitute preliminary information in the creation of a database of

arthropods associated with sheep carcasses, which in turn can be used to solve veterinary legal cases involving domestic and wildlife small ruminant species such as goats, impala, springbok, and nyala, amongst others.

Keywords: medico-veterinary legal cases, postmortem interval estimate, wildlife forensics

3.2 Background

According to the Endangered Wildlife Trust's (EWT) red data of South African mammals, about 17% are considered to be threatened by extinction and 10% are deemed to be near threatened (Steyn, 2018). Furthermore, according to the IUCN, the rate in which these animal species are becoming extinct is 1000 times more than the expected natural rate (Rolo *et al.*, 2013). The major contributing factor to this loss is significantly influenced by human actions (Rolo *et al.*, 2013) and wildlife-related crimes ranging from illegal possession of controlled species, animal cruelty, animal neglect, illegal wildlife trade and poaching (Tobe and Linacre, 2010).

There has been an observed increase in number of reported cases of game and wildlife poaching in South Africa (Lubbe *et al.*, 2019) commonly for meat, fur, trophies, and for organs which have a high black-market value in certain communities or regions due to certain beliefs and practices (Anderson, 1999). As a result, many game and wildlife species including elephants and rhinos have consequently become endangered due to extinction and/or extirpation. For example, more than 1000 rhinos were killed between 2013 and 2017, and elephant poaching has also increased over the past years with more than 100 000 African elephants killed between 2014 and 2017 (Actman, 2019). Considering that South Africa holds approximately 80% of rhino population worldwide (Anderson and Jooste, 2014; Lubbe *et al.*, 2019) the current state of poaching where rhinos are killed daily is concerning (Lubbe *et al.*, 2019).

According to Lockdown and Arkow (2016), several cases of animal cruelty have been identified and these include cases of animal neglect, animal hoarding, dog fighting, blunt force trauma, poisoning, animal sexual abuse among others. However, most of cases that are reported to human law enforcement agencies are due to animal neglect (i.e. lack of food and water, lack of shelter, lack of necessary veterinary care, lack of sanitation) and maltreatment (i.e. assault, drowning, abandonment, trapping, poisoning, restriction of movement, shooting) (Patronek, 1997; Lockdown and Arkow, 2016). Animal neglect may be incidental, short term and easily resolved whereas other cases may be long term, large scale and chronic (Lockwood, 2013; Lockdown and Arkow, 2016). In many cases when animal carcasses are discovered it is difficult to determine the time elapsed since the neglect or death (Defilippo *et al.*, 2020), and without this evidence or information, it becomes difficult to pursue a case. As a result, many cases of animal cruelty are not followed up (Defilippo *et*

al., 2020) and the culprits get away with crime and more animals continue to be killed.

While forensic entomology has been used in medico-legal investigation to provide useful information such as the presence of toxin or drug, post-mortem interval (PMI) estimation, corpse transfer, detection of wounds, evidence of trauma and neglect or abuse cases(Amendt *et al.*, 2011; Rolo *et al.*, 2013; Brundage and Byrd, 2016) , the last few years have shown an increasing interest in developing and applying forensic entomology to solve legal cases of poaching and illegal killing of wildlife (Anderson, 1999; Watson and Calton, 2003; Rolo *et al.*, 2013; Wilson *et al.*, 2014) and domestic animals (McGarry *et al.*, 2017; Defilippo *et al.*, 2020). Subsequently, several attempts have been made to apply forensic entomology to cases of wildlife crime (Rolo *et al.*, 2013), neglect or abuse of domesticated animals (McGarry *et al.*, 2017).

This study explored the application of forensic entomology using domestic sheep (*Ovis aries*) as an animal model for domestic and wild small ruminants to document insect species that may be associated with each stage of animal decomposition and assess their potential in PMI estimation taking into account the geographical location and seasonal effects in KwaZulu-Natal province of South Africa.

3.3 Materials and Methods

3.3.1 Study location

The study was conducted in Ukulinga research and training farm at the University of KwaZulu-Natal (29.6627° S, 30.4050° E) (Figure 3.1). The farm is situated in Pietermaritzburg, under uMgungundlovu district of KwaZulu-Natal province, South Africa. This area is characterized by warm to hot summers and mild winters, which are often accompanied by irregular frost (Kiala *et al.*, 2017). The average monthly temperature ranges from 13.2°C to 21.4°C, with an annual mean temperature of 17°C (Mills and Fey, 2004; Everson *et al.*, 2013; Kiala *et al.*, 2017). The area receives an annual precipitation of 680 mm in over 106 days per annum (Kiala *et al.*, 2017) and its falls under the Southern Tall Grassveld and mainly herbaceous as a result of long-term burnings (Mills and Fey, 2004; Kiala *et al.*, 2017). The cold season is experienced from the month of May to end August, whilst the warm season is from September to April. The cold season trial of this study was conducted from June to August, with measured average temperature ranging from 18°C to 19°C, and the warm season trial was conducted from November to January, with measured average temperature ranging from 21°C to 23°C.

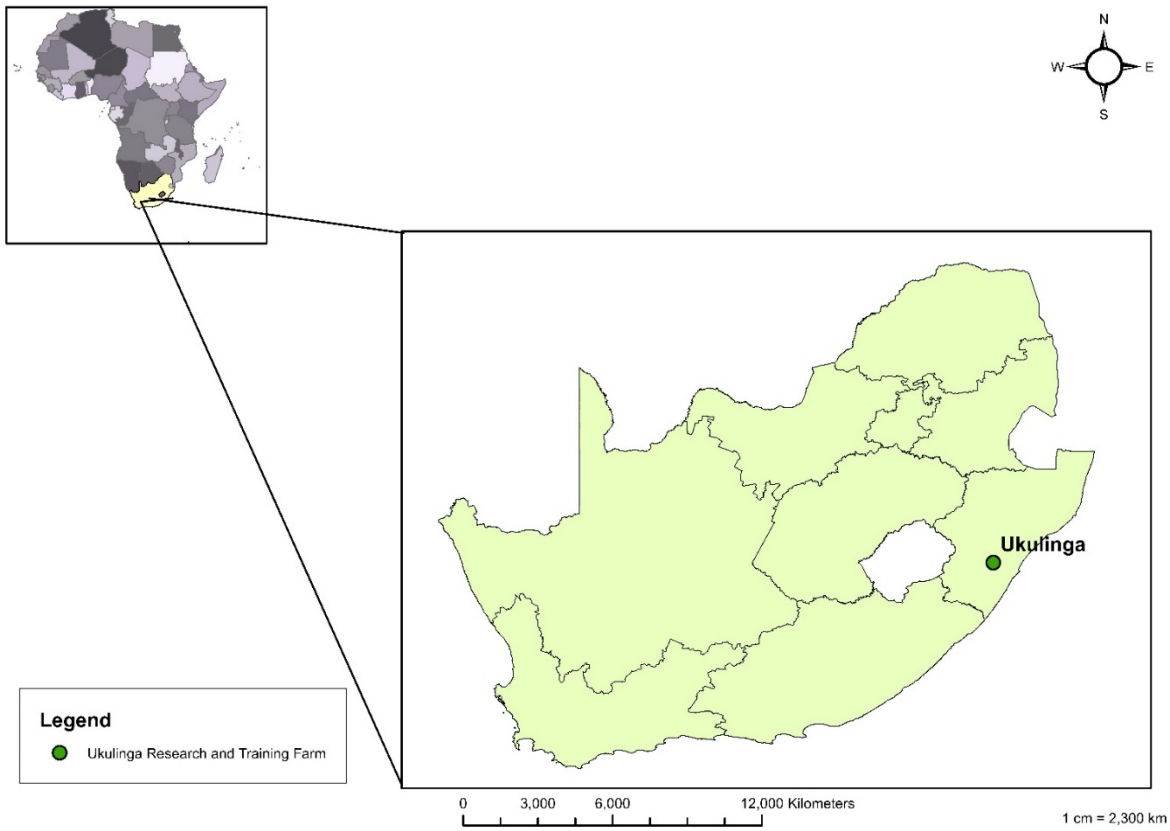

Figure 3.1: Location of the study in the KwaZulu-Natal province of South Africa.

3.3.2 Study animals

Two medium sized healthy Merino sheep with an average weight of 80 kg (live weight) were purchased from University of KwaZulu-Natal Ukulinga farm, and one sheep carcass was used for the study in each season (cold season and warm season).

3.3.3 Sampling procedures

Experimental sheep were humanely killed, followed by three knife stabs around the neck region to create wounds that mimic the cases of an illegally killed animal. Carcasses were then placed in metal cages (100 cm x 100 cm x 100 cm) covered with mesh wire to prevent predators but allowed free movement of insects. The day when the animals were killed was recorded as day 0 and marked the initiation of each trial and each seasonal experiment which lasted 59 days.

Stages of decomposition of carcasses were determined as described by Martinez *et al.* (2007) and Wolff *et al.* (2001). The following information was collected through visual observation and recordings from the carcass and its surroundings on each day of sample collection; physical changes of the carcasses, odor and intensity and the presence of arthropods during each stage of decomposition at given days and times as shown in Table 3.1. Temperature of carcass and that of the

soil surrounding the carcass were measured once a day at 09:00 am using an infrared thermometer. Adult arthropods found on and around the carcasses were collected using either fly traps which were hanged on the cages or picking them directly by hands and the collected adult arthropods were then counted and recorded from 9:00 – 11:00 am, and preserved in 70% ethanol for further identification. This was done for all the different stages of decomposition. The number of eggs and larvae of arthropods on- and around the carcass during the first three 3 stages (fresh, bloated, active stages) of decomposition were not determined to avoid disturbance to maggots establishing on the carcasses, which could consequently interfere with decomposition rate of the carcass leading to inaccurate results. Therefore, this study only sampled adult arthropods.

3.3.4 Morphological and molecular identification of collected adult arthropods

Collected adult arthropods were morphologically identified using identification keys as described by (Byrd and Castner, 2001; Kolver, 2009; Iqbal *et al.*, 2014; Lutz *et al.*, 2018; Lubbe *et al.*, 2019; BugGuide, 2020)' Molecular techniques were used to confirm arthropod species identification using mitochondrial primers described by Folmer *et al.* (1994) and Zhuang *et al.* (2011) (results shown in chapter 5).

3.3.5 Data analysis

Counts of collected arthropods taxa and diversity, species abundance, soil temperature and carcass body temperature were recorded on Microsoft Excel and summarized in tables and figures. A chi-square/Fisher exact test was used to test for seasonal difference in abundance and diversity of arthropods groups. The difference was considered significant if the p-value was $p \leq 0.05$ with GraphPad.

3.4 Results

Five stages of decomposition of the sheep carcass were observed and categorized based on the physical appearance of the body and carcass temperature as described by Wolff *et al.* (2001) (Table 3.1); namely i. Fresh (0-1 days) ii. Bloated (2-6 days) iii. Active (7-12 days) iv. Advanced (13-51 days) and v. Dry (52-58 days) and they lasted for 2, 5, 6, 40 and 7 days respectively.

Table 3.1: Summary of the physical characteristics of sheep carcass at different stages of decomposition and the sampling time during the warm and cold season.

Stages of decomposition	**Period (days)**	**Sampling time**	**Physical changes**	**Odor**	**Presence of arthropods**
Fresh (0-1 days)	2	9:00 – 11:00	None	None	Yes
Bloated (2-6 days)	5	9:00 – 11:00	i. Swelling of the abdomen	i. Foul odor	Yes
Active (7-12 days)	6	9:00 – 11:00	i. Carcass deflated ii. Significant tissue loss	i. Odor was still present and prominent	Yes
Advanced (13-51 days)	40	9:00 – 11:00	i. Significant tissue loss ii. limbs collapsed into resting position iii. Legs and rib bones were visible	i. Slight odor was present	Yes
Dry (52-58 days)	7	9:00 – 11:00	i. Gut contents dried out ii. Skin and wool dried out	None	Yes

a. Fresh stage (0-1 days post death)

No physical changes of the carcass were observed at this stage, with exception to a sharp decrease in body temperature from 38 to 27 °C and 26 to 16 °C during warm and cold season respectively (Figures 3.2 and 3.3).

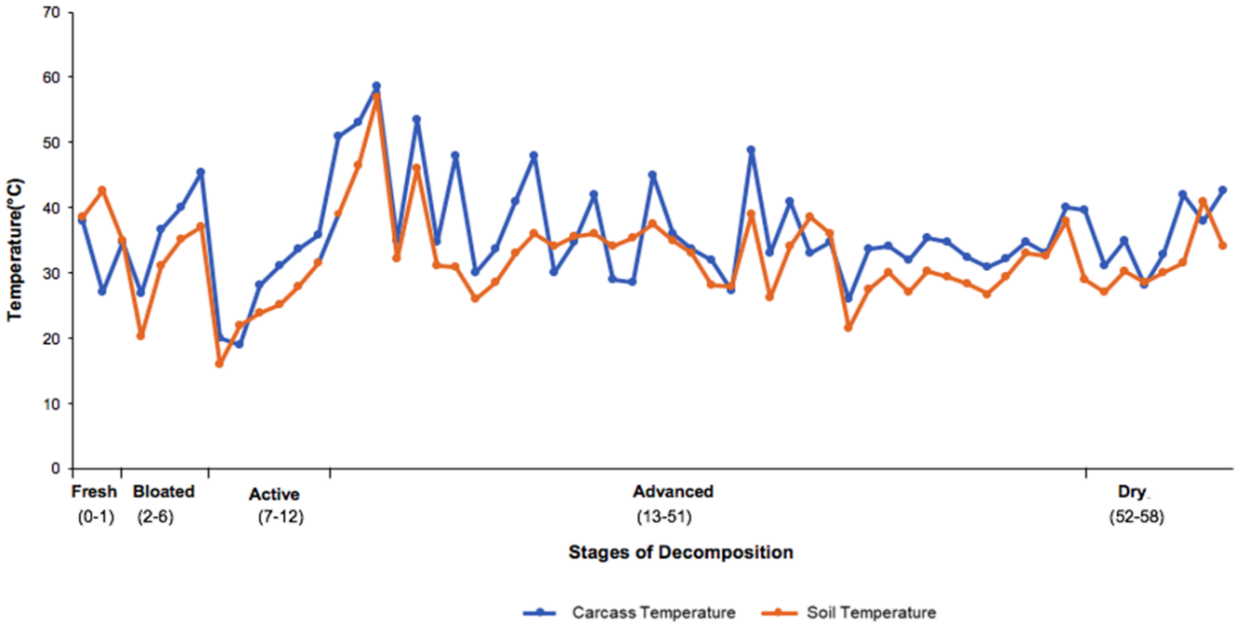

Figure 3.2: Comparison of carcass body temperature and soil temperature at different stages of decomposition during the warm season.

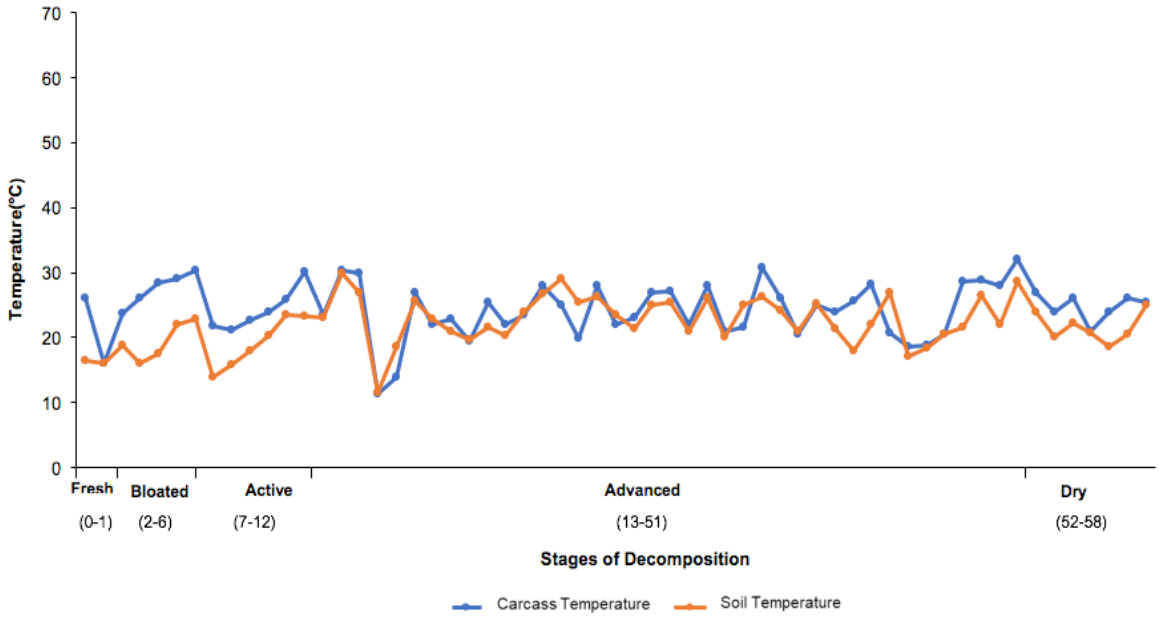

Figure 3.3: Comparison of carcass body temperature and soil temperature at different stages of decomposition during the cold season.

