The identification of Diptera of the grave and their succession patterns during winter and summer in central South Africa, with reference to forensic applications

Sylvia Shalomé van der Merwe

Submitted in fulfilment of the requirements in respect of the Master's degree qualification in Entomology in the Department of Zoology and Entomology in the Faculty of Natural and Agricultural Sciences at the University of the Free State

January 2016

Supervisor: Dr. Sonja L. Brink

Co-supervisor: Prof. Linda Basson

Written declarations

I, Sylvia Shalomé van der Merwe, declare that the Master's Degree research dissertation or interrelated, publishable manuscripts/published articles, or coursework Master's Degree mini-dissertation that I herewith submit for the Master's Degree qualification in Entomology at the University of the Free State is my independent work, and that I have not previously submitted it for a qualification at another institution of higher education.

I, Sylvia Shalomé van der Merwe, hereby declare that I am aware that the copyright is vested in the University of the Free State.

I, Sylvia Shalomé van der Merwe, hereby declare that all royalties as regards intellectual property that was developed during the course of and/or in connection with the study at the University of the Free State, will accrue to the University.

_____

Sylvia Shalomé van der Merwe

Acknowledgements

I would like to extend a special thank you to the following people:

To my Mother and Father, Christobel and Leonard vd Merwe, thank you for supporting my on-going long pursuit of knowledge.

To my love, Jason Botham, thank you for always supporting me and encouraging my journey in discovery.

To Doctor Henry Disney, a special thank you for lending your expertise to assist with identification of dipteran specimens.

To Doctor Marieke Gryzenhout, thank you for your concurrent collaborative work on fungal growth on below ground carcasses.

To the University of the Free State Animal Unit, thank you for treating all test animals with respect and carrying out euthanasia of test animals in an ethical manner.

> A special thank you to Mr. Seb Lamprecht and Mr Riaan van Zyl for your personal help with all ethics and animal related issues.

To the Department of Zoology and Entomology and its lecturers, thank you for your support and funding.

> Thank you to both of my study co-ordinators/supervisors for undertaking the study.
>
> A special thank you to Professor Schalk vdm Louw and Doctor Vaughn Swart for your motivation, support and advice during the study.

To the National Research Foundation, thank you for funding the research.

Thank you to everyone else that contributed to the study, no matter how large or small.

Abstract

Forensic entomology is a specialist branch of forensic sciences in which information about insects is used to draw certain conclusions when investigating medico-legal cases involving both humans and wildlife. The primary application of insects collected from a crime scene is to determine the Post-Mortem Interval (PMI). One of the principal cornerstones in the effective application of forensic entomology is the correct identification of species found at a crime scene. The estimation of PMI using succession and developmental data is dependent upon the specific species that were present on a cadaver. The aim of this study was to identify the members of the Diptera that are capable of colonising buried carrion within a Free State grassland area, as well as to compare below ground dipteran data with above ground dipteran succession patterns.

The field experiments, conducted in order to determine what species of Diptera are capable of reaching buried carrion during winter and summer months, as well as to determine the succession patterns of Diptera on buried remains, were conducted on the west fields of the University of the Free State campus, in central South Africa. The area is characterised by dry mild winters and warmer summers, with occasional rainfall periods. A total of seven pigs (*Sus scrofa* Linnaeus 1758) were used during each season, which consisted of one above ground control carcass and six separate below ground carcasses buried at 60 cm. Below ground carcasses were left for predetermined periods of time and were excavated on separate occasions over each 120 day trial period to monitor dipteran occurrence, colonisation and dipteran interactions.

Members of Diptera were found to colonise carcasses at 60 cm during both winter and summer trials. Higher dipteran species diversity was noted on the buried winter carcasses in comparison to the buried summer carcasses. Four species of Diptera, *Megaselia scalaris* (Loew 1866) (Phoridae), *Conicera tibialis* Schmitz 1925 (Phoridae), *Muscina stabulans* (Fallén 1817) (Muscidae) and *Leptocera* Olivier 1813 sp. (Sphaeroceridae) were seen to occur on winter carcasses, with first colonisation taking place from day 21 of the winter trial. Three species of the Diptera, *M. scalaris*, *Leptocera* sp., and *Sarcophaga* Meigen 1826, were seen to colonise buried summer carcasses, with first

colonisation taking place from day 21 of the summer trial. Dipteran faunal composition on buried carcasses was noted to be different to that of Diptera on above ground carcasses during both the winter and the summer trials. Statistical analysis showed that there was a significant difference between above ground and below ground dipteran faunal similarity.