A total of ten arthropod taxa were collected and recorded during the warm season (Figure 3.4) and included *Chrysomya marginalis* (*Ch. marginalis*), *Chrysomya putoria* (*Ch. putoria*), *Chrysomya albiceps* (*Ch. albiceps*), *Chrysomya chloropyga* (*Ch. chloropyga*), *Lucilia cuprina* (*L. cuprina*), *Musca domestica* (*M. domestica*), *Sarcophaga calcifera* (*S. calcifera)*, *Dermestes maculatus* (*D. maculatus),* *Thanatophilus micans* (*T. micans*) and *Othophagus crassicollis* (*O. crassicollis)* (Table 3.2). However, during the cold season only seven taxa were recorded and included *Ch. marginalis, C. putoria, C. albiceps, Ch. chloropyga, L. cuprina, M. domestica* and *Sarcophaga calcifera* minus *D. maculatus, T. micans* and *O. crassicollis* which were recorded during the warm season. Flies from the families Calliphoridae, Muscidae and Sarcophagidae were the first to colonize the carcasses during both warm and cold seasons. These included *Ch. marginalis, Ch. putoria, Ch. albiceps, Ch. chloropyga, L. cuprina, M. domestica* and *S. calcifera* (Table 3.2). These species were more abundant during the warm season (n = 89) as compared to the cold season (n = 35) and there was no statistical significance observed (p = 0.527) (Table 3.3). Furthermore, *M. domestica* was the most abundant species in the cold season (n = 10), whilst *Ch. albiceps* and *M. domestica* were in the warm season (n = 20).

Arthropods eggs were observed on the carcass on day one post exposure during the warm seasons, although the species of arthropods which laid the eggs were not identified.

Table 3.2: Succession pattern of arthropods species during the five stages of decomposition of sheep carcass during warm and cold seasons at Ukulinga research and training farm in the province of KwaZulu-Natal, South Africa.

Order	Family	Species	Fresh (0-1 days)	Bloated (2-6 days)	Active (7-12 days)	Advanced (13-51 days)	Dry (52-58 days)
Diptera	Calliphoridae	*Chrysomya marginalis*					
Diptera	Calliphoridae	*Chrysomya putoria*					
Diptera	Calliphoridae	*Chrysomya albiceps*					
Diptera	Calliphoridae	*Chrysomya chloropyga*					
Diptera	Calliphoridae	*Lucilia cuprina*					
Diptera	Muscidae	*Musca domestica*					
Diptera	Sarcophagidae	*Sarcophaga calcifera*					
Coleoptera	Cleridae	*Necrobia rufipes*					
Coleoptera	Dermestidae	*Dermestes maculatus*					
Coleoptera	Silphidae	*Thanatophilus micans*					
Coleoptera	Scarabaeidae	*Onthophagus crassicollis*					
Coleoptera	Scarabaeidae	*Onthophagus sp.*					
Coleoptera	Meloidae	*Hycleus lunatus*					

Key: Black fill, warm season; black dotted, cold season.

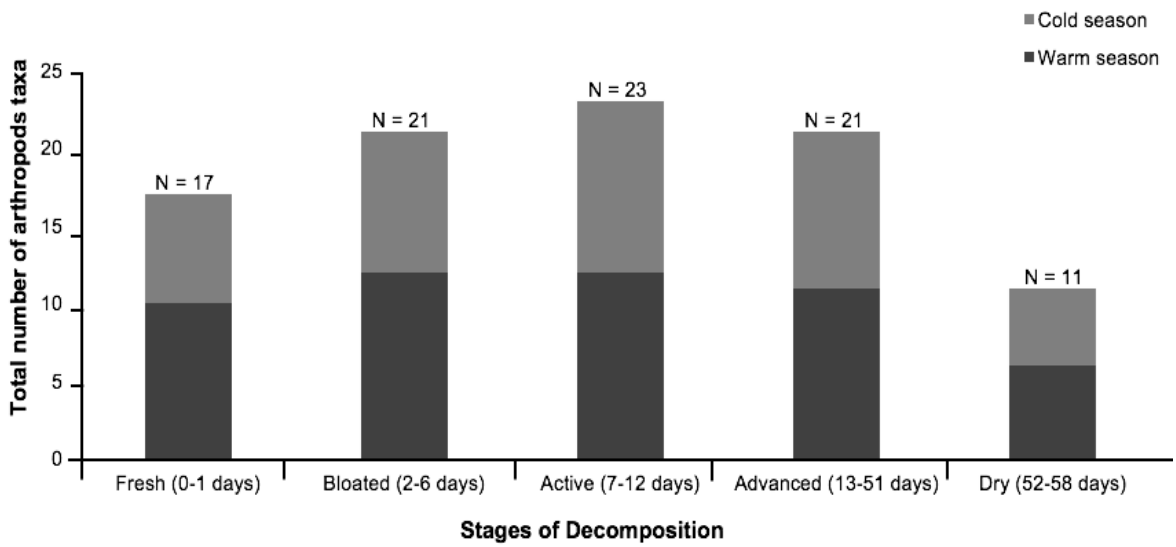

Figure 3.4: Taxa richness recorded during decomposition of sheep carcassin the warm and cold season.

b. *Bloated stage (2-6 days post death)*

This stage was characterized by the swelling on the abdominal region of both carcasses, and a foul odor was emitted from the carcass. The carcass temperature on the first day of bloated stage during the warm season was 34.6 °C, which was an increase from 27 °C recorded on the last day of fresh stage. The temperature then declined on the second day of the bloat stage, followed by gradual increase until the last day (45.3 °C) of the warm season (Figure 3.2). Similar trend was observed during the cold season, where the carcass temperature increased from the fresh stage, starting the bloat stage at 23.7 °C and increased until the last day of this stage to 30.4 °C (Figure 3.3). There was an increase in the taxa richness by 2 species (*Onthophagus sp.* and *Hycleus lunatus*) during warm season (n = 12) and by an addition of *D. maculatus* and *Onthophagus sp.* during the cold season (n = 9) (Figure 3.4).

The following arthropod species recorded during fresh stage persisted to the bloat stage; *Ch. marginalis, Ch. putoria, Ch. albiceps, Ch. chloropyga, L. cuprina, M. domestica, S. calcifera* and *D. maculatus* during both seasons (Table 3.2). However, there was an observed significant increase in the number of these arthropods colonizing the carcass during this stage as compared to the fresh stage (p = 0.986).

Chrysomya albiceps (n = 44), *M. domestica* (n = 40) and *Ch. marginalis* (n = 30) were more abundant during warm and cold seasons (*Ch. albiceps* (n = 22), *M. domestica* (n = 27) and *Ch. marginalis* (n = 20) respectively (Table 3.3).

Table 3.3: Abundance of adult arthropods associated with different stages of decomposition of sheep carcasses during warm and cold seasons.

Ecological category	**Order**	**Family**	**Genus/Species**	**Fresh** (0-1 days)		**Bloated** (2-6 days)		**Active** (7-12 days)		**Advanced** (13-51 days)		**Dry** (52-58 days)		**Total**	
				CS	**WS**	**CS**	**WS**	**CS**	**WS**	**CS**	**WS**	**CS**	**WS**	**CW**	**WS**
Necrophagous	Diptera	Calliphoridae	*Chrysomya marginalis*	8	12	20	30	13	36	6	21	0	4	47	103
Necrophagous	Diptera	Calliphoridae	*Chrysomya putoria*	4	6	12	18	6	12	1	8	0	0	23	44
Necrophagous	Diptera	Calliphoridae	*Chrysomya albiceps*	6	20	22	44	12	28	2	18	0	0	42	110
Necrophagous	Diptera	Calliphoridae	*Chrysomya chloropyga*	2	5	8	13	5	16	0	3	0	0	15	37
Necrophagous	Diptera	Calliphoridae	*Lucilia cuprina*	3	18	12	22	1	6	1	0	0	0	17	46
Necrophagous	Diptera	Muscidae	*Musca domestica*	10	20	27	40	18	21	9	17	7	12	71	110
Necrophagous	Diptera	Sarcophagidae	*Sarcophaga calcifera*	2	8	9	19	6	25	2	9	2	0	21	61
Total				**35**	**89**	**110**	**186**	**61**	**144**	**21**	**76**	**9**	**16**	**236**	**511**
Chi-square/Fisher exact p-value				**p = 0.527**		**p = 0.986**		**p = 0.228**		**P = 0.158**		**p = 0.209[a]**		**Nd**	
Predators	Coleoptera	Cleridae	*Necrobia rufipes*	0	0	0	0	11	20	16	22	9	11	36	53
Necrophagous	Coleoptera	Dermestidae	*Dermestes maculatus*	0	10	4	22	17	34	22	36	25	44	68	146
Predators	Coleoptera	Silphidae	*Thanatophilus micans*	0	4	0	9	0	11	4	22	0	26	4	72
Total				**0**	**14**	**4**	**31**	**28**	**65**	**42**	**80**	**34**	**81**	**108**	**271**
Chi-square/Fisher exact p-value				**Nd**		**p = 0.84[a]**		**p = 0.713[a]**		**p = 0.064**		**p = 0.74[a]**		**Nd**	
Coprophagous	Coleoptera	Scarabaeidae	*Onthophagus crassicollis*	0	2	0	13	3	22	8	26	11	29	22	92
Coprophagous	Coleoptera	Scarabaeidae	*Onthophagus sp.*	0	0	4	12	1	4	0	6	0	0	5	22

Total				**0**	**2**	**4**	**25**	**4**	**26**	**8**	**32**	**11**	**29**	**27**	**114**
Chi-square/Fisher exact p-value				**Nd**		**p = 0.312[a]**		**p = 0.141[a]**		**p = 0.485**		**P = 0.462**		**Nd**	
Incidental	Coleoptera	Meloidae	*Hycleus lunatus*	0	0	0	6	0	0	0	0	0	0	0	6
Total				**0**	**0**	**0**	**6**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**6**

Abbreviations: CS, cold season; WS, warm season; [a]Fisher's exact; Nd, Not done; Significant at $p < 0.05$

Hycleus lunatus and Onthophagus sp. were recorded during the bloated stage of the warm season (Table 3.2). However, *D. maculatus* and *Onthophagus sp.* were the first beetle species to appear on the carcass also during the cold season (Table 3.2).

c. *Active stage (7-12 days post death)*

At this stage the carcass deflated, and a significant amount of soft tissue loss was observed from both carcasses at the end of this stage. The carcass temperature at the beginning of this stage (day 7 post death) during the warm season was 20 °C, then declined to 19 °C on the second day of the active stage, and subsequently increased until the last day of this stage (Figure 3.2). Similar trend in temperature change was observed during the cold season whereby the active stage began with a body temperature of 21.8 °C, which then declined to 21.2 °C on the second day and then increased until the last day of this stage (Figure 3.3).

The number of arthropod taxa remained the same (n = 12) during the warm season, whereas it increased from 9 to 11 during the cold season (Figure 3.4). This stage was associated with the presence of *Ch. marginalis, Ch. putoria, Ch. albiceps, Ch. chloropyga, L. cuprina, M. domestica* and *S. calcifera* (Table 3.1). The number of arthropod species collected during both cold and warm season decreased compared to the bloated stage, with exception to *Ch*. *marginalis* and *S. calcifera* which increased from 30 – 36 and 19 – 25 during the warm season (Table 3.3). There was an observed increase in the number of *D. maculatus* during both season, and *O. crassicollis and T. micans* during the warm season. However, *Othophagus sp.* decreased in both seasons, whilst *H. lunatus* cleared from the carcass and there was an introduction of *Necrobia rufipes* in both seasons, and *O. crassicollis* in the cold season (Table 3.3).

d. Advanced stage (*13-51 days post death*)

At this stage, carcasses showed a significant loss of tissue, and by the end of this stage limbs had collapsed into resting position, and legs and rib bones were now visible. Since most of the soft tissue was lost, body temperature was the same with surrounding atmospheric temperature most of the time (Figure 3.2 and 3.3). *Chrysomya marginalis, Ch. putoria, Ch. albiceps, Ch. chloropyga, L. cuprina, M. domestica* and *S. calcifera* were found on the carcass, however their number and activity declined (Table 3.2). There was a decrease in the number of arthropod taxa during the warm (n = 11) and cold season (n = 10) (Figure 3.4). *Chrysomya chloropyga* and *L. cuprina* were not collected from this stage during the cold and warm season, respectively. A lot of dead flies and larvae were observed during this stage. There was an increase in the number of *N. rufipes, D. maculatus, micans, O. Crassicollis and Othophagus sp.* (Table 3.3). However, during the cold season *T. micans* appeared for the first time and *Onthophagus sp.* Dissapeared from the carcass

e. *Dry stage* (*52-58 days post death*)

At this stage gut contents of both carcasses were dried out and the skin and wool were completely dry with no moisture. As with the advanced stage, the temperature of both carcasses was equivalent to that of the surrounding environment (Figure 3.2 and 3.3). There was a sharp decrease on the total number of arthropods taxa collected during the warm season (n=6) and the cold season (n = 5) (Figure 3.4). Species of *Ch. putoria, Ch. albiceps, Ch. chloropyga and L. cuprina* did not appear on the carcasses during this stage (Table 3.2). However, *Ch. marginalis* appeared on the carcass during the warm season and adult flies of *M. domestica* were observed during both seasons, although in low in numbers (Table 3.3). *Necrobia rufipes* decreased in number during both seasons, whilst *D. maculatus, O. Crassicollis and T. micans* increased. However, *T. micans* did not appear on the carcass during the cold season and *Onthophagus sp.* disappeared from the carcasses during both warm and cold seasons (Table 3.3).

3.5 Discussion

The decomposition pattern and time taken by the carcass exposed to warm season was the same as that of the carcass exposed to the cold season. This was not expected as most studies show that carcasses exposed to the summer season decompose faster than that one exposed to winter (Bass, 1996; Gilbert, 2014). The observed similar period of decomposition of carcasses between seasosns in this study may have been due to rainfall experienced mostly when the warm season experiment was taking place. According to Lyu et al. (2016) rainfall wets the carcass and expel fly maggots from the carcass (Singh and Bala, 2019), and as a result affect the rate at which the carcass decomposes.

This study shows that the decomposition periods correspond with those reported by Wolff *et al.* (2001) who used pig as an animal model. However, the dry stage in our study was shorter. In the first stage of decomposition, a sharp decrease in the carcass temperature was observed in both seasons. A similar observation was made by Martinez *et al.* (2007) who recorded a sharp decrease in body temperature of the carcass, reaching levels below the ambient temperature in pigs (*Sus scrofa* L.). Dipteran families Calliphoridae, Sarcophagidae and Muscidae are of great significance in the early stages of decomposition (Mabika *et al.*, 2014). According to Goff (1993) and Martinez *et al.* (2007), Calliphoridae, Sarcophagidae families are typically necrophagous, and mainly feed on the carcass tissue and are important in estimating PMI in forensic investigations due to the predictable time they appear on the carcass. In this study, Calliphoridae (*Ch. marginalis, Ch. putoria, Ch. albiceps, Ch. chloropyga, Lucilia cuprina)*, Sarcophagidae (*S. calcifera)* and Muscidae (*M. domestica*) species were the first to colonize the carcass in both warm and cold season. Mabika *et al.* (2014) and Martinez *et al.* (2007) confirmed that Calliphoridae and Muscidae are primary colonizers in rabbit and pigs respectively. However, the authors also recorded Sarcophagidae family as secondary colonizers.

Although arthropod family and genus from this study were similar to those reported by Martinez *et al.* (2007) and Shi *et al.* (2009) from pig and rabbit respectively, the species collected in these studies varied from the species collected from the present study.

Results further show that *D. maculatus, T. micans and O. crassicollis* were also the first beetle species to colonize the sheep carcass and persisted throughout the five stages of decomposition during the warm season. According to Villet *et al.* (2011), *D. maculatus* species are common and active during summer, and this could explain the absence of these species on the carcass during the first stage of decomposition during the cold season. As with the study conducted by Early and Goff (1986) on cats, *D. maculatus* beetles in this study were first collected during the bloated stage during the cold season. Considering that Mabika *et al.* (2014) collected these *Dermestes* spp. during decay stage, it is safe to conclude that the succession pattern of this species may be due to seasonal peaks rather than the decomposition stages (VanLaerhoven and Anderson, 1999). According to Midgley *et al.* (2010), *T. micans* beetles have proven to be useful indicators in forensic entomology. As observed in this study, this beetle species arrived soon after death in the warm season and hence has forensic potential like flies from the family Calliphoridae and Sarcophagidae (Ridgeway *et al.*, 2014). Surprisingly, this species was rare during the cold season.

According to Kelly *et al*. (2009), the bloated stage is associated with swelling of the abdomen due to the build-up of gases and bacterial action. This normally results in an increase in the body temperature of a carcass (Wolff *et al.*, 2001; Martinez *et al.*, 2007). Similar observations were made in this study, where the abdomen region of the sheep carcasses resembled a balloon and body temperatures increased. According to Martinez *et al.* (2007), the increase in temperature may also be due to insect activity and putrefaction processes. Emission of the foul odor from the carcass coincided with the increase in number of necrophagous species (Calliphoridae, Muscidae and Sarcophadidae) visiting the carcasses. As the foul odor became more pronounced, the numbers of *Ch. marginalis, Ch. putoria, Ch. albiceps, Ch. chloropyga, L. cuprina, M. domestica, S. calcifera* also increased and this might be related to the effect of the odor in attracting these flies.

There was an observed increase in taxa richness during the bloated stage on both seasons in this study and Shi *et al.* (2009) observed similar trend in rabbit whereby the highest taxa richness was recorded during the bloated stage in summer in Guangzhou, China. The increase in taxa richness may also be due to the observed new beetle species in our study. Furthermore, the number of arthropods collected were more abundant during this stage compared to other stages in the two seasons, with the highest number of arthropods collected during the warm season. However, Shi *et al.* (2009) obtained the highest number of arthropod species on rabbit carcass during spring and winter in China, and similar observation were made by Tantawi *et al.* (1996) on the same animal species in Egypt.

As decomposition progressed to the active stage, the carcasses started deflating and at the end of this stage there was a great tissue loss, this was due to a high feeding activity of insect maggots (*Ch. marginalis, Ch. albiceps, Ch. Chloropyga and L. cuprina*) on the carcass tissues. These findings corresponded with to those reported by Kelly *et al.* (2011), where a pig carcass deflated during the active stage due to the active feeding rate of maggots of *Ch. marginalis, Ch. albiceps, Ch. Chloropyga, L. cuprina* and Sarcophagidae species. At the beginning of this stage, there was a notable decline in carcasses body temperature, which subsequently increased from day three until the last day of this stage. However, Martinez *et al.* (2007), also observed a low carcass temperature which only increased in the last days of this stage. According to Kelly *et al.* (2011), the decline in body temperature coincides with the release of gases by the body as the skin tears up (Kelly *et al.*, 2011). Wolff *et al.* (2001) noted that the small increase in the temperature, also observed in this study, may be due to larval activity as the body burst. The taxa richness remained the same as the bloated stage during the warm season and increased in the cold season. However, the number of flies collected decreased during this stage in both seasons, which coincided with the introduction and increase in the number of beetles in cold and both seasons, respectively. These observations were supported by previous studies which noted that Dipteran species normally increase in numbers during the initial stages of decomposition, whilst Coleoptera species only increase in numbers as decomposition advances (Campobasso *et al.*, 2001; Kelly *et al.*, 2008).

As decay advances into the dry stage, the carcasses lost significant amount of tissue, gut contents, skin and wool were dry with no moisture. As a result, the carcasses body temperature corresponded with surrounding atmospheric temperature most of the time. Similar observations were made by Wolf *et al.* (2001) who recorded body temperature which was almost similar to that of the environment during the advanced and dry stages of decomposition. The taxa richness and number of flies continued to decrease, with only *Ch. marginalis* and *M. domestica* persisting to the dry stage. Rosa *et al.* (2011) also observed similar trend whereby Dipteran species from the families Calliphoridae, Sarcophagidae, Muscidae and Fanniidae decreased in numbers from the advanced decay stage as compared to the first three stages of decomposition. This could be due to the decreased attraction of necrophagous dipteran species to the carcass when the tissues are dry (Tantawi *et al.*, 1996).

Overall, *Ch. marginalis*, *Ch. albiceps* and *M. domestica* were the most dominant fly species, collectively contributing 63.2 % of the collected flies in warm season, and 68.9 % in the cold season. Studies also showed that *Ch. Marginalis* (Braack, 1986) and *Ch. Albiceps* (Gilbert, 2014) occurred predominantly in summer in the Kruger National Park and Gauteng highveld respectively. Surprisingly, Gilbert (2014) reported that *Ch. albiceps* in Gauteng province only occurred in summer, whereas in

our study this *Chrysomya* was one of the dominant species in both seasons. Braack (1986) reported that *M. domestica* was more abundant in spring. *Dermestes maculatus and T. micans* contributed more (60.9 %) to the total number of beetles collected throughout the study during the warm season. However, only *D. maculatus* was more predominant in the cold season. Villet *et al.* (2011) reported that *D. maculatus* is more common in summer, and Braack (1986) recorded the highest number in late autumn, Braack (1987) later reported that *D. maculatus* beetles are normally present and utilizes carcass-habitat throughout the year which explains the observed abundance of this species in both season in this study. Furthermore, Braack (1986) recorded the highest number of *T. micans* collection in summer. These results further highlights that the relationship between taxa richness and temperature is still debatable (Shi *et al.*, 2009), and the presence of these species in abundance may indicate that they are endemic in this area. Therefore, these species can be investigated further as potential indicator species of PMI estimates in this area and considered in cases of relocation or movement of a carcass after death.

The results support previous reports that Dipteran families of Calliphoridae, Sarcophagidae and Muscidae are of great significance as potential indicators of early stages of decomposition. Studies have shown that within these families, arthropods from the genera *Chrysomya*, *Lucilia*, *Sarcophaga* and *Musca* colonize sheep carcass as early as the fresh stage just like any other animal model previously studied (pigs, rabbit, cats and impala). However, the species within these genera may differ depending on geographical location, season, and animal species. The sheep carcass from this study attracted *Ch. marginalis, Ch. putoria, Ch. albiceps, Ch. chloropyga* from the *Chrysomya* genus, *L. cuprina* from *Lucilia*, *S. calcifera* from *Sarcophaga* and *M. domestica* from *Musca* genus in both cold and warm seasons. Additionally, Coleoptera species *D. maculatus, T. micans, O. crassicollis* also colonized the carcass during the first stages of decomposition. Results also show that *Chrysomya marginalis, M. domestica, D. maculatus, T. micans and O. crassicollis* persisted on the carcass throughout all five stages of decomposition during the warm season. Whereas, in the cold season only *M. domestica* and *S. calcifera* persisted on the sheep carcass throughout all five stages of decomposition. Arthropod taxa collected on the carcass during both seasons were similar, minus *Hycleus lunatus* which was unique to the bloated stage of the warm season.

3.6 Conclusion

Results from this study constitute preliminary data in the creation of a database of arthropods associated with sheep carcass, which in turn can be used to solve medico-veterinary legal case involving small domestic ruminant such as goats, and small-medium wildlife ruminants such as impala, springbok, deer, nyala, amongst others. There is need for future research using other animal species such as cattle as animal models mimic medium to large domestic and wildlife ruminant carcasses and

document dipteran and coleopteran species occurring taking into account different geographical regions in South Africa in order to assess their value in PMI estimation.

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3.8 References

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CHAPTER 4: Insect succession and decomposition pattern on pig (*Sus scrofa domesticus*) carrion during warm and cold seasons in Kwazulu-Natal Province of South Africa

4.1 Abstract

Certain insect species display a strong habitat/location and seasonal trends/associations with carrion, which make them valuable as potential indicator species to determine post-mortem interval (PMI), the location and/or season of death of human or animal. Two medium sized Large-white pigs were humanely killed and used for cold and warm season. Five stages of decomposition and their duration fresh (0-1day), bloated (2-6 days), active (7-12 days), advanced (13-51 days) and dry (52-58 days) stages were observed on pig carcasses during the warm and cold seasons. The most abundant carrion fly species found on the carcass during warm season were *Chrysomya marginalis* (n = 111), *Chrysomya albiceps* (n = 99) and *Musca domestica* (n = 131). The same species were the most abundant in the cold season (n = 55), (n = 34) and (n = 81) respectively, although in lower numbers than in the warm season. The highest number of Diptera (necrophagous) individuals were observed and collected during the bloated stage in both seasons (n = 152) and cold season (n = 92). However, the difference in the number of necrophagous arthropods collected during both seasons were not statistically significant (P = 0.150). Among the collected Coleoptera species, *Dermestes maculatus* (n = 112) and *Necrobia rufipes* (n= 62) were the most abundant found during the warm season and the same species were the most abundant during the cold season *(*n = 66) and (n = 48) respectively. Colonization by *D. maculatus* on the pig carcass during the warm season started during the fresh stage and persisted throughout all stages of decomposition during the warm season. In the cold season colonization started from the active stage of decomposition. *Necrobia rufipes* appeared on the carcass during the active stage and persisted throughout all stages during both seasons. The arrival time and association of these species with different stages of decomposition during the warm and cold season pointed to their value in estimating the PMI in forensic investigations in the locality of KwaZulu-Natal, South Africa. Consequently, they can potentially be useful in the estimation of PMI and and other cases of criminal investigations. As a result, we recommend that more research to be conducted in different geographical locations of South Africa and develop a database of insect species that are endemic in the locations which can be related to cases that need application of forensic entomology.

Keywords: Diptera, coleoptera, species identification, seasonality, stages of decomposition

4.2 Background

Forensic entomology is the scientific study of the use of insects and other arthropods associated with a carrion in forensic investigations (Wolff *et al.*, 2001; Haskell *et al.*, 2008). This study has been accepted and used in various courts of law worldwide (Amendt *et al.*, 2007; Amendt *et al.*, 2011), and is now recognized as an important investigative tool in many developed countries (Haskell *et al.* 2008, Kokdener, 2016; Tembe and Mukaratirwa, 2020). Medico-legal entomology deals with insect evidence collected at the crime scene, and this field has been gaining more recognition than urban and stored-product forensic entomology globally (Sukontason *et al.*, 2007; Haskell *et al.*, 2008).

In medico-legal entomology, insects and other arthropods are commonly used to confirm relocation of carcasses or post-mortem transfer (Sumodan, 2002), postmortem interval (PMI), cause and manner of death, chemical and drug verification, child abuse and neglect and other related cases of a forensic investigation (Amendt *et al.*, 2007; Kokdener, 2016). However, the application of forensic entomology evidence in forensic investigation requires accuracy and consideration of several factors, such as understanding the role of different insect species and their colonization process throughout carcass decomposition; the effect of temperature, seasons and climatic zones on carrion associated species.

According to Parry *et al.* (2016), carrion-feeding insect species have been observed to vary in diverse environments. This variation may be due to factors such as habitat (Matuszewski *et al.*, 2013), availability of food, the presence or absence of other insect species (Williams, 2002), and season (Richards *et al.*, 2009). Therefore, studying, recording and comparing the different insect species found in a specific geographic area/location in different seasons can provide valuable information on the availability, abundance and diversity of carrion-feeding insect species, subsequently improving the existing knowledge and understanding of their adaptive responses to different environmental conditions. This knowledge is of great importance in the field of forensic entomology (McIntyre, 2000; Kitching, 2013) and is still lagging in many southern African countries including South Africa (Tembe and Mukaratirwa, 2020).

Matuszewski *et al.* (2013), observed that certain insect species display a strong habitat and seasonal associations, which make them valuable as potential indicator species to determine the location and season of death. Although the effect of seasons and other environmental factors on the decomposition rate and succession pattern of arthropods have been previously studied in other countries including few regions of South Africa, the seasonal colonization pattern and activity of many carrion-associated arthropods to determine PMI and relocation of a carcass still remains inadequately

studied in many regions of South Africa, including KwaZulu-Natal province which is ecologically different from other previously studied regions in South Africa. Therefore, this study aimed at determining the dipteran and coleopteran species associated with pig carcasses and their sequence of arrival and colonization immediately after death and throughout decomposition during the warm and cold season.

4.3 Materials and Methods

4.3.1 Study location

The study location has been described in chapter 3.

4.3.2 Sampling method

Two pigs (*Sus scrofa domesticus*) with average live weight of 100 kg were humanely Killed and the carcasses were manipulated as described in chapter 3. Carcasses were immediately placed in metallic cages (100 cm x 100 cm x 100 cm) covered with mesh wire which protected the carcass from scavengers such as rats and other small vertebrates but allowing free movement of insects. The post-mortem changes of the carcass and the presence of Dipteran and Coleoptera species throughout all five stage of decomposition were recorded and are described in Table 4.1. Sampling procedure, morphological and molecular identification of collected dipteran and coleopteran species has already been described in chapter 3.

4.3.2 Data analysis

To verify the statistical difference between the abundance of Dipteran and Coleopteran groups and species collected in different seasons a Chi-square/Fisher exact test was performed and $p < 0.05$ was considered statistically significant.

Table 4.1: Summary of the physical characteristics of decomposition of pig (*Sus scrofa domesticus*) carcass, sampling times and the presence of Dipteran and Coleopteran species during the warm and cold season

Stages of decomposition	Period (days)	Sampling time	Physical changes	Foul odor	Presence of Dipteran and Coleopteran species
Fresh (0-1 days)	2	9:00 - 11:00	- Soft torsos - Flexible limbs	- None	Yes
Bloated (2-6 days)	5	9:00 - 11:00	- Body colour darkened	- Foul odor present	Yes
Active (7-12 days)	6	9:00 -11:00	-Skin peeling commenced	- Foul odor and intense	Yes
Advanced (13-51 days)	40	9:00 -11:00	-Great peeling of the skin -Skin began to dry out - Great removal of the soft tissue	- Foul odor reduced	Yes
Dry (52-58 days)	7	9:00 -11:00	- little moisture - Skin was dry	- None	Yes

4.4 Results

Five decomposition stages were observed in the pig carcasses during the two seasons (warm and cold season) based on the postmortem changes of the carcass and categorized as described by Wolff *et al.* (2001). These were i. fresh ii. bloated iii. active iv. advanced and v. dry (Figure 4.1 A and B).

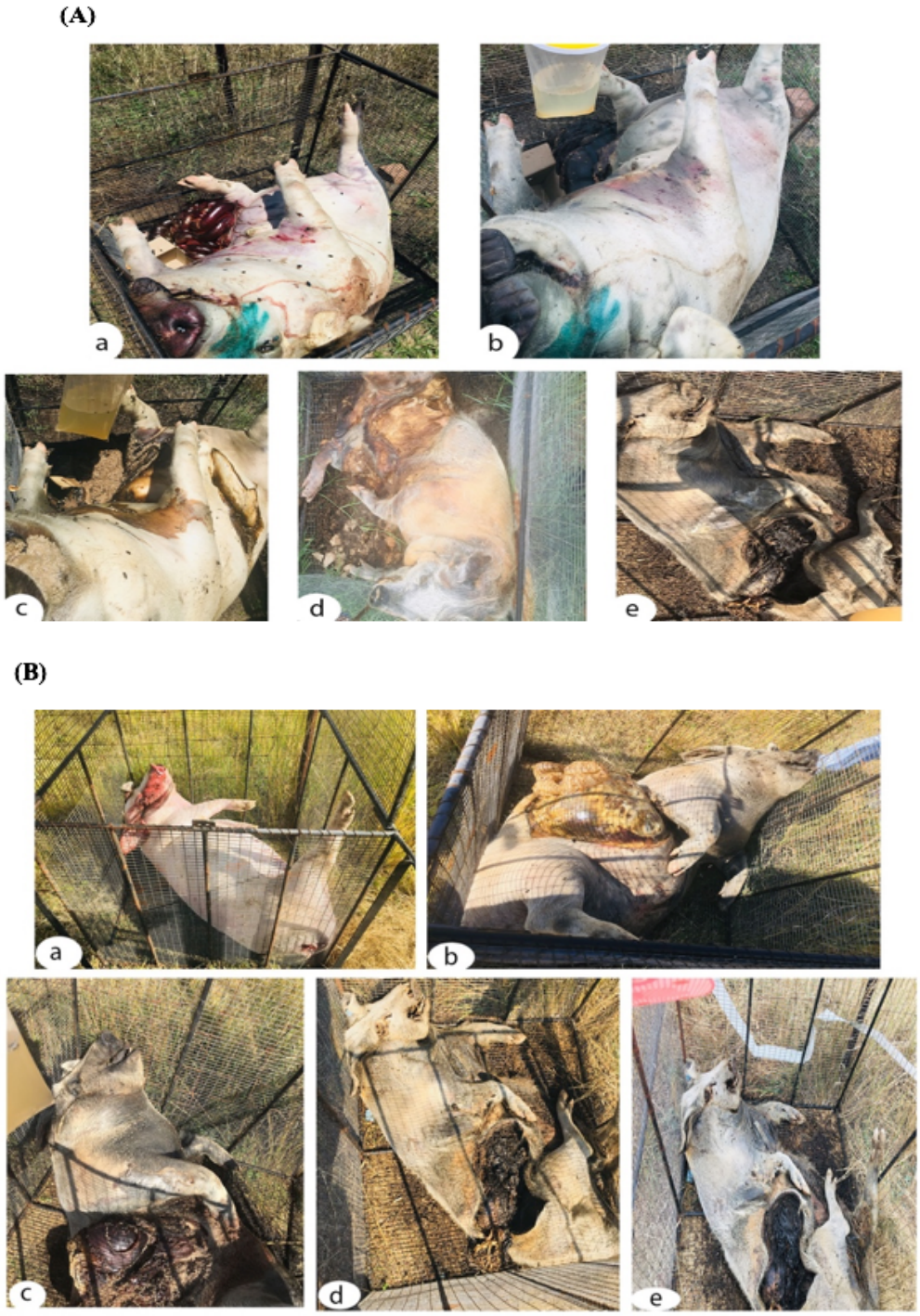

Figure 4.1: Decomposition stages of pig carcass during the warm season (A) and cold season (B). Images (a-e) represents different stages of decomposition: (a) fresh stage, (b) bloated stage, (c) active stage (d) advanced stage, (e) dry stage.

a. Fresh stage (0-1 days)

The fresh stage commenced soon after the animals were humanely killed, and it was associated with soft torsos and flexible limbs and no foul odor (Figure 4.1 A and B). There was an observed decrease in the body temperature from 37 °C to 24 °C during the warm season and from 25.4 °C to 15.4 °C during the cold season (Figure 4.2 A and B).