Preservation experiments on immature Diptera of the grave for identification purposes showed that the standard preservation mediums used for insects of forensic importance, such as ethanol solution and formaldehyde solution, are not effective. Preservation of adult samples was successful in 70% ethanol solution. Breeding trials under laboratory conditions proved effective in breeding larval instars found on buried carcasses through to adulthood for identification. Due to the difficulty in preservation of immature individuals of Diptera of the grave, and the ease of breeding larvae of these dipteran species, identifications were done on adult specimens. Adult Diptera were described based on taxonomic criteria, including wing venation, setal hairs and the hypopygium of male phorid specimens, and a simplified identification key was successfully compiled using morphology of the adult Diptera of the grave.

KEYWORDS: Diptera, Burial, Identification, Morphology, Preservation, Breeding, Morphological Key, Forensic Entomology, Phoridae, Soil

Uitreksel

Forensiese entomologie is ‘n spesialisafdeling van forensiese wetenskap waar inligting aangaande insekte gebruik word om sekere afleidings aangaande medies-regsgeldige sake te maak waar beide mense en diere betrek is. Die primêre aanwending van insekte wat op ‘n misdaadtoneel versamel is, is om die Post-Mortem Interval (PMI) te bepaal. Een van die grondslag beginsels in die doeltreffende aanwending van forensiese entomologie is die noukeurige identifikasie van spesies wat op die misdaadtoneel aangetref word. Die PMI-bepaling deur middel van suksessie- en ontwikkelingsdata, is afhanklik daarvan om die spesifieke spesies wat op die kadawer teenwoordig is te gebruik. Die doel van die huidige studie was om verteenwoordigers van die Diptera te identifiseer wat in staat is om aas wat begrawe is te koloniseer binne ‘n Vrystaat-graslandgebied, sowel as om die data van ondergrondse Diptera met die suksessiepatrone van bogrondse Diptera te vergelyk.

Die veldeksperimente, uitgevoer om te bepaal watter spesies van Diptera in staat is om aas wat begrawe is tydens winter- en somermaande te bereik, sowel as die bepaling van die suksessiepatrone van Diptera op oorskot wat begrawe is, is uitgevoer op die westelike velde van die Universiteit van die Vrystaat-kampus in sentraal Suid-Afrika. Hierdie gebied word deur droë, gematigde winters en warmer somers, met toevallige reënvalperiodes gekenmerk. ‘n Totaal van sewe varke (*Sus scrofa* Linnaeus 1758) is tydens elke seisoen gebruik, wat uit een bogrondse kontrole-karkas en ses aparte karkasse begrawe op 60 cm bestaan het. Karkasse wat begrawe is, is vir voorafbepaalde periodes gelaat en dan weer opgegrawe op verskillende tye oor ‘n 120-dae proefperiode ten einde die voorkoms, kolonisering en interaksies van Diptera te monitor.

Verteenwoordigers van Diptera is bevind om karkasse op 60 cm tydens beide die winter- en somerproewe te koloniseer. Hoër spesieverskeidenheid van Diptera is op die winterkarkasse wat begrawe is gevind, in vergelyking met die somerkarkasse wat begrawe is. Vier Diptera spesies, *Megaselia scalaris* (Loew 1866) (Phoridae), *Conicera tibialis* Schmitz 1925 (Phoridae), *Muscina stabulans* (Fallén 1817) (Muscidae) en *Leptocera* Olivier 1813 sp. (Sphaeroceridae) is op

die winterkarkasse waargeneem, met eerste kolonisering vanaf dag 21 tydens die winterproef. Drie Diptera spesies, *M. scalaris*, *Leptocera* sp., en *Sarcophaga* Meigen 1826, is op somerkarkasse wat begrawe is waargeneem, met eerste kolonisering wat op dag 21 van die somerproef voorgekom het. Die fauna-samestelling van Diptera op karkasse wat begrawe was, is bevind om te verskil van dié van Diptera op bogrondse karkasse tydens beide die winter- en somerproewe. Statistiese analise het getoon dat daar ‘n wesenlike verskil tussen die Diptera-ooreenkoms bogronds en ondergonds was.

Preserverings-eksperimente op onvolwasse Diptera-van-die-graf vir identifikasie-doeleindes het getoon dat die standaard preserveringsmediums wat vir insekte van forensiese belang gebruik word, soos etanol- en formaldehiedoplossings, nie effektief was nie. Die preservering van volwasse monsters was wel suksesvol deur van 70% etanol gebruik te maak. Uitbroeiproewe onder laboratoriumtoestande het getoon om effektief te wees in die uitbroei van larwale instars na volwassenes vir identifikasie vanaf karkasse wat begrawe was. As gevolg van die probleme wat ondervind is om onvolwasse individue van Diptera-van-die-graf te preserveer, en die gemak om larwes van hierdie Diptera-spesies uit te broei, is identifikasies uitgevoer deur van volwasse eksemplare gebruik te maak. Volwasse Diptera-eksemplare is beskryf gebaseer op taksonomiese kriteria wat vlerkbearing, seta-hare en die hipopigium van manlike eksemplare van die Phoridae ingesluit het. Verder is ‘n vereenvoudigde identifikasie-sleutel suksesvol opgestel deur van die morfologie van volwasse Diptera-van-die-graf gebruik te maak.

SLEUTELWOORDE: Diptera, grafte, identifikasie, morfologie, preservering, uitbroei, morfologiese sleutel, forensiese entomologie, Phoridae, grond

Table of contents

Chapter 1: Introduction

“Succession is no doubt one of the most important and widespread of the phenomena discovered by ecologists up to the present time” – V.E. Sheford 1911

1.1 Background

Forensic entomology is a specialist branch of forensic sciences in which information about insects is used to draw certain conclusions when investigating medico-legal cases involving both humans and wildlife (Gennard 2007). Although developments in forensic entomology have been increasing during recent years (Amendt *et al*. 2011; Corréa *et al*. 2014), forensic entomology is not a new science (Byrd & Castner 2010). Analysis using insects has been looked into since the 13th century, first recorded being used by a Chinese lawyer named Sung Tźu in China (Benecke 2001). Illustrations of maggot-covered corpses have been recorded since the 16th century in Europe. (Klotzbach *et al*. 2004). Despite the early observations made throughout the years, it was not until the 1960's that forensic entomology became an accepted discipline in the United States when it was discovered that carrion insects display predictable succession patterns and species development rates (Byrd & Castner 2010).

1.1.1 Usage of insect in forensics

Insects have been found in almost every type of habitat around the world, making them some of the most versatile and resilient groups of living organisms on earth (Byrd & Castner 2010). In order to survive in a variety of environments, insects have adopted various feeding behaviours, including scavenging behaviour and detrivorous feeding (Triplehorn & Johnson 2005). Scavengers and decomposers play a large role to ensure cycling of nutrients and the return of organic matter to an ecosystem (Braack 1987). Forensic carrion feeders can be considered as decomposers because they assist in the breakdown of carrion by feeding on dead animal matter (Richard 2001 unpubl.). Members of the Calliphoridae are primarily considered as important carrion feeders. Blowflies (Diptera: Calliphoridae) have a worldwide distribution of over 1 600 species (Marshall 2012). Adult blowflies are known for their conspicuous metallic green, black or blue colouration (McAlpine *et al*. 1987; Byrd & Castner 2010; Marshall 2012). Many species belonging to the family Calliphoridae breed in mammalian

carrion, showing the significance of this family in forensic entomology (Kurahashi & Kirk-Spriggs, 2006). According to an article by Lui and Greenberg (1989), calliphorids and some related flies are often the most important consumers of carrion, whether it is below ground or above ground carrion. Due to this important function, these organisms play a major ecological role in the decomposition process and are increasingly useful in forensic entomology as a vital means of calculating the Post Mortem Interval (PMI) in homicide cases (Lui & Greenberg 1989). Members of Diptera are some of the major feeders of carrion as their larvae prominently feed on corpses, therefore studies thus far have concentrated on the investigation of the developmental rate of different forensically important Diptera, the accurate identification of forensically important Diptera, as well as the occurrence of species on carrion under different conditions (Gennard 2007; Byrd & Castner 2010; Benbow *et al*. 2015).