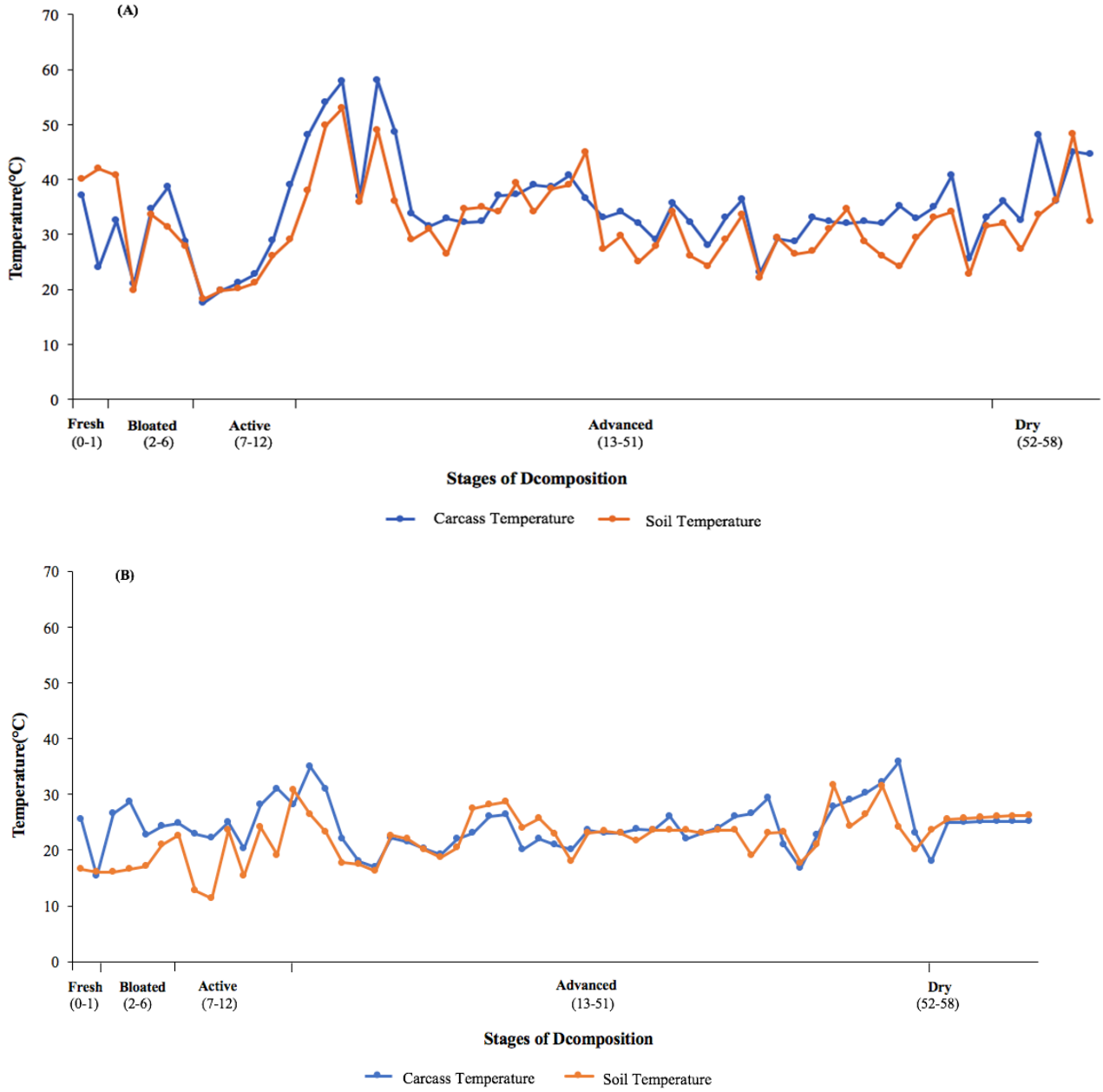

Figure 4.2: Comparison between body temperature of a pig carcass and soil temperature during different stages of decomposition in the warm (A) and cold season (B).

Seven arthropod taxa (Dipteran and Coleopteran) were recorded during both warm and cold season (Figure 4.3). *Chrysomya marginalis, Ch. putoria, Ch. albiceps, Ch. chloropyga, L. cuprina, M. domestica* and *S. calcifera* were the first species to arrive and colonize the carcasses within few minutes of death in both warm and cold seasons (Table 4.2). A total of 76 necrophagous species including *Chrysomya marginalis (Ch. marginalis), Chrysomya putoria (Ch. putoria), Chrysomya albiceps (Ch. albiceps), Chrysomya chloropyga* (*Ch. chloropyga*), *Lucilia cuprina* (*L. cuprina*), *Musca domestica* (*M. domestica*) and *Sarcophaga calcifera* (*S. calcifera*) were observed associated with this stage of decomposition in the warm season (Table 4.3). During the cold season, the fresh stage consisted of 34 necrophagous flies, *Ch. marginalis, Ch. putoria, Ch. albiceps, Ch. chloropyga, L. cuprina, M. domestica* and *S. calcifera* (Table 3). These species were observed to be more abundant during the warm season (n = 76) as compared to the cold season (n = 34, Table 4.3). *Musca domestica* (n = 22*),* and *Ch. albiceps* (n = 16*)* were more abundant during the warm season, whereas *M. domestica* was the most abundant species in the cold season (n = 11, Table 4.3). Statistically there was no significance in the abundance of necrophagous species collected during different seasons (p = 0.082) (Table 4.3).

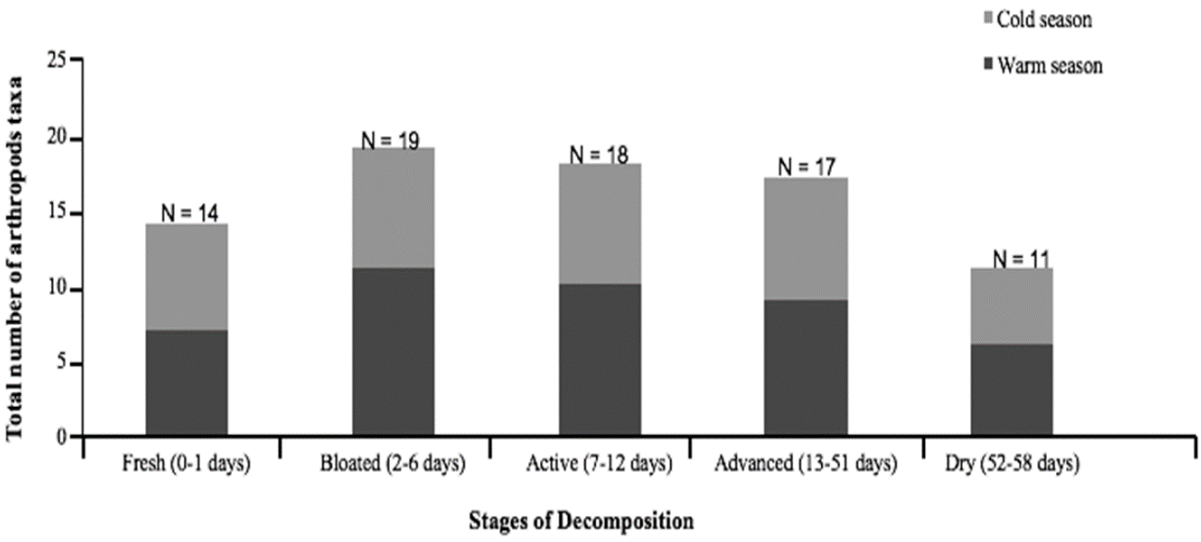

Figure 4.3: Arthropods taxa richness at different stages of a decomposing pig carcass during the warm and cold season.

Table 4.2: Succession pattern of different arthropods (Diptera and Coleoptera) species attracted during the five different stages of decomposition of pig carcass during the warm and cold seasons at Ukulinga research and training farm in the province of KwaZulu-Natal, South Africa

Ecological category	Order	Family	Species	Fresh (0-1 days)	Bloated (2-6 days)	Active (7-12 days)	Advanced (13-51 days)	Dry (52-58 days)
Necrophagous	Diptera	Calliphoridae	*Chrysomya marginalis*					
Necrophagous	Diptera	Calliphoridae	*Chrysomya putoria*					
Necrophagous	Diptera	Calliphoridae	*Chrysomya albiceps*					
Necrophagous	Diptera	Calliphoridae	*Chrysomya chloropyga*					
Necrophagous	Diptera	Calliphoridae	*Lucilia cuprina*					
Necrophagous	Diptera	Muscidae	*Musca domestica*					
Necrophagous	Diptera	Muscidae	*Atherigona soccata*					
Necrophagous	Diptera	Sarcophagidae	*Sarcophaga calcifera*					
Predators	Coleoptera	Cleridae	*Necrobia rufipes*					
Necrophagous	Coleoptera	Dermestidae	*Dermestes maculatus*					
Predators	Coleoptera	Silphidae	*Thanatophilus micans*					
Coprophagous	Coleoptera	Scarabaeidae	*Onthophagus crassicollis*					
Incidental	Coleoptera	Meloidae	*Hycleus lunatus*					

Key: Black fill arrow, warm seasons; Balck doted arrow, cold season.

Table 4.3: Abundance of adult Dipteran and Coleopteran species associated with different stages of pig carcasses during different stages of decomposition during the cold and warm season

Ecological category	**Order**	**Family**	**Genus/Species**	**Fresh** (0-1 days)		**Bloated** (2-6 days)		**Active** (7-12 days)		**Advanced** (13-51 days)		**Dry** (52-58 days)		**Total**	
				CS	**WS**	**CS**	**WS**	**CS**	**WS**	**CS**	**WS**	**CS**	**WS**	**CS**	**WS**
Necrophagous	Diptera	Calliphoridae	*Chrysomya marginalis*	6	10	22	35	18	40	9	26	0	0	55	111
Necrophagous	Diptera	Calliphoridae	*Chrysomya putoria*	2	5	4	7	0	0	0	0	0	0	6	12
Necrophagous	Diptera	Calliphoridae	*Chrysomya albiceps*	4	16	14	38	9	30	7	15	0	0	34	99
Necrophagous	Diptera	Calliphoridae	*Chrysomya chloropyga*	5	8	11	13	3	9	0	5	0	0	19	35
Necrophagous	Diptera	Calliphoridae	*Lucilia cuprina*	5	11	9	15	5	7	0	0	0	0	19	33
Necrophagous	Diptera	Muscidae	*Musca domestica*	11	22	26	38	26	42	13	19	5	10	81	131
Necrophagous	Diptera	Muscidae	*Atherigona soccata*	0	0	2	0	0	0	0	0	0	0	2	0
Necrophagous	Diptera	Sarcophagidae	*Sarcophaga calcifera*	1	4	4	6	1	3	2	7	1	4	9	24
Total				**34**	**76**	**92**	**152**	**62**	**131**	**31**	**72**	**6**	**14**	**225**	**445**
Chi-square/Fisher exact p-value				**p = 0.082[a]**		**p = 0.150[a]**		**p = 0.116[a]**		**P = 0.102[a]**		**p = 0.001[a]***		**p = 0.134[a]**	
Predators	Coleoptera	Cleridae	*Necrobia rufipes*	0	0	0	0	10	13	18	22	20	27	48	62
Necrophagous	Coleoptera	Dermestidae	*Dermestes maculatus*	0	0	0	11	18	28	22	34	26	39	66	112
Predators	Coleoptera	Silphidae	*Thanatophilus micans*	0	0	0	4	0	9	2	13	0	18	2	44
Total				**0**	**0**	**0**	**15**	**28**	**50**	**42**	**69**	**46**	**84**	**116**	**218**
Chi-square/Fisher exact p-value				**Nd**		**p = 0.800[a]**		**p = 0.624[a]**		**p = 0.093[a]**		**p = 0.594[a]**		**p = 0.440[a]**	
Coprophagous	Coleoptera	Scarabaeidae	*Onthophagus crassicollis*	0	0	0	5	0	12	4	15	5	10	9	42
Total				**0**	**0**	**0**	**5**	**0**	**12**	**4**	**15**	**5**	**10**	**9**	**42**

Incidental	Coleoptera	Meloidae	*Hycleus lunatus*	0	0	0	4	0	0	0	0	0	0	0	4
Total				**0**	**0**	**0**	**4**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**4**

CS, cold season; WS, warm season; Nd, not done; [a]Fisher's exact; *Significant at $p < 0.05$

b. *Bloated stage (2-6 days)*

At this stage, the body color of both carcasses changed from white and became darkened, a foul odor was being emitted from the carcasses (Figure 4.1 A and B). In the warm season, the carcass temperature at the beginning of bloated stage was 32.5 °C, an increase from 24 °C recorded on the last day of the fresh stage (Figure 4.2 A). On the second day of this stage the temperature then decreased to 21°C, followed up by an increase to 38.6 °C until the fourth day of the stage. On the last day of the bloated stage, the carcass temperature decreased to 28.7°C (Figure 4.2 A). A different trend was observed during the cold season, where during first two days of bloated stage the body temperature increased to 26.5 °C and 28.7 °C respectively, then decreased on the third day to 22.7°C. The body temperature then increased to 24.8 °C until the last day of this stage (Figure 4.2 B). The body temperature was slightly higher than that of soil temperature, during both seasons (Figure 4.2A and B). The number of arthropods taxa increased in both the warm (n = 11) and cold season (n = 8, Figure 4.3). The Dipteran species observed and recorded during the fresh stage persisted on the carcasses to the bloated stage in both seasons (Table 4.2). However, there was an additional species *Antherigona soccata* (*A. soccata*), found on the carcass during the cold season. *Antherigona soccata* and *Ch. putoria* did not pass through this stage (Table 4.2).