Adult members of Diptera are usually identified according to different morphological traits and defining characteristics varies between suborder, superfamilies, families, genera and species. The principal characteristics conventionally used in identifying members of Diptera are those of the legs, wings and arrangements of bristles and setal hairs of the head and thorax (Triplehorn & Johnson 2005), thus relying on external morphology. According to Triplehorn & Johnson (2005), defining traits may vary between families and also includes the use of the structures on the head, the size and shape of dipteran specimens or shape of body structures, as well as colour of the insect. Members of the family Sarcophagidae are characterised by their wing venation, hairs on the thorax, as well as colour stripes on the dorsal side of the thorax (Triplehorn & Johnson 2005; De Carvalho & De Mello-Patiu 2008). Members of the family Sphaeroceridae are typically characterised by their wing venation as well as the features of the hind tarsi (Triplehorn & Johnson 2005; De Carvalho & De Mello-Patiu 2008). Muscidae adults are defined by their wing venation, setal hairs on the hind legs and hairs on the thorax (McAlpine *et al*. 1981; Triplehorn & Johnson 2005). Members of the family Phoridae are defined by traits of the wings, characteristics of the hind legs, as well as by their general appearance (McAlpine *et al*. 1981; Triplehorn & Johnson 2005; De Carvalho & De Mello-Patiu 2008). As defining characters to genus and species level need to be

unique to a genus or species, focus is often shifted to more distinct features for identification. Characters such as setal hairs on the hind and mid legs, the form of the genitalia in male specimens, and structure of the abdomen may be examined to distinguish between different genera and species (McAlpine *et al*. 1981; Disney 1983; Disney 1989; Dong & Yang 2015).

Carrion breeding flies, like blowflies and flesh flies, are reared in laboratory conditions for the purpose of identification as well as for PMI estimation. Flies of forensic importance are successfully reared on carrion tissue mediums as sufficient protein meals are made available for growth, development and subsequent reproduction (Byrd & Castner 2010). These specimens can be preserved and analysed for crime scene use. It is widely accepted that preservation of entomological evidence from a crime scene is successful in a 70% ethanol solution as this method is recommended by several sources, including Gennard (2007), Byrd & Castner (2010) and Tomberlin & Benbow (2015). However, a study by Day & Wallman (2008) showed variability in preservation methods used on two Calliphoridae species, suggesting that different preservation methods could be species specific and should be investigated for each species and each instar of the same species as these may react differently to different preservation mediums. It is therefore imperative that suitable preservation methods should be investigated for each species, including species representatives of Diptera of the grave.

The primary application of insect information collected for forensic analysis is to determine the PMI. In regular investigations, PMI estimations are done using pathology information within the first 48 hours of death (Greenberg & Kunich 2002). After this time frame, the use of entomological evidence can contribute to the calculation of a more accurate PMI estimate. Entomological evidence, such as members of the Calliphoridae and Sarcophagidae, is used to determine the PMI (Greenberg & Kunich 2002). In addition to developmental rates of forensically important Diptera, the correct identification of species is necessary for accurate estimation of PMI (Smith 1986). According to Kolver (2009 unpubl.), the basic building blocks of the correct application of forensic entomology can be considered as a four-fold system. Forensically important insects need to be identified in an accurate and reliable way. In conjunction with

correct identification, the life history of each forensically important species is required. It is also vital for investigators to understand the ecology, interactions and association of these species with a decomposing body to establish a species' role on carrion. Finally, an investigator should consider deviations from past findings, as no two cases are fully identical due to differences in the species involved, the geographic location of a case and the microclimate and environment on and around a corpse.

Successional patterns of carrion-feeding insects, especially Diptera can aid in establishing a PMI in a forensic case (Pastula 2012 unpubl.). Forensically important Diptera typically move from a more complex insect community in an ecosystem to a simpler community and are attracted to different stages of decay (VanLaerhoven & Anderson 1999). The temporary niche created by a corpse provides an isolated food source for specialised feeders away from the larger insect community (Byrd & Castner 2010; Tomberlin & Benbow 2015). Since species of forensic importance do not all have the same behavioural patterns and colonisation times (Gennard 2007), arrival patterns of arthropod species and the differential utilisation of carrion by species results in predictable successional patterns (Byrd & Castner 2010).

Due to the difficulty in obtaining human cadavers for study, many studies use simulated scenarios, instead of real life cases, in order to determine successional patterns and faunal composition. Experimentally obtained evidence has been investigated in order to help estimating PMI in real cases as well as prove the validity of experimental studies applicable to real cases. In a study by Arnaldos *et al*. (2005), entomological evidence collected in real crime scene cases were compared to the species found in an experimental study to determine the sarcosaprophagous faunal composition of an area in Spain. From the results, it was concluded that, although many species can be found, it is important to determine the faunistic composition of isolated areas in order to determine the forensically important species of an area. The significance of doing an experimental study was also confirmed as it was found to be directly applicable to actual forensic cases, proving that experimental studies are sufficient for sarcosaprophagous faunal analysis for forensic application.

1.1.2 Insects in burial cases

Arrival and detection of a body may be affected by barriers and initial access to carrion. Access of a cadaver to dipteran individuals is considered to be the second most important variable affecting decomposition rate of a human cadaver, after temperature (Mann *et al*. 1990). Factors such as clothing and wrapping (Kelly 2006 unpubl.), as well as soil cover over a cadaver (Carter *et al.* 2007) may act as a barrier and restrict access to a decomposing body, delaying colonisation of a cadaver (Byrd & Castner 2010). Physical barriers can be intentional or unintentional, often altering dipteran colonisation and decomposition of a cadaver (Pastula 2012 unpubl.). The decomposition of human cadavers, for example, has differential decomposition patterns in different environments (Pope 2010 unpubl.). In some murder cases, the bodies of victims are left where they are killed, but there are usually attempts to conceal a body or otherwise dispose of evidence (Gunn & Bird 2011). A very common method of concealing a murder victim is burial, but owing to the size of a victim, the means to get a body to an "easy" dumping site without detection, as well as the effort and time it takes to dig to any depth, most victims are buried in shallow graves (Gunn & Bird, 2011). Burial is a popular physical barrier used by criminals to dispose of evidence (Pastula 2012 unpubl.). However, despite the predilection of using soil as a 'concealer', very little data exists for enclosed spaces (Pope 2010 unpubl.).

Forensic taphonomy is the study of burial crime cases and includes the study of ceremonial graves as well as clandestine graves (Pokines & Symes 2013). The word *taphos,* from the word taphonomy, is of Greek derivation and means tomb or grave. Forensic taphonomy has been used for many to assist in the identification of victims, to determine the cause of a victim's death, as well as to assist in the estimation of PMI (Nawrocki 1996). The study of soil crime scene cases and tombs encompasses a variety of fields, including the studies of Botany, Anthropology, Pathology and Entomology (Carter 2011). The detection of graves in forensic cases, for example, uses a multidisciplinary approach to find clandestine graves in different areas, including using signs of entomological indicators, soil scavenging patterns as well as cues such as gaseous expulsion. In a study by Davenport *et al*. (1992), 14 discipline-based methods were used to

test the detection of buried pig carcasses in Douglas County, Colorado, over a long period of time. The use of biological indicators, such as insects and scavengers, as well as other factors such as thermal feedback, gaseous release and the soil characteristics after long-term exposure to climatic conditions could prove useful in crime scene cases, depending on the environmental conditions. Although forensic taphonomy is a growing field around the world, very few studies have been done to describe the effects of different grave environments on entomological evidence.

Burial cases have been investigated mainly in areas in the northern hemisphere, investigating the effects of different factors on decomposition. In a study by Turner *et al*. (2013), a soil type analysis was done on four types of soil in Turkey, and the effects of these soil types on decomposition on pig carcasses was recorded during generally warmer months (May-November). It was found that different soil types affected the presence of insects able to colonise a buried carcass as well as the decomposition rate of carcasses. It has been hypothesised that different ratios in soil type, which is regularly found in nature, can affect insect colonisation and decomposition of a buried cadaver (Pokines & Symes 2013; Turner *et al*. 2013). Although many studies have been done in regards to burial archaeology and cemetery studies in Australia and South Africa, no entomological studies have been done in regards to investigating dipteran composition on buried carrion in the southern hemisphere.