There was an increase in the total number of necrophagous flies collected on the pig carcasses during the warm season (n = 152) and cold season (n = 92) (Table 4.3). However, the difference was not significant (n = 0.150, Table 4.3). It was further observed that the recorded numbers of *M. domestica* (n = 38), *Ch. albiceps* (n = 38), and *Ch. marginalis* (n = 35) were higher during warm season than in the cold season (*M. domestica* (n = 26), *Ch. albiceps* (n = 14) and *Ch. marginalis* (n = 22) (Table 4.3). In addition to the dipteran species collected collected during this stage, 24 beetles were collected comprising four species *Dermestes maculatus* (n = 11), *Thanatophilus micans* (n = 4), *Onthophagus crassicollis* (n = 5) and *Hycleus lunatus* (n = 4) during the warm season. However, *H. lunatus* did not persist through this stage.

c. Active stage (*7-12 days*)

The foul odor from decomposing tissues from the carcasses was more intense, exudates were discharged by the body and the skin peeling commenced during this stage (Figure 4.1 A and B). During the warm season, the carcass temperature at the beginning of the active stage was 17.5 °C, which subsequently increased until the last day to 38.9 °C (Figure 4.2 A). During the cold season, the active stage began with the carcass body temperature of 22.8 °C, which then slightly declined to 22.2 °C on the second day of the active stage and increased to 31°C until the last day of the stage (Figure 4.2B). There was a slight decrease in the total number of arthropods observed and collected during the warm season

(n = 10), however in the cold season the total number of arthropods taxa remained the same (n = 8, Figure 4.3). The total number of necrophagous dipteran species collected during this stage decreased from 152 to 131 during the warm season and from 92 to 62 during the cold seasons (Table 4.3). In both seasons, *Ch. marginalis, Ch. albiceps, Ch. chloropyga, L. cuprina, M. domestica, S. calcifera* continued to persist on the carcasses (Table 4.2). However, *Ch. chloropyga* did not persist from this stage during the cold season and *L. cuprina* during both seasons (Table 4.2). There was an increase in the total number of beetles at this stage during the warm season (Table 4.3). The number of flies collected during both seasons decreased, with the exception of *Ch*. *marginalis* and *M. domestica* which increased in the warm season (Table 4.3). However, the number of *M. domestica* remained constant (n = 26) during the cold season (Table 4.3). An increase in the number of *D. maculatus* (n = 28), *T*. *micans* (n = 9) and *O. Crassicollis* (n= 12) was observed during the warm season. Furthermore, there was an introduction of *D. maculatus* in the cold season and *Necrobia rufipes* during both seasons (Table 4.3).

d. *Advanced stage (13-51 days)*

This stage was characterized by a massive peeling and drying out of the skin, and loss of the soft tissue of both carcasses (Figure 4.1 A and B). For most of the time during the advanced stage, the temperature of both carcasses was the same with that of the surrounding soil temperature (Figure 4.2 A and B). The total number of arthropods taxa decreased (n = 9), whereas it remained the same during the cold season (n = 8, Figure 4.3). Species of *Ch. marginalis, Ch. albiceps, M. domestica* and *S. calcifera* persisted on the pig carcasses during both seasons and *Ch. chloropyga* during the warm season only (Table 4.2). However, their numbers on the carcasses were reduced (Table 4.3). The total number of necrophagous species collected from both carcasses declined at this stage in comparison to the previous stages in both warm (n = 720) and cold (n = 31) seasons (Table 4.3). The total number of predator arthropods taxa continued to increase during this stage in both warm (n = 69) and cold seasons (n = 42) (Table 4.3). There was an increase in the number of *N. rufipes* and *D. maculatus* during warm (n= 22, n = 34) and cold seasons (n = 18, n = 22) respectively and *O. crassicollis* (n = 15) during the warm season (Table 4.3). Additionally, *T. micans* (n = 2) and *O. crassicollis* (n = 4) appeared on the pig carcass for the first time during the cold season (Table 4.2 and 4.3).

e. *Dry stage (52-58 days)*

Both carcasses had dry skin during this stage of decomposition (Figure 4.1 A and B). A similar pattern of body temperature was observed as in the advanced stage, where body temperature was similar to that of the soil temperature (Figure 4.2 A and B). *Musca domestica* and *S. calcifera* persisted on pig carcasses during both warm and cold seasons (Table 2). There was a decrease in the total number of arthropods

taxa recorded and collected during the warm (n = 6) and cold season (n = 5, Figure 4.3). There was a decrease in the total number of coprophagous species (n = 10, Table 4.3) and a slight decrease during the cold season (n = 5, Table 4.3), and statistically there was an observed difference in the abundance of coprophagous arthropods collected during different seasons (p = 0.001, Table 4.3). Species of *N. rufipes* and *D. maculatus* persisted on the carcasses and increased in numbers during the warm (n = 27, n = 39) season and cold season (n = 20, n = 26) respectively, whereas *O. crassicollis* increased in number from 4 to 5 during the cold season and declined from 15 to 10 during the warm season (Table 4.3). *Thanatophilus micans* increased (n = 18) during the warm season and disappeared from the pig carcass during the cold season (Table 4.3).

Results of molecular analysis are reported in chapter 5.

4.5 Discussion

The decomposition time taken by the carcass exposed to warm season was the same as that of the carcass exposed to the cold season. This was not expected as most studies show that carcasses exposed to the warm seasons decompose faster than that of the winter experiments (Bass, 1996; Gilbert, 2014). In this study the observed similar time of decomposition between carcasses may be due to rainfall, as it was raining most of the time in the warm season and according to Lyu *et al.* (2016) rainfall is a major factor that affect the rate at which the carcass decomposes because rain wet the carcass and expel fly maggots from the carcass (Sing and Bala, 2019). This study also showed that the duration of the decomposition stages was similar to that described by Wolf *et al.* (2001) in pig carcass with an exception of the dry stage in this study which was shorter than that of Wolf *et al.* (2001).

During the first stage (fresh) of decomposition, the carcasses had soft torsos (abdomen and thorax) and flexible limbs and there was no odor during both warm and cold seasons. However, there was an observed decrease in the body temperature of carcasses in both warm and cold seasons. Similar observations were made by Kelly *et al.* (2008) and Kelly *et al.* (2011), where the fresh stage of pig (*Sus scrofa* L.) carcasses were associated with soft torso and flexible limbs with no odor during the winter and summer experiments in South Africa. Furthermore, Grisales *et al.* (2010), made similar observations where a body temperature of the pig (*Sus scrofa* L.) carcass decreased from 38 °C to 22 °C during the fresh stage of decomposition. Species from the families Calliphoridae (*Ch. marginalis, Ch. putoria, Ch. albiceps, Ch. chloropyga, L. cuprina*), Muscidae (*M. domestica*) and Sarcophadidae (*S. calcifera* were the first colonizers in both carcasses mainly for feeding and breeding purposes. Similarly, Shi *et al.* (2009), confirmed Sarcophagidae species as primary colonizers of the rabbit carcass in warmer temperatures and tropical areas in Guangzhou, China. However, Mabika *et al.* (2014) and Martinez *et al.* (2007) recorded Sarcophagidae family as secondary colonizers and Calliphoridae and Muscidae as primary

colonizers in rabbit and pig carcasses respectively. According to Mabika *et al.* (2014), species from these families play an important role during the early stages of decomposition, and due to the predictable sequence of arrival on the carcass they are potential indicators of PMI and determining clues in cases of criminal investigations especially if the body tissue is still fresh (Padonou *et al.* 2017, Tembe and Mukaratirwa *et al.* 2020).

The bloated stage of both carcasses was associated with a change in body color from white to dark, with a foul odor. Similar observations were made by Kelly *et al.* (2011) where during the bloated stage the pig carcass body color darkened. Verheggen *et al.* (2017), reported that the bloated stage of vertebrate carcasses is characterized by the presence of perceived odor. Mabika *et al.* (2014) also observed that the bloated stage of the rabbit was associated with odor. Furthermore, during the bloated stage we observed an increase in the body temperature of the carcasses of which according to Martinez *et al.* (2007), the increase in temperature may be due to high insect activity taking place during this stage. It was further observed that during both seasons the carcass body temperature was slightly higher than that of the soil temperature. Similar observations were made by Payne (1965), whereby the temperature of the pig carcass was slightly higher than the soil temperature during the bloated stage. Furthermore, as the carcasses released foul odor, the numbers of *Ch. marginalis, Ch. putoria, Ch. albiceps, Ch. chloropyga, L. cuprina, M. domestica* and *S. calcifera* visiting the carcasses also increased during both seasons and according to Verheggen *et al.* (2017), the odor plays a crucial role in attracting necrophagous insect species.

The results further showed that Coleoptera species *D. maculatus, T. micans, O. crassicollis* and *H. lunatus* were the first beetle species to visit the pig carcass during bloated stage and persisted throughout all five stages of decomposition during the warm season with exception of *H. lunatus*, which did not pass through the bloated stage. The presence of these beetles on the carcass as early as bloated stage supports the findings of other several studies that reported the early arrival of Coleoptera species on carcasses (Early and Goff, 1986; Braack, 1987; Mayer and Vasconcelos, 2013; Singh and Bala, 2019). According to VanLaerhoven and Anderson (1999), the presence of these beetles during the bloated stage may be due to seasonal peaks appearance rather than the decomposition stages (Mabika *et al.*, 2014). This could also explain the absence of these species on the carcass during the bloated stage of decomposition during the cold season. The occurrence of *H. lunatus* in low numbers only in the warm season during the bloated stage may indicate that the beetles could have accidentally landed on the carcass, as they are normally associated with crops than dead bodies and their role in decomposing carcasses is still not clear.

A total number of arthropods taxa (Dipteran and Coleopteran) richness increased during this stage in the warm and cold season. Similarly, Shi *et al.* (2009) observed and reported the highest number

of taxa richness during the bloated stage of the rabbit in Guangzhou, China. Furthermore, the number of dipteran species collected during the bloated stage were more abundant compared to other stages during both cold and warm season and the dominant species were *Ch. albiceps*, *M. domestica* and *Ch. marginalis*. However, the highest number of Dipteran species were collected during the warm season. Similar results were reported by Keshavarzi *et al.* (2019), where the highest number of arthropods were observed during the bloated stage of albino rats and the dominant species were *Ch. albiceps*, *Calliphora vicina* and *S. africa* during the autumn season.

The active stage was also characterized by the presence of foul odor of decay and skin peeling. However, as the insect maggots fed on the carcass the foul odor became more pronounced. These results correspond with those reported by Kelly *et al.* (2008) and Kelly *et al.* (2011), where the active stage of a pig carcass was characterized by a strong odor which became prominent as maggots fed on the carcass and persisted until the carcass started deflating. Furthermore, the observed skin peeling of carcasses might have occurred due to the skin drying out and erupting/tearing (Kelly *et al.* 2008). The early days of this stage was also characterized by a decrease in carcasses body temperature, which subsequently increased from day two until the last day of this stage in the warm season, however in the cold season it decreased on the second day and subsequently increased until the last day of decomposition. Martinez *et al.* (2007), also observed low body temperature of pig carcass that increased in the last days of this stage. The observed increase in carcass body temperature may be due to insect larval activity as the body burst (Wolf *et al.* 2001) or peel which allows insect maggots to feed underneath it (Kelly *et al.*, 2011). Additionally, according to Kelly *et al.* (2011), the observed slightly decrease in carcass body temperature may be due to the emission of gases by the body as the skin erupted.

Chrysomya chlorophaga was slightly more in numbers during the warm season as compared to the cold season and it persisted on the carcass up to the advanced stage in the warm season, whereas it did not pass through this stage during the cold season. Similarly, Williams and Villet (2019), found *Ch. chloropyga* in high numbers during November (warm season) in Eastern Cape, South Africa and stated that this species seems to be restricted by the maximum and minimum temperature excesses. This might explain the observed low numbers in both seasons. The number of flies collected during the active stage, decreased in both seasons, coinciding with the introduction of *N. rufipes* and *D. maculatus* and increase of *D. maculatus* and *O. crassicollis* in cold and warm seasons, respectively. Campobasso *et al.* (2001) and Kelly *et al.* (2008) also noted that Dipteran species usually increase in numbers during the early stages of decomposition, whereas Coleoptera species only increase in numbers as the decomposition progresses.

The advanced stage of decomposition was characterized by a reduction in foul odor and the carcasses showed great peeling of the skin and the skin began to dry out and there was significant loss of soft tissue. The carcass body temperature was consistent with that of the surrounding soil temperature. These findings correspond with that of Wolf *et al.* (2001), where the recorded pig body temperature was almost similar to that of the environment surrounding the carcass during both advanced and dry stages of decomposition. The number of *Chrysomya* species (*Ch. marginalis, Ch. albiceps* and *Ch. Chloropyga*) continued to decrease and did not persist through this stage during both seasons. However, only *M. domestica* and *S. calcifera* remained on the carcass up to the dry stage. Similar observations were made by Rosa *et al.* (2011), where the numbers of fly species from the families Calliphoridae, Sarcophagidae and Muscidae decreased from the advanced decay stage as compared to the early stages of a decomposing pig. This might be explained by the association and attraction of most Dipteran species to the carcass when the tissues are still soft (Goff, 1993) and the presence of strong foul odor (Verheggen *et al.* 2017). The number of *N. rufipes* and *D. maculatus* continued to increase during both seasons and *T. micans* and *O. crassicollis* during the warm season of this study. Kelly *et al.* (2011) also observed *N. rufipes* and *D. maculatus* associated with the advanced stage of decomposition in all seasons and *T. micans* in warmer seasons. Furthermore, in the study of Kelly *et al.* (2008), *D. maculatus* were observed in high numbers during this stage, however, *N. rufipes* remained in the same numbers as in the active stage of decomposition. The presence of these species in numbers during this stage could be due to their preference towards the dry skin (Mashaly *et al.*, 2018).

During the dry stage of decomposition, the carcass showed little moisture and dry skin and the foul odor was reduced. Subsequently, most of the time the body temperature of the carcass was consistent with that of the surrounding soil temperature. This stage was mainly dominated by the presence of *D. maculatus* and *N. rufipes* followed by *T. micans* and *O. crassicollis* in the warm season. However, in the cold season this stage was dominated by *D. maculatus* and *N. rufipes*. These observations were supported by Mayer and Vasconcelos (2013) and Mashaly *et al.* (2018), where *D. maculatus* and *N. rufipes* were reported to be strongly associated with the dry stages of a decomposing pig (Arnaldos *et al.*, 2004). Due to their feeding preference these species can be used as potential indicators of PMI in forensic investigation.

As in other previously reported studies, *Ch. marginalis, Ch. albiceps* and *M. domestica* were most dominant and abundant in this study throughout both seasons, although there were more in numbers in the warm season as compared to the cold season. Kelly *et al.* (2011) showed that *Ch. marginalis, Ch. albiceps* and *M. domestica* were the dominant species in the summer and autumn experiments and *Calliphora vicina* and *Ch. chloropyga* during the winter season in Bloemfontein. However, the absence of *Calliphora vicina* in this study may be due to differences in geographic regions

and climate (Shin *et al.*, 2015). Williams and Villet (2019) also noted that *Ch. albiceps* occurred most of the year, although reduced in numbers from July to September in Eastern Cape province of South Africa. *Chrysomya albiceps* was also found in high numbers on the pig carcass during the spring and summer in Argentina (Horenstein *et al.* 2007). Furthermore, Braack (1986) also reported that *Ch. marginalis* was more abundant in summer in the Kruger National Park of South Africa. However, Gilbert (2014), showed that *Ch. albiceps* appeared only during summer whereas in this study this species was one of the dominant species during both cold and warm seasons. Coleoptera species, *N. rufipes* and *D. maculatus* were the most dominant species in both warm and cold seasons, and *T. micans* was the third dominant species. However, *T. micans* and *O. crassicollis* were present in less numbers during the cold season. Similarly, Kelly *et al.* (2008), reported that species of *N. rufipes* and *D. maculatus* were the most dominant Coleoptera species present and breeding on the carcasses through all seasons and only recorded *T. micans* during the warm season. Villet *et al.* (2011) reported that *D. maculatus* is more common in summer, and Braack (1986) reported their highest occurrence in late autumn. Braack (1987) reported that *D. maculatus* beetles are usually present and uses the carcass-habitat throughout the year and this could explain their observed high numbers in both seasons in this study. Although high number of flies were collected in warm season in this study, these findings point out that there is still a huge debate on the correlation concerning temperature and arthropods taxa richness (Shi *et al.*, 2009).

According to the classification of ecological relationship between the insects and carcasses by Smith (1986), Goff (1993) and Martinez *et al.* (2007), the following ecological categories were also observed in this study; necrophagous species which mainly feed on the soft tissues of the carcass and those species were *Chrysomya* species, *L. cuprina*, *M. domestica* and *S. calcifera.* These species have been reported to be of forensic value because of their predictable time of appearance and hence may be used to estimate PMI in forensic investigation, predator species which prefer feeding on the decaying tissues and the larvae and pupae of necrophagous species and these species are *N. rufipes*, *D. maculatus* and *T. micans*. This group is considered as the second most significant group of species associated with the carcass decomposition and they are also significant in forensic investigations and coprophagous species which feed mainly on the faecal material or excrement from the carcass and only *O. crassicolli*s species were recorded in this category. Lastly incidental species which according to Villet *et al.* (2011), constitutes any organism (airborne or mobile terrestrial insect) that may land on the carcass unintentionally and according to Braack (1986) this group occurs in low numbers and in this study this specie was recorded as *H. lunatus*.

4.6 Conclusion

The pig carcass was ideal as a model in determining insect succession and decomposition pattern during warm and cold seasons in KwaZulu-Natal Province of South Africa. Consequently, the

succession and decomposition pattern observed could potentially be useful in forensic investigation related to the estimation of PMI. We recommend that similar research be conducted at other geographical locations of South Africa with a different ecological system to build a database of Dipteran species of forensic value which are endemic in these areas.

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4.8 References

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CHAPTER 5: Molecular identification and diversity of adult arthropod carrion community collected from pig (*Sus scrofa domesticus*) and sheep (*Ovis aries*) carcasses within the same locality during different stages of decomposition in the KwaZulu-Natal province of South Africa.