Mégnin (1887, 1894) and Motter (1898) were among the first to note that buried cadavers had their own specialist group of insect fauna, suggesting that burial insect succession differed from above ground cases. This was later confirmed by later studies conducted by Schmitz (1928) and Chu & Wang (1975). In a study by Pastula (2012 unpubl.), faunal compositions on buried carcasses were determined at depths of 30 cm and 60 cm in East Lansing, Michigan. It was concluded that specific members of the families Muscidae, Sarcophagidae and Phoridae were role-players in succession of buried carcasses at both 30 cm and 60 cm within the area during summer months, depending on the climatic conditions as well as soil composition. This was found to be significantly different to the above ground faunal analysis of the study. However, it was suggested that studies should be conducted in other regions in order to

accurately apply insect results for Post-Burial Interval (PBI) estimation as species distribution and behaviour, environmental conditions, and climate may differ.

Seasonality may play a role in the colonisation of Diptera on buried carrion. In a study conducted in the United Kingdom by Turner & Wiltshire (1999) during winter months, cold conditions combined with the type of soil proved to inhibit the decomposition process and limited accessibility of buried carcasses to insects. In contrast to winter, summer shows a general increase in dipteran diversity and abundance with an increase in temperature (Baumgartner 1988). Studies by VanLaerhoven & Anderson (1999) and Pastula (2012 unpubl.) showed prominent dipteran activity during summer months, which contrasts the results from winter months obtained by Turner & Wiltshire (1999). It could be deduced that Diptera of the grave are therefore more prevalent during summer months than winter months. However, this is dependent on region and environmental conditions (Byrd & Castner 2010).

Long-term colonisation of buried remains has been found in several cases. In a case reported by Martín-Vega *et al*. (2011), buried bodies in Spain were exhumed from the Guadalajara cemetery in the Castile La Mancha Region in order to improve the osteological collection of the Department of Zoology and Physical Anthropology of the University of Alcalá, Spain. Upon excavations of the human cadavers that had been buried for 18 years, empty pupal cases and adult Diptera of the species *Conicera tibialis* Schmitz 1925 were discovered on the bodies. *Conicera tibialis* has been found to be associated with buried remains for several months and, in some cases, for years after a corpse had been buried (Byrd & Castner 2010).

In a recent observation by Karapazarlioglu & Disney (2015), a species of Phoridae, *Conicera similis* (Haliday 1833), was noted for the first time on buried carrion in areas of Turkey. This was the first record of this species being involved on buried carrion. In a study by García-Rojo *et al*. (2013), the species was first established as a forensically important species on a human corpse when it was found among other forensically important species during a peculiar case where a body was found in a cistern tank in the Iberian Peninsula in Spain.

Members of *Conicera* Meigen 1830 have been considered to be of potential importance especially in burial cases. From the aforementioned studies, it can be inferred that representatives of Phoridae are the most prominent members of Diptera involved in burial cases. However, little is known about the diversity of burial insect fauna and may mean by implication that new forensically important species may be established by future studies.

At present, various forensic entomology studies have shown that a number of dipteran species are capable of colonising a body buried directly into the soil, including members of Phoridae, Muscidae, Sphaeroceridae, Sarcophagidae and Calliphoridae (Payne *et al*. 1968; Turner & Wiltshire 1999; VanLaerhoven & Anderson 1999; Pastula 2012 unpubl.). These families can be described as follows:

Adult Phoridae flies have been found to be highly versatile and have been found in a variety of above ground environments. Adult Phoridae are commonly associated with decaying plant matter (Borror *et al*. 1989), however larvae of many phorid species typically develop in a variety of decomposing organic matter (Byrd & Castner 2010). The family Phoridae are particularly associated with late stages of decomposition, typically where other dipteran species as well as coleopteran species are both found on carrion (Gennard 2007). In below ground cases, one particular species, *C. tibialis*, has been found to locate buried carrion using olfactory cues (Gennard 2007) and complete several generations below ground without emerging from the soil (Coyler & Hammond 1951; Martín-Vega *et al*. 2011).

The biology and habits of muscid flies in above ground cases have been noted to vary considerably as adult individuals may feed on decaying plant and animal matter, plant pollen and nectar, animal excreta, or even blood (Mullen & Durden 2002; Gennard 2007; Byrd & Castner 2010). Many species of Muscidae are closely associated with humans and are considered of both medical and forensic importance (Mullen & Durden 2002). In above ground cases, muscid flies are often seen to arrive after Sarcophagidae and Calliphoridae. Female members of the Muscidae often lay their eggs in natural body openings of a

cadaver, within wound sites of a cadaver, or within the folds of fluid-soaked clothing (Byrd & Castner 2010).

Sarcophagidae, commonly known as flesh flies, have been noted to be attracted to above ground carrion under a variety of conditions, including carrion exposed to sun and shade, moist and dry carrion, as well as carrion indoors and outdoors (Byrd & Castner 2010). Flesh flies have often been found on indoor cadavers and are associated with a cadaver throughout early and late stages of decay (Aldrich 1916; Hall & Doisy 1993). Female flesh flies deposit living first instar larvae on decomposing carrion, known as larviposition, instead of ovipositing eggs onto a cadaver. Although Sarcophagidae can be found throughout a variety of decomposition stages, flesh flies are often seen on a cadaver when Calliphoridae are absent (Byrd & Castner 2010).

Sphaeroceridae, commonly known as lesser dung flies, are found in association with excreta as well as animal remains. In above ground cases, the presence of soiled clothing on human cadavers is thought to greatly increase the chance of finding Sphaeroceridae on a cadaver (Byrd & Castner 2010). Sphaerocerid flies may also be found on above ground carrion, garbage refuse, seaweed and algae, fungal growth, and other decaying organic matter (Buck 1997; Triplehorn & Johnson 2005; Byrd & Castner 2010). Over 20 species of Sphaeroceridae have been reared from various fungi, showing the nature of some sphaerocerids as fungivores (Jakovlev 1994).

Calliphoridae, commonly known as blow flies, are the most widely sampled Diptera associated with decaying carrion. A number of species of this family are extensively used in forensic investigations (Byrd & Castner 2010). Few instances of colonisation by species of this family have been recorded on shallow buried carrion, with *Calliphora vicina* Robineau-Desvoidy 1830 found being able to detect decaying volatiles from shallow graves (Paczkowski *et al*. 2012). Despite this, no such colonisation by calliphorid species has been documented on buried carrion at 60 cm depth.

For above ground cases, carrion communities have been documented during recent years for different South African regions. African carrion communities have been found to contain representatives of the same insect families that

usually occur on other continents, and include species of the orders Diptera, Coleoptera, Hymenoptera and Lepidoptera (Villet 2011). Blowflies (Diptera: Calliphoridae) are regarded as the primary forensic feeders in South Africa. In regards to forensically important dipteran species in southern Africa, at least six Afrotropical Calliphoridae species are regarded as primary indicators in forensic investigations, including (in order of prevalence on decomposing corpses): *Chrysomya chloropyga* (Wiedemann, 1818), *Chrysomya albiceps* (Wiedemann, 1819), *Lucilia cuprina* (Wiedemann, 1830), *Chrysomya marginalis* (Wiedemann, 1830), *Calliphora croceipalpis* Jaennicke, 1867, & *Chrysomya megacephala* (Fabricius) (Kurahashi & Kirk-Spriggs, 2006). Further species include members of the Sarcophagidae, Muscidae, and Piophilidae (Kelly 2006 unpubl.; Hoffman 2014 unpubl.).

Other species of Phoridae that occur in Afrotropical regions have been found to be of forensic importance in the Northern hemisphere. For example, first reports of *Megaselia curtineura* (Breus 1909), an Oriental and Afrotropical phorid species, was made in 2009, stating its occurrence on human corpses in Penang, Malaysia (Thevan *et al.* 2009). Later, a study by Kumara *et al.* (2012) found similar results, with *M. curtineura* being among 16 species found on human remains in north Peninsular Malaysia. Similarly, Phoridae species *Dohrniphora cornuta* (Bigot 1857), a widespread species of Phoridae (Barnes 1990), has been thought to be of forensic importance due to its frequent occurrences in forensic cases (Disney *et al.* 2014). *Megaselia rufipes* (Meigen 1804), a cosmopolitan Phoridae species (Gonzalez-Vainer *et al*. 2012), has been found to readily breed on carrion and has been associated with forensic cases in the northern hemisphere (Disney 2005; Boehme *et al*. 2010). Although all of the aforementioned dipteran species are widely distributed and occur in the southern hemisphere (Disney 1994), no studies have established their importance in burial cases.