5.1 Abstract

The current study aimed at molecular identification and diversity of adult arthropods carrion collected from pig and sheep carcasses during different stages of decomposition in the cold and warm season in KwaZulu-Natal province of South Africa. Adult arthropods found on and around the carcasses were collected using either fly traps or picking them directly by hand. Molecular analyses confirmed the identification of twelve (12) arthropod taxa collected from both sheep and pig carcasses during the cold season. Results showed that 11/12 arthropod species were common in both sheep and pig carcasses, with exception to *Onthophagus vacca* and *Atherigona soccata* species which were unique to sheep and pig carcasses respectively. However, during the warm season, the sheep carcass attracted more (n=13) taxa as compared to the pig carcass. The difference in the arthropod species was due to the presence of *O. vacca* which was also unique to the sheep carcass during this season. Furthermore, there was an addition of a beetle species *Hycleus lunatus*, which was collected from both sheep and pig carcasses but unique to the warm season. The pig carcass attracted more dipteran species during both warm (n = 1519) and cold season (n =779) as compared to sheep carcass during the warm (n =511) and cold season (n =229). In contrast, Coleoptera species were more abundant on the sheep carcass during the warm season (n = 391) and cold season (n = 135) as compared to the pig carcass in both warm season (n =261 and cold season (n = 114). Overall, more flies and beetles were collected on both sheep and pig carcasses during the warm season (n = 2682) as study, further highlighted that variations in temperature due to different seasons influenced the observed difference in the abundance of collected arthropod between seasons.

Keywords: Arthropods, beetles, flies, diversity, cold season, warm season

5.2 Background

Arthropods are some of the major contributors to the community structure and ecosystems due to their high diversities, high densities which may result from their high reproductive rates, as well as their ability to occupy several trophic categories within communities (Azwandi *et al.*, 2013). According to Castner (2001), insects are the largest groups of arthropods with the most numerous and diverse species on the planet.

Anderson (2001) reported that immediately after death of an animal, insect species primarily from the families Calliphoridae and Sarcophagidae are the first to colonize the body and develop at a

predictable rate or sequence if the environmental conditions are suitable. As the carcass decomposes, through chemical, physical and biological changes, which in turn attracts a sequence of insect colonization until nothing of nutritional value remains on the carcass (Anderson, 2001).

According to Amendt *et al.*, (2004), there are four categories of insects species that can be found on a decomposing carcass; firstly necrophagous species which use the carcass for feeding or oviposition, secondly, the predators which are insects feeding on other insects or arthropods as a food source, the omnivores species such as ants, beetles and wasps, which feeds on the carcass and its colonizers, and lastly, other species such as spiders, springtails, which uses the carcass to build up their environment. The first two categories consist of flies and beetles, which are major groups of insects which are attracted to carrions and may provide valuable information in forensic investigations (Amendt *et al.*, 2004; deSouza *et al.*, 2008).

Although most successional studies have been conducted on flies (Diptera), in the previous years there has been an increased interest in beetles (Coleoptera) that are of forensic value (Kulshrestha and Satpathy, 2001; Midgley et al, 2010; Hu *et al.*, 2020). As a result, studies on beetles of forensic value are on the increase which include species of family Dermestidae (Zanetti *et al.*, 2010; Martin-Vega *et al.*, 2017), Silphidae (Midgley and Villet 2009; Fratczak and Matuszewski, 2014) Cleridae (deSouza *et al.*, 2008; Hu *et al.*, 2020), Staphylinidae (Fratczak and Matuszewski, 2014; Watsonhorzelski, 2012), and Histeridae (Caneparo *et al.*, 2017). Succession experiments have shown that these beetles arrive at corpses at different stages of decay (Watson and Carlton, 2005; Wang *et al.*, 2017; Matuszewski *et al.*, 2019) and their biological habits and feeding preferences differ (Hu *et al.*, 2020). For example, species of Dermestidae are mainly found on the carcass during the late decay stage of decomposition and prefer feeding on the dry skin tissues of animal carcass (Amendt *et al.*, 2010; Hu *et al.*, 2020). Histeridae species are predators, and only feed on other immature insects that are found on the carcass (Byrd and Castner, 2009; Hu *et al.*, 2020). As such, these differences render them as potentially valuable in forensic entomology (Hu *et al.*, 2020).

According to Anderson (2000a), the pattern of carrion colonization by Dipteran and Coleopteran species mainly depends on habitat, seasons, bio-geoclimatic zone, microclimate and the state of decomposition (Amendt *et al.*, 2004; Joseph *et al.*, 2011) and it is predictable within these parameters. This predictable time and sequence of arrival of insect on the carcass, allows forensic entomologists to determine or predict the period of time the insect is on the carcass and consequently the time since death, also known as postmortem interval (PMI) in forensic investigations (Anderson, 2001). Furthermore, insect or arthropod species recovered from the carcass may be used to determine the cause and manner of death, the transfer of the body between different locations after death, presence of drugs or poisons in a decomposing body and the linking of suspects with the crime/death

scene in forensic investigations (Sukontason *et al.*, 2007; Vanin *et al.*, 2008; Joseph *et al.*, 2011; Mise *et al.*, 2013). And therefore, correct identification of these arthropod species is a crucial element in forensic investigation (Chen *et al.*, 2004).

Arthropods species identification is mainly achieved through use of morphological and/or molecular techniques (Rolo *et al.*, 2013). However, differentiation of some arthropods species especially at larval stage using morphological approaches is still challenging (Amendt *et al.*, 2004; Zehner *et al.*, 2004; Boehme *et al.*, 2012). But on the other hand, DNA analysis method can accurately identify different arthropod species at all developmental stages (Oliveira *et al.*, 2011; Kokdener, 2016). As a result, they have been widely used to identify arthropods species that are of importance in forensic investigations, consequently providing accurate PMI estimation (Park *et al.*, 2013). Hence, this study used molecular techniques to identify arthropod species that are of forensic importance.

Although many different animal models ranging from pigs (Wolf *et al.*, 2001; Kelly *et al.*, 2011; Abajue *et al.*, 2017; Odo and Iloba, 2020a), rabbits (deSouza *et al.*, 2008; Shi *et al.*, 2009; Mabika *et al.*, 2014; Odo and Iloba, 2020b), elephants (Coe, 1978) and rats (Mouna *et al.*, 1997; Carter *et al.*, 2008; keshavarzi *et al.*, 2019) have been used in forensic entomological studies worldwide, these results only represent what is happening in monogastrics rather than ruminants. Given the high rate of veterinary legal cases involving illegally killing or poaching of small domestic ruminant and small-medium wildlife ruminants, this study aimed at molecular identification and comparing seasonal arthropods community diversity and abundance between pig (monogastrics) and sheep (ruminants) carrion within the same locality during different stages of decomposition in KwaZulu-Natal Province of South Africa.

5.3 Materials and methods

5.3.1 *Study location*

Study site has been described on chapter 3 of the thesis.

5.3.2 *Study animals and sampling procedure*

Study animals and sampling procedures are described in chapter 3 and 4.

5.3.3 *Morphological identification*

Morphological identification of collected dipteran and coleopteran species has already been described in chapter 3 and 4.

5.3.4 *DNA extraction and amplification*

Specimens were grouped into different taxa based on the morphological identification. From each taxon two to four representative specimens were selected for confirmation of species using molecular techniques. Two or three legs, depending on size of each dipteran fly specimens, were carefully dissected out from the whole specimen and DNA was extracted using DNeasy Tissue Mini-Prep Kit (Zymo Research Cooperation) according to the manufacturer's instructions. DNA were amplified using the universal mitochondrial primers LCO1490 (5'-ggtcaacaaatcataaagatattgg-3') and HCO2198 (5'taaacttcagggtgaccaaaaaatca-3') (Folmer *et al.*, 1994). PCR reactions were performed in a final volume of 25 µl containing the following PCR mixture: 2 µl of each primer (10 µM), 12.5 µl PCR Master Mix (2X) (Thermo Scientific), 5.5 µl sterile water and 4-5 µl of genomic DNA extract. Amplification was performed under thermal conditions: 95°C for 7 min, followed by 35 cycles of (60s at 95°C, 60s at 55°C, 60s at 72°C) and final extension for 7 minutes at 72°C.

For the Coleopteran species, a separate PCR was performed to amplify the mitochondrial primers (F: 5'-CAGATCGAAATTTAAATACTTC-3' and R: 5'-GTATCAACATCTATTCCTAC-3') (Zhuang *et al.*, 2011). PCR amplification was performed in a 25 µL reaction volume, each containing 5 µL of genomic DNA, 12.5 µL PCR Master Mix (2X) (Thermo Scientific), 2 µL (10 µM) of each primer and 3.5 µL sterile water under the thermal cycling conditions: 94 °C for 3 min, followed by 35 cycles of 94 °C for 30s, 30s of annealing temperature at 50 °C, 72 °C for 30s and lastly final extension at 72 °C for 5 min. Fragments were separated by electrophoresis in 1 % agarose gel stained with ethidium bromide, at 80V for one hour and aamplifications were detected at 710 bp and 272 bp for the insects and beetles respectively. Amplicons were sent to Inqaba biotech industries (Pty) Ltd. (Pretoria, South Africa) for Sanger sequencing.

5.3.5 *Sequence and phylogenetic analysis*

Sequences were assembled and manually edited using Bio Edit (Hall, 1999). Basic local alignment search tool (BLAST) of the NCBI (National Centre of biotechnology) was used to identify the closest matches available on the database. Sequences were aligned with the homologous sequences obtained from the GenBank databased using the MUSCLE option of MEGA 7 (Kumar *et al.*, 2016). The sequences were trimmed to a common nucleotide length of 450 for the flies and 240 for beetles. The jModeltest (Posada, 2008) was used to determine the best model of nucleotide substitution to use in neighbor-joining, maximum likelihood and Bayesian inference analyses. The GTR+G model was selected for the datasets. To determine the evolutionary relationships between the arthropods, the Neighbor-joining (NJ) and maximum likelihood (ML) phylogeny trees were generated using PAUP*4.0 (Swofford, 1998), and the nodal support values were estimated using 1000 bootstrap pseudo-replicated. Bayesian

analyses were run using four Markov chains on MrBayes 3.1.2 (Huelsenbeck and Ronquist, 2001), sampling every 100 generations for 5 million generations or until the standard deviation of the split frequencies was less than 0.01. The first 500,000 trees were discarded as burnin. The Bayesian inference phylograms were generated with 50% majority-rule consensus and the nodal support indicated as posterior probabilities.

5.4 Results

5.4.1 Morphological identification of arthropod species

Morphological identification based on the identification key described by (Tourle *et al.,* 2009; Iqbal *et al.*, 2014; Lutz *et al.*, 2018) classified collected dipteran flies into five genera; *Chrysomya*, *Lucilia*, *Musca*, *Sarcophaga* and one unidentified species, and five Coleoptera families (Cleridae, Dermestidae, Silphidae, Scarabaeidae and Meloidae) (chapter 3 and 4).

5.4.2 Molecular identification of arthropod species

Sequence analysis of the arthropods based on the mitochondrial gene confirmed our previous morphological identification in chapters 3 and 4 with identification of the following blow fly species from the genera *Chrysomya* (*Ch. marginalis; Ch. putoria, Ch. albiceps, Ch. chloropyga*), *Lucilia* (*L. cuprina*), *Musca* (*M. domestica*) and further identified *Sarcophaga* and the unidentified genera up to species level (Figure 5.1). Sequence analysis of the arthropods based on the mitochondrial gene confirmed the classification and identification the blow fly species from the genera *Chrysomya* (*Ch. marginalis; Ch. putoria, Ch. albiceps, Ch. chloropyga*), *Lucilia* (*L. cuprina*), *Musca* (*M. domestica*) and further identified *Sarcophaga* and the unidentified genera up to species level. Blast search showed that *Chrysomya* isolate from this study showed homologies of 100 % with the GenBank *Ch. marginalis* (AB112862 – South Africa, KM434354 – Egypt), *Ch. putoria*, *Ch. albiceps* (JQ246661 and EU418540 - South Africa) and *Ch. chloropyga* (KF919011 – France, KM407601 – Egypt). These sequences of isolated were deposited on GenBank under the accession numbers MZ476261 – MZ476266, and MZ476272 – MZ476274. *Lucilia cuprina* isolate (MZ476269) showed a homology of 99 - 100 % (AB112852 – Australia, FR719165 – Kenya), with *M. domestica* (MZ476267) showing showing a homology of 99 - 100 % to Brazilian (AY526196) and South Korean (JX861433) respectively. The *Sarcophaga* isolates (MZ476270 – MZ476271) identified as *S. calicifera* with a 99 % homology to a GenBank isolate from Burundi (KU746555).

Phylogeny analysis yielded a phylogenetic tree showing paraphyletic relationship between the dipteran species (Figure 5.1). *Chrysomya* species formed their own well-supported clade by neighbor-joining and Bayesian inference showing the evolutionary relationship between the four *Chrysomya*

species, and further supporting the identification of 9 isolates from this study as *Ch. marginalis*, *Ch. putoria*, *Ch. albiceps* and *Ch. chloropyga*. The remaining four genera namely *Lucilia*, *Musca*, *Sarcophaga* and *Atherigona* showed strongly supported clades showing a strong and close relationship within genera and species, and further authenticating the identification of the different species collected from the pig and sheep carcasses. However, these genera showed a weak-moderately supported paraphyletic relationship to one another. The unidentified genera (P-B2) formed a strong supported clade confirming the identification of this isolate as *A. soccata*, and this species was unique to the pig carcass during the bloated stage.

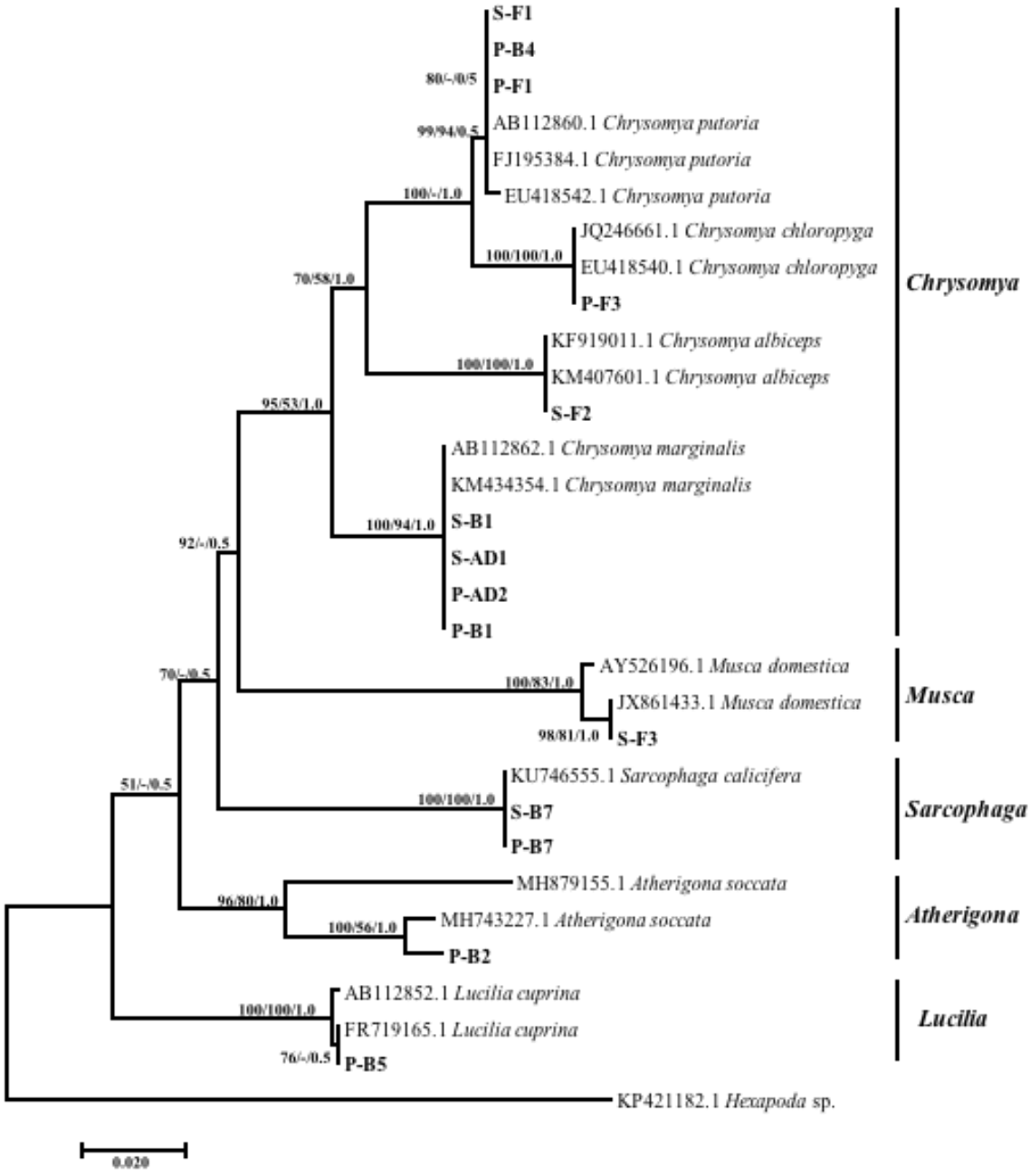

Figure 5.1: Tree based on the mitochondrial Cytochrome oxidase subunit 1 region illustrating relationships between experimental Diptera isolates collected from sheep and pig carcasses, and the close matches downloaded from the NCBI GenBank and outgroups. The sample ID alphabets represents: P- pig, S – sheep, F – fresh, B- bloated, AD- advanced stages. Support values indicated at the nodes are, in order: neighbor joining bootstrap value, maximum likelihood bootstrap value, Bayesian inference posterior probability.

Molecular analysis of the mitochondrial region of the beetles yielded sequences with a shorter nucleotide length. BLAST and phylogenetic analyses showed that 16 isolates from this study identified as *Dermestes maculatus* (De Geer) with a percentage homology ranging from 98 - 99 %. These isolates formed a monophyletic sister clade to *Onthophagus* species (Fig. 2), and their sequences were deposited into GenBank under the accession numbers MZ485937 – MZ485952. Within the *Onthophagus* clade, two weakly supported monophyletic clades were formed supporting four isolates from this study as either *Onthophagus crassicollis* or *O. vacca*. Specimens identified as *O. crassicollis* were collected from both pig and sheep carcasses and showed homology of 98.68 % with an isolate from the United Kingdom (KU739447) by BLAST analysis. Their sequences were deposited into GenBank under the accession numbers MZ485955 - MZ485956. *Onthophagus vacca* specimens from this study (MZ485953 - MZ485954) showed a lower homology of 96.18 % to the Australian isolate (KC294241), and this species was unique to sheep carcass. Lastly, isolate P-B21, which identified as *H. lunatus*, showed a homology of 98.48 % to the Italian isolate (MN849997). This isolate (MZ485957) formed a well-supported clade by neighbor-joining method with the Italian isolate and a strong supported clade with other *Hycleus* species (Figure 5.2).

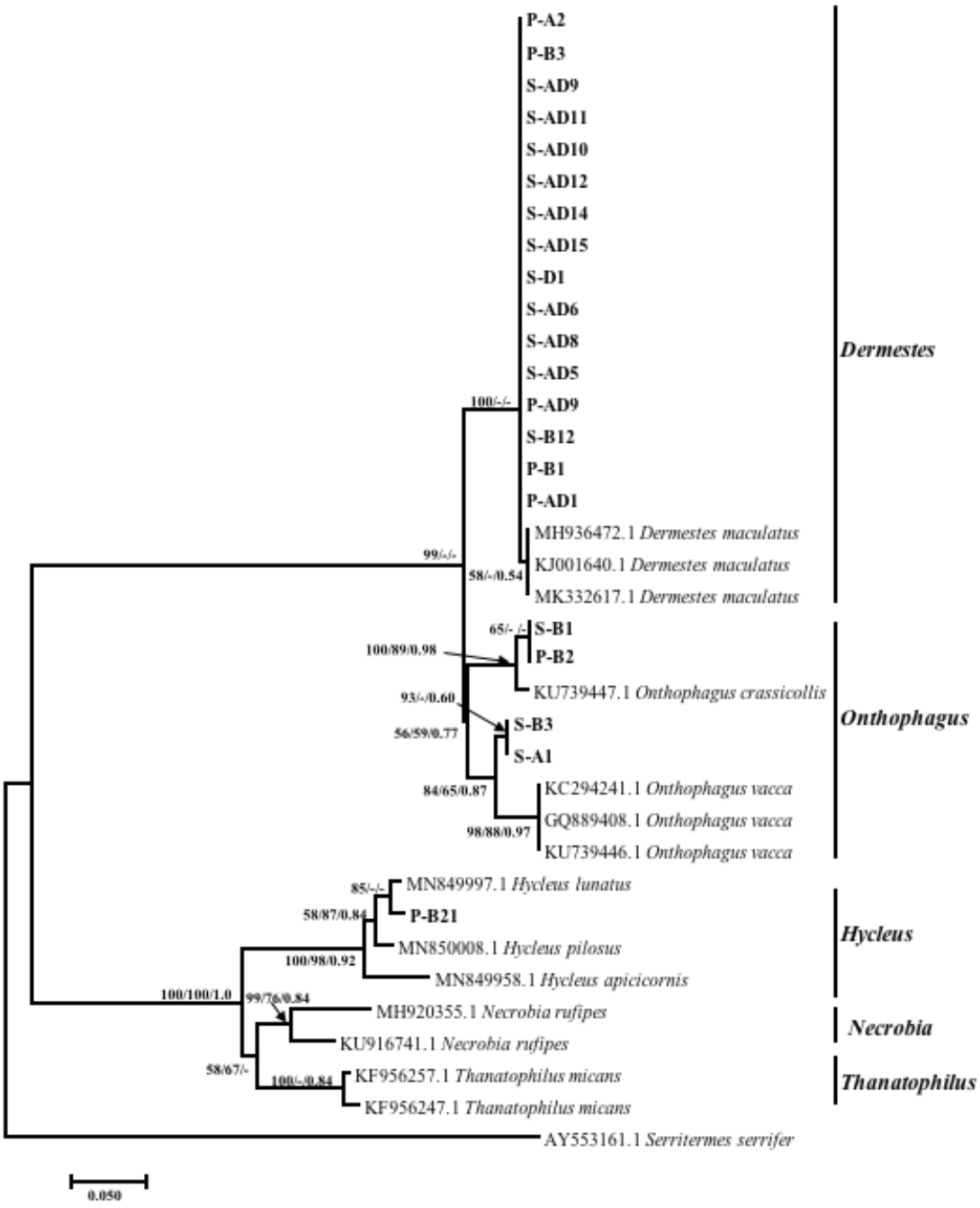

Figure 5.2: Tree based on the mitochondrial region illustrating relationships between experimental Coleoptera isolates collected from sheep and pig carcasses, and the close matches downloaded from the NCBI GenBank and outgroups. The sample ID alphabets represents: P- pig, S – sheep, B- bloated, A- active, AD- advanced stages. Support values indicated at the nodes are, in order: neighbor joining bootstrap value, maximum likelihood bootstrap value, Bayesian inference posterior probability.

5.4.3 Arthropod diversity at different stages of a decomposing pig and sheep carcass during a cold season.

Both sheep and pig carcasses were colonized by the same dipteran species during the first two stages (fresh and bloated) of decomposition; namely *Chrysomya marginalis*, *Ch. putoria*, *Ch. albiceps*, *Ch. chloropyga*, *Lucilia cuprina*, *Musca domestica* and *Sarcophaga calcifera*, plus *Atherigona soccata* flies which were only found on the pig carcass during the bloated stage (Table 5.1). No beetles were collected on the pig carcass during these two stages of decomposition. However, there was a first collection of *Dermestes maculatus* and *O. vacca* on the sheep carcass during the bloated stage.

As decomposition progressed to the active stage, there was an observed decrease in the number of fly taxa in pig carcass, which coincided with the arrival of more beetle species. This decrease in fly taxa included disappearance of *Ch. putoria* and *A. soccata* from the pig carcass from this stage, with the appearance of *Necrobia rufipes* and *D. maculatus*. The number of fly taxa on the sheep carcass remained the same, but the beetle taxa collected increased with the addition of *Onthophagus crassicollis* and *N. rufipes*. The following species were confirmed and observed succession pattern during the advanced stage: the presence of *Thanatophilus micans* on both sheep and pig carcass, clearing of *L. cuprina* on pig carcass, and *Ch. chloropyga* on both sheep and pig carcasses. *Musca domestica* and *S. calcifera* were the only Diptera species which remained on both carcasses to the dry stage. *Necrobia rufipes, D. maculatus* and *O. crassicollis* were also the only Coleoptera species found in the carcasses during the last stage of decomposition.

Table 5.1: Succession pattern of adult insect species during different stages of pig and sheep carcass decomposition during cold season located in Pietermaritzburg, KwaZulu-Natal province of KwaZulu-Natal province of South Africa.

Ecological category	**Order**	**Family**	**Genus**	**Presence/absence of insects at different stages of pig and sheep carcass decomposition**									
				Fresh (0-1 days)		**Bloated** (2-6 days)		**Active** (7-12 days)		**Advanced** (13-40 days)		**Dry** (41-50 days)	
				Pig	Sheep	Pig	Sheep	Pig	Sheep	Pig	Sheep	Pig	Sheep
Necrophagous	Diptera	Calliphoridae	*Chrysomya marginalis*	+	+	+	+	+	+	+	+	-	-
Necrophagous	Diptera	Calliphoridae	*Chrysomya putoria*	+	+	+	+	-	+	-	+	-	-
Necrophagous	Diptera	Calliphoridae	*Chrysomya albiceps*	+	+	+	+	+	+	+	+	-	-
Necrophagous	Diptera	Calliphoridae	*Chrysomya chloropyga*	+	+	+	+	+	+	-	-	-	-
Necrophagous	Diptera	Calliphoridae	*Lucilia cuprina*	+	+	+	+	+	+	-	+	-	-
Necrophagous	Diptera	Muscidae	*Musca domestica*	+	+	+	+	+	+	+	+	+	+
Necrophagous	Diptera	Muscidae	*Atherigona soccata*	-	-	+	-	-	-	-	-	-	-
Predator	Diptera	Sarcophagidae	*Sarcophaga calicifera*	+	+	+	+	+	+	+	+	+	+
Predator	Coleoptera	Cleridae	*Necrobia rufipes*	-	-	-	-	+	+	+	+	+	+
Necrophagous	Coleoptera	Dermestidae	*Dermestes maculatus*	-	-	-	+	+	+	+	+	+	+
Predator	Coleoptera	Silphidae	*Thanatophilus micans*	-	-	-	-	-	-	+	+	-	-
Coprophagous	Coleoptera	Scarabaeidae	*Onthophagus. vacca*	-	-	-	+	-	+	-	-	-	-
Coprophagous	Coleoptera	Scarabaeidae	*Onthophagus crassicollis*	-	-	-	-	-	+	+	+	+	+

5.4.4 Arthropod diversity at different stages of a decomposing pig and sheep carcass during a warm season.

All Diptera species were found on both sheep and pig carcasses during the fresh and bloated stages (Table 5.2). There were no beetles colonizing the pig carcass during the fresh stage, however *D. maculatus*, *T. micans* and *O. crassicollis* beetles were found on the sheep carcass during this stage. Colonization of beetles on the pig carcass commenced during the bloated stage where *D. maculatus*, *T. micans*, *O. crassicollis*, *Hycleus lunatus* were observed. The same beetle species were confirmed on the sheep carcass on bloated stage, with an additional *O*. *vacca*.

As the colonization advances to the active stage, the species of flies found in and around the pig carcass started decreasing with *Ch. putoria* as the first species to leave the carcass, followed by *L. cuprina* during the advanced stage. *Lucilia cuprina* was the first fly species to leave the sheep carcass during the advanced stage. The introduction of *N. rufipes* on the sheep and pig carcasses during the active stage coincided with the disappearance of *H. lunatus* from both carcasses. Therefore, *D. maculatus*, *T. micans*, *O. crassicollis* and *N. rufipes* beetles persisted on both sheep and pig carcasses up to the dry stage, whilst *O. vacca*. persisted only up to the advanced stage. During the dry stage, both sheep and pig carcasses remained with two fly species each, with *M. domestica* the common species on both carcasses. *Chrysomya marginalis* was found on the sheep carcass whilst *S. calcifera* was found on the pig carcass.

Table 5.2: Succession pattern of different insects attracted to different stages of a decomposing pig and sheep carcass during a warm season located in Pietermaritzburg, KwaZulu-Natal province of South Africa.

Ecological category	**Order**	**Family**	**Genus**	**Presence/absence of insects at different stages of pig and sheep carcass decomposition**									
				Fresh (0-1 days)		**Bloated** (2-6 days)		**Active** (7-12 days)		**Advanced** (13-40 days)		**Dry** (41-50 days)	
				Pig	Sheep	Pig	Sheep	Pig	Sheep	Pig	Sheep	Pig	Sheep
Necrophagous	Diptera	Calliphoridae	*Chrysomya marginalis*	+	+	+	+	+	+	+	+	-	+
Necrophagous	Diptera	Calliphoridae	*Chrysomya putoria*	+	+	+	+	-	+	-	+	-	-
Necrophagous	Diptera	Calliphoridae	*Chrysomya albiceps*	+	+	+	+	+	+	+	+	-	-
Necrophagous	Diptera	Calliphoridae	*Chrysomya chloropyga*	+	+	+	+	+	+	+	+	-	-
Necrophagous	Diptera	Calliphoridae	*Lucilia cuprina*	+	+	+	+	+	+	-	-	-	-
Necrophagous	Diptera	Muscidae	*Musca domestica*	+	+	+	+	+	+	+	+	+	+
Necrophagous	Diptera	Sarcophagidae	*Sarcophaga calcifera*	+	+	+	+	+	+	+	+	+	-
Predator	Coleoptera	Cleridae	*Necrobia rufipes*	-	-	-	-	+	+	+	+	+	+
Necrophagous	Coleoptera	Dermestidae	*Dermestes maculatus*	-	+	+	+	+	+	+	+	+	+
Predator	Coleoptera	Silphidae	*Thanatophilus micans*	-	+	+	+	+	+	+	+	+	+
Coprophagous	Coleoptera	Scarabaeidae	*Onthophagus. vacca*	-	-	-	+	-	+	-	+	-	-
Coprophagous	Coleoptera	Scarabaeidae	*Onthophagus crassicollis*	-	+	+	+	+	+	+	+	+	+
Incidental	Coleoptera	Meloidae	*Hycleus sp*	-	-	+	+	-	-	-	-	-	-

5.4.5 Arthropod taxa richness from pig and sheep carcass during cold and warm seasons

The overall number of arthropods taxa collected from the pig and sheep carcasses were equal (n = 12) during the cold season (Table 5.1). Whilst, more (n= 13) arthropod taxa were collected from the sheep carcass during the warm season (Table 5.2). The highest number of taxa were found during the bloated stage of the pig carcass (n = 11) and both bloated and active decomposition stage of sheep carcass (n = 12) in warm season (Table 5.2). The overall number of Diptera species taxa found on the pig carcass during the cold season was eight (8) whilst only seven (7) taxa were found on the sheep carcass (Table 5.1). The difference in Diptera species taxa found between the two animal models was caused by the presence of *Atherigona soccata* species, which was unique to the bloated stage of pig carcass during cold season only. Although the pig carcass generally attracted more dipteran taxa, reduction in the number of taxa on the carcass started as early as the advanced stage whilst most species left the sheep carcass mainly during the dry stage. The sheep carcass presented five coleopteran taxa, which was more than the four found on the pig carcass during the cold season. The difference is attributed by the presence of *O*. *vacca* which was unique to the sheep carcass during the active and advanced stages of decomposition.

Both carcasses attracted the same amount of dipteran taxa (n = 7) during the warm season (Table 5.2). As with the cold season, the pig carcass was the first animal model which presented an early reduction of dipteran taxa which started as early as the active stage with the disappearance of *Ch. putoria*. Seven coleopteran taxa were found on the sheep carcass whilst pig presented only six taxa. The difference was contributed by the presence of *Onthophagus* sp. which was also unique to the sheep carcass during the warm season as well. The results also showed the presence of *H. lunatus*, which was unique to the bloated stage of both sheep and pig carcasses only during the warm season.

5.4.6 Arthropod abundance from pig and sheep carcass during cold and warm seasons

The pig carcass attracted more dipteran species (i.e. more flies were collected on and around the pig carcass) during the warm (n = 1519) and cold (n = 779) seasons as compared to sheep carcass during the warm (n = 511) and cold (n = 229) season (Table 5.3). Coleoptera species were more abundant on the sheep carcass during the warm (n = 391) and cold (n = 135) seasons as compared to the pig carcass in both warm (n = 261) and cold (n = 114) seasons (Table 5.3). Generally, more flies and beetles were collected on both sheep and pig carcasses during the warm season as compared to the cold season (n =1272) (Table 5.3).

Chrysomya species and *M. domestica* were dominant on the pig carcass on both seasons, contributing more than 90% of the total number of flies collected on and around the pig carcass during

the warm and cold season respectively. The sheep carcass was mainly dominated by *Ch. marginalis*, *Ch. albiceps* and *M. domestica* which contributed more than half of the total number of flies collected on and around the carcass. *Dermestes maculatus* and *N. rufipes* were the most dominant beetle species on both pig and sheep carcasses during the cold season. These two species contributed about 89% and 77% of the total number of flies collected on the sheep and pig carcasses respectively. The same species were dominant on the pig carcass during the warm season, and these two beetle species contributed 65.5% of the total beetle species collected. However, the sheep carcass was dominated more by *D. maculatus*, *N. rufipes*, *T. micans* and *O. vacca* which was unique to the sheep carcass. These species were dominant on the sheep carcass, making up 79 % of the beetles collected on the sheep carcass during the warm season.

Table 5.3: Summary of seasonal abundance of carrion-feeding insect species collected from pig and sheep carcasses located in Pietermaritzburg, KwaZulu-Natal province of South Africa during dry and warm season

Order	**Family**	**Species**	**Cold season**		**Warm season**	
			Pig	**Sheep**	**Pig**	**Sheep**
Diptera	Calliphoridae	*Chrysomya marginalis*	210	47	356	103
Diptera	Calliphoridae	*Chrysomya putoria*	87	23	202	44
Diptera	Calliphoridae	*Chrysomya albiceps*	150	50	303	110
Diptera	Calliphoridae	*Chrysomya chloropyga*	91	15	276	37
Diptera	Calliphoridae	*Lucilia cuprina*	38	17	60	46
Diptera	Muscidae	*Musca domestica*	191	71	298	110
Diptera	Muscidae	*Atherigona soccata*	2	0	0	0
Diptera	Sarcophagidae	*Sarcophaga calcifera*	10	21	24	61
Total Diptera			**779**	**244**	**1519**	**511**
Coleoptera	Cleridae	*Necrobia rufipes*	37	36	59	53
Coleoptera	Dermestidae	*Dermestes maculatus*	66	68	112	146
Coleoptera	Silphidae	*Thanatophilus micans*	2	4	44	72
Coleoptera	Scarabaeidae	*Onthophagus. vacca*	0	5	0	22
Coleoptera	Scarabaeidae	*Onthophagus crassicollis*	9	22	42	92
Coleoptera	Meloidae	*Hycleus lunatus*	0	0	4	6
Total Coleoptera			**114**	**135**	**261**	**391**
Total (Diptera and Coleoptera)			**893**	**379**	**1780**	**902**

5.5 Discussion

Morphological identification of flies, supported by molecular analysis based on the mitochondrial gene identified *Ch. marginalis; Ch. putoria, Ch. albiceps, Ch. chloropyga*, *L. cuprina*, *M. domestica*, *S. calcifera* and *A. soccata*. Following several unsuccessful attempts with various mitochondrial and nuclear markers, a successful amplification of the beetles' species was obtained using primers as described by Zhuang *et al.* (2011). However, molecular analysis showed low resolution of the beetle specimens due to the short nucleotide sequence size obtained and failure to obtain usable sequences for specimens identified morphologically as *T. micans* and *N. rufipes* (Byrd and Castner, 2001; Kolver, 2009; BugGuide, 2020).

Previous studies showed that arthropod species composition varies per carcass type/animal model (Watson and Calton, 2003; Azwandi *et al.*, 2013). Watson and Calton (2003) observed a difference in arthropod species composition between black bear, white-tailed deer, alligator and swine. Similar observations were made by Azwandi *et al.* (2013) who reported differences in number of taxa obtained in rat (n = 47), rabbit (n = 55) and monkey (n = 54). Combined morphological and molecular techniques in this study also showed a difference in arthropod taxa observed during the warm season whereby the sheep carcass attracted more arthropod taxa (n = 13) compared to the pig carcass (n = 12). This difference in the number of arthropod taxa from these two animal models was observed to be attributed to the presence of beetle species *O. vacca* which was unique to the sheep carcass.

In contrast, there were no observed variation in the number of in insect taxa (n = 12) collected and identified on pig and sheep carcasses during the cold season. Although the number of insect species collected from these carcasses were similar, 11 (*Ch. marginalis, Ch. putoria, Ch. albiceps, Ch. chloropyga, L. cuprina, M. domestica, S. calcifera, D. maculatus*, *T. micans*, *O. crassicollis* and *N. rufipes*) of the 12 taxa collected were common to both pig and sheep carcasses, with two insect species observed to vary per animal type. This includes a Muscidae species, *Atherigona soccata* which was only collected on the pig carcass during the cold season, and a Scarabaeidae species *O. vacca* which was only collected on the sheep carcass on both cold and warm seasons. Similar observation was made by Calton and Watson (2003) although on different animal species, who documented 11 arthropod families which were present on the black bear, white-tailed deer, and swine carcasses, however, absent from the alligator carcass. Considering that both sheep and pig carcasses shared the same ecological niche, it can be concluded that these two arthropod taxa are unique to their respective animal models because every animal model has its own physical characteristics that are not only limited to the difference in size but also affected by factors such as the diet of the animal and the thickness of their furs among other factors (Azwandi *et al.*, 2013).

Studies have showed that insects get attracted to the carcass immediately after death (Nuorteva, 1977; Erzinclioglu, 1983; Smith, 1986; Anderson and VanLaerhoven, 1996; Dillon, 1997). According to Byrd and Castner (2001), Calliphorid blow flies are often the primary colonizers and are attracted to a carcass over a great distance by odor, and in South Africa, this is marked by flies of the genus *Chrysomya* (Braack, 1981). The same observations were made in this study where both sheep and pig carcasses regardless of the season were assembled by blow flies from the genus' *Chrysomya* and *Lucilia* along with the flesh flies (*Sarcophaga*) and house fly (*Musca*) within the first stage of decomposition. This observation is in line with Byrd and Castner (2001) report that species from the families Calliphoridae (*Chrysomya* and *Lucilia*), Muscidae (Musca) and Sarcophagidae (*Sarcophaga*) are most encountered species on carrions and serve as most useful evidence in forensic investigations. However, their species often vary depending on factors such as geographic region or biogeoclimatic zones (Byrd and Castner, 2001).

There was an observed difference in the arrival time of the Coleoptera species communities between animal models and seasons. Assemblage of beetles were observed to begin earlier on the sheep carcass as compared to the pig carcass, and during the warm season as compared to the cold season. During the cold season, the first coleopteran species were observed and collected on the sheep carcass during the bloated stage and the active stage on the pig carcass. In the cold season, the highest number of coleopteran taxa were collected during the active (n = 4) and advanced (n = 4) stages for sheep carcass and during the advanced stage (n = 4) on the pig carcass. During the warm season, beetles were collected from the sheep carcass as early as the fresh stages, whilst on the pig carcass observation and collection of beetles only began during the bloated stage. The highest number of beetle taxa were on both sheep (n = 5) and pig (n = 4) carcasses observed during the bloated, active decay and advanced decay stages.

According to Byrd and Castner (2001), seasonality and/or relative abundance of certain insect species and their ability of colonize the carcass remains during different decomposition stages in different seasons provide valuable information such as determining the season of death. In this study, coleopteran species *H. lunatus* was collected on the sheep and pig carcasses during the warm season only. Furthermore, the dipteran species *A. soccata* was collected on the pig carcass during the cold season. These species may potentially serve as valuable indicators for determining season of death, and the animal species with regards to *A. soccata*. However, the lower number of individuals collected may also indicate the high probability of accidental colonization.

In conclusion, season displayed an influence on collected arthropod taxa richness and abundance. The highest number of taxa (n = 13) were recorded from the sheep carcass during the warm season. The overall number of arthropods were collected during the warm season as compared

to cold season. The observed difference in arthropod abundance between seasons in this study might have been influenced by temperature. More dipterans were collected from the pig carcass during both warm and cold seasons, as compared to the sheep carcass. In contrast, sheep carcasses attracted more Coleopterans during both warm and cold as compared to the pig carcasses in warm and cold seasons. According to Azwandi *et al.* (2013), the difference in assemblages of arthropod communities between animal models may be due to the amount food provided by the animal type, for instance, the pig carcasses (average weight of 100 kg) in this study which was larger than the sheep carcasses (average weight of 80 kg) provided more food in terms of body tissue and fluids to the necrophagous species.

5.6 Acknowledgements

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CHAPTER 6: General discussion, conclusion, and recommendations for future research

6.1 General discussion

This study aimed at determining the arthropod species that are attracted to carrion and during different stages of decomposition of sheep and pig carcasses during the warm and cold season in KwaZulu-Natal province of South Africa and further determine arthropod species that are potential indicators of PMI and relocation of a carcass in forensic investigations.

Previous experimental studies with pigs and other laboratory animals have shown that Diptera species from the families Calliphoridae, Sarcophagidae and Muscidae are the primary colonizers of carrion (Kokdener, 2016; with Byrd and Castner, 2001). In South Africa, *Lucilia* spp, *Ch. marginalis*, *Ch. albiceps*, *Ch. chlorophyga* have been reported to be the the first insect species to appear on the carcass (Kolver, 2009). In this study, during the early stages of decomposition (fresh and bloated stages) for both pig and sheep carcasses, *Ch. marginalis, Ch. albiceps, Ch. putoria, Ch. chloropyga and L. cuprina* also with *M. domestica* (house flies) and *S. calcifera* (flesh flies) were recorded. These species have been reported to be of forensic value in medico-legal investigations (Byrd and Castner, 2001; Kolver, 2009).

Beetles are secondary decomposers and some species have been reported to appear on carcasses at later stages of decomposition (Mabika *et al.*, 2014; Kokdener, 2016). This makes beetles, suitable forensic indicators of the later stages of decomposition. However, in this study, beetles were attracted during the fresh stage of the sheep and bloated stage of the pig carcass during the warm season. The presence of beetle species at early stages may be due to seasonal peaks, as well as to feed on dipteran insects found on the cracsses. Furthermore, there was an observed difference in the arrival time and pattern of beetles between two animal models during the warm season. *Dermestes maculatus*, *T. micans* and *O. crassicollis* beetles arrived on the sheep carcass during the fresh stage. However, there were no beetle species associated with the pig carcass during the fresh stage. This may also indicate that the sheep carcass may hace specifically attracted beetle species earlier than the pig carcass and further studies ar e needed to confirm this phenomenon.

As the decomposition progressed to the active stage of decomposition flies (necrophagous) began to decrease from both pig and sheep carcasses. However, the persistence of some insect species on the pig and sheep differed. For instance, *Ch. putoria* was the first species that disappeared on the pig carcass during the active stage and followed by *L. cuprina* during the advanced stage in the warm season. Whereas, on the sheep carcass *Lucilia cuprina* was the first fly species that disappeared on the carcass during the advanced stage in the warm season. In both pig and sheep carcasses *N. rufipes* first appeared on the carcasses during the active stage and persisted up to the dry stage during

the warm season. Additionally, species of *D. maculatus*, *T. micans*, and *O. crassicollis* were found persisting on the sheep and pig carcasses up to the dry stage, whereas *Onthophagus* sp. persisted on the carcasses only up to the advanced stage of the sheep carcass and it was only unique to the sheep carcass. In the last stage of decomposition both carcasses attracted only two dipteran species, i.e. *M. domestica a*nd *Ch. Marginalis* for the sheep carcass, whilst pig carcass attracted *M. domestica* and *S. calcifera*.

In the cold season, both the sheep and pig carcasses attracted same dipteran species during the fresh and bloated stage of decomposition. These species included *Ch. marginalis*, *Ch. putoria*, *Ch. albiceps*, *Ch. chloropyga*, *L. cuprina*, *M. domestica* and *S. calcifera,* with the exception of *A. soccata* species which only occurred during the bloated stage of the pig carcass. This observation indicates that regardless of the animal model, blow flies, mainly *Chrysomya* species and *Lucilia* species along with *Sarcophaga species* and house fly are the first insects to arrive on the carcass during the early stages of fresh and bloated decomposition and as such they may be recognized as potential indicators of the early stages of decomposition in forensic investigations in our locality of study. Additionally, no coleopteran species were associated with the pig carcass during both fresh and bloated, whereas *D. maculatus* and *Onthophagus sp* were first observed and collected during the bloated stage of the sheep carcass.

As the active stage commenced following the bloated stage, the number of necrophagous flies decreased and *Ch. putoria* and *A. soccata* disappeared from the pig carcass and this coincided with the arrival of coleopteran species (*N. rufipes* and *D. maculatus*) species during this stage. These species are known to feed on necrophagous specie, skin and hair of the carcass. On the other hand, the necrophagous species and predators found during the bloated stage were still present on the sheep carcass, but there were two additional beetles (*O. crassicollis* and *N. rufipes*) collected from the sheep carcass during the cold season. From these observations the persistence of necrophagous species on the pig and sheep carcasses was not the same during this stage, however, the arrival time *of N. rufipes* between two animal models was similar in the cold season. During the advanced stage of decomposition *L. cuprina* cleared only from the pig carcass, and *Ch. chloropyga* cleared from both both sheep and pig carcasses in the cold season. *Necrobia rufipes, D. maculatus, T. micans,* and *O. crassicollis* persisted on both pig and sheep carcasses during the cold season. In the last stage (dry) of decomposition *M. domestica* and *S. calcifera* were the only fly species which remained on the pig and sheep carcass and *N. rufipes, D. maculatus* and *O. crassicollis* were the only beetles found on both pig and sheep carcasses during the cold season.

Results showed that both Coleoptera and Diptera species were more active and dominant during the warm season as compared to the dry season. This observation supports the previous

studies who reported that seasonal changes have an influence on the abundance of arthropod species, with most arthropod species being more abundant during warm seasons than the cold seasons (Kelly *et al.*, 2011; Parry *et al.*, 2016). As a result, it can be concluded that season had a strong influence on collected insects in this study in terms of species abundance in both animal models. Furthermore, Insect species vary with different seasons and the knowledge on the presence or absence of certain arthropods species during different seasons can provide useful information in forensic investigations in relation to determination of the season of death. In this study, the occurrence of *H. lunatus* only on the sheep and pig carcasses during the warm season and the occurrence of *A. soccata* on the pig carcass in the cold season only. This indicates that these species are only on specific to a season, and they may be use as potential indicators for determining season of death, and the animal species since *A. soccata* was found only on the pig carcass. However, these species were observed in lower numbers and the assumption was they might have also colonized the carcass accidentally.

In overall, families Calliphoridae and Muscidae were the dominant dipteran species observed and collected during both cold and warm season in both animal models. The species *Ch. marginalis* and *Ch. albiceps and M. domestica* were the most common and dominant Calliphoridae species during both the cold and warm season on both carcasses (sheep and pig). Coleoptera species Dermestidae (*D. maculatus*) and Silphidae (*T. micans*) were the most dominant on the sheep carcasses during the warms season. However, cold season was mainly dominated *D. maculatus*. On the pig carcass, Coleoptera species, *N. rufipes* and *D. maculatus* were dominant in both warm and cold seasons. additionally, on both animal types these Coleoptera species were more in numbers during the active up to the dry stage of decomposition. Furthermore, results showed that more flies were attracted to the pig carcass in both warm and cold seasons, as compared to the sheep carcass which in contrast, attracted more beetles throughout warm and cold season as compared to the pig carcasses during both warm and cold seasons. The observed difference in the attraction of these insects to different animal models may have been attributed by the amount food provided by each animal type. For example, the pig carcasses were larger than the sheep carcasses and it provided more food to especially to the flies as compared to the sheep.

Insect species vary with geographic regions and some display strong preferences towards certain regions. Thus, knowledge on the distribution of insect species and their preferred locations are useful in the estimation of the carcass relocation between different geographic areas (Sumadon, 2002; Kokdener, 2016). The observed insect succession and decomposition pattern on both pig and sheep carcasses in this study has a potential to provide useful information in relation to the estimation of PMI and relocation of carcasses in cases of wildlife poaching and animal neglect and cruelty amongst others in this country.

6.2 General conclusion and recommendations for future research

To date, the field of forensic entomology has grown tremendously, and is now recognized as a useful tool, providing evidence in investigation cases of suicide, homicide, among others. However, there is a paucity of the use of forensic entomology in the medico-veterinary field/cases involving domestic animals and wildlife in South Africa and other sub-Saharan African countries. Our findings are in support of the previous studies (Byrd and Castner, 2001; Martinez *et al*., 2007; Mabika *et al*., 2014; Kokdener, 2016) that dipteran families of Calliphoridae (blowflies), Sarcophagidae (flesh flies) and Muscidae (house flies) are the early decomposers to arrive on a carcass and are useful indicators of the early stages of decomposition in forensic investigations regardless of the season and/or animal type. The observed seasonal differences on species abundance, highlighted that the abundance of arthropods was mainly influenced by different the climate conditions. Results from this study has generated preliminary data in the creation of a database of arthropods associated with both pig and sheep carrion during different seasons. Consequently, this database can be improved by adding more information on the experiemnetal animal species for use to solve medico-veterinary legal cases related to the estimation of PMI and relocation of the carcass. Furthermore, these results, will further raise awareness of the importance of research in forensic entomology in different geographical localities in South Africa and other sub-Saharan African countries and its application inmedico-veterinary legal cases.

Both pig and sheep carcasses were approriate as models in determining the succession pattern of insects and decomposition stages during the warm and cold seasons in KwaZulu-Natal Province of South Africa. However, there is need for future research using other experimental animal species such as cattle, goats, impala, deer, nyala amongst others as animal models to mimic both medium to large domestic and wildlife ruminant species and document dipteran and coleopteran species taking into account the different geographical regions in South Africa in order to assess their value in PMI estimation. This will build a database of dipteran and coleopteran species of forensic value which are endemic in these areas. Furthermore, few studies have investigated the nocturnal oviposition in southern Africa and therefore future studies on nocturnal oviposition are recommended as nocturnal oviposition may be useful in the determination of PMI.

6.3 References

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