No studies have been done in the South African region on burial faunal composition. A pilot study was conducted during the summer of 2013 in the Bloemfontein area, Free State, to determine the capability of species of potential forensic importance to reach below ground carrion in the region. Halved pig carcasses, used as bait, were buried at 30 cm in the natural clay soil

environments of a central Free State grassland field and were monitored over a 21 day period. It was deduced that insects are in fact capable of colonising carrion at a depth of 30 cm, and it was hypothesised that members of Diptera first colonised the below ground bait between day 3 and day 7 after burial.

The findings of the pilot study confirmed that certain species within the area possesses the ability to reach buried carrion and play a role in arthropod succession. However, although this proved true for bait at 30 cm, the effect of depth of a grave on insect succession still remained unclear. It was hypothesised that varying depths, different soil types and seasonal change may have an effect on the entomological evidence found on a buried body.

South Africa is considered as having one of the highest malicious crime rates in the world, with an average of between 15 000 and 17 000 murder cases reported between 2010 and 2014 (S.A.P.S. 2015). However, many murder cases go unreported due to criminals disposing of victims in a variety of ways. Given the fact that forensic sciences is still a growing field in the region, many studies in regards to forensic entomology, among other fields, have to be explored further in South Africa in order to fill the gaps in information that is needed to effectively use findings in real life cases.

1.2 Aims and objectives

The aim of this study was to identify the members of Diptera that are capable of colonising carrion at a depth of 60 cm within a Free State grassland area during summer and winter months. A secondary aim was to compare the data with above ground species of forensic importance.

The objectives for the study, together with hypothesised outcomes, are listed as follows:

1. To identify a method of preservation and breeding of sampled Diptera of the grave for identification purposes.
    i. Immature representatives of Diptera will show successful preservation in 70% ethanol solution.

ii. Sampled members of the Diptera will be capable of breeding on organ or muscle tissue due to their carrion feeding.

2. To identify adult Diptera of the grave based on morphological characteristics.

iii. Identification of Diptera of the grave can be done using external morphological characteristics.

3. To compile a user-friendly method of identifying Diptera of the grave for use by crime scene investigators.

4. To document differences in dipteran succession in above ground and below ground cases.

iv. Members of Diptera in the Bloemfontein area are capable of reaching carrion at a depth of 60 cm.

v. Burial faunal composition will contain different species to that of above ground fauna.

vi. Members of Phoridae would be the most dominant on buried carrion.

5. To document differences in dipteran succession during summer and winter months.

vii. Burial members of Diptera will be more abundant on buried carrion during summer months than during winter months.

Chapter 2: Materials and Methods

“Though this be madness, yet there is method in it”.

- William Shakespeare, Polonius in Hamlet

2.1. Study site

Both summer and winter seasonal studies were conducted in the Free State area in central South Africa. The study site was located in Bloemfontein on the University of the Free State grounds (Figure 2.1.1). Each seasonal study was conducted on two different sectors of an open, 24 hectare grassland field area ($29^0$8'S; $26^0$10'E, ± 1560m above sea level) away from built up areas.

The area was characterised by summers with measured temperatures ranging from 21°C to 43°C and winters with temperatures ranging from 12°C to 27°C. Annual expected rainfall for the region ranged from 350-400 mm and is concentrated over the summer months (MacKellar *et al*. 2014; SANBI 2014).

The grassland contained several grass species, with *Themeda triandra* Forsk. dominating the area, and a few scattered trees in the area were noted to be of the species *Vachellia karroo* (Hayne) and *Rhus rehmanniana* Engl. (Table 2.1.1).

A few vertebrates such as birds and grazing mammal species were present at the experimental site during the study trial period (Figure 2.1.2, Table 2.1.2).

Table 2.1.1: List of prominent plant species within the study area, including common names of each species

Species name	Common name
Tree species	
Vachellia karroo (Hayne) [formally *Acacia karroo* (Banfi & Galasso 2008)]	Sweet thorn tree
Rhus rehmanniana Engl.	Blunt-leaved currant
Grass species	
Themeda triandra Forsk.	Red grass or red oat grass
Aristida congesta Roem & Schult.	Buffalo grass or stickgrass
Chloris virgata Sw.	Blackseed grass or feathertop grass
Eragrostis curvula (Schrad.) Nees.	African lovegrass
Eragrostis lehmanniana Nees.	Lehmann lovegrass
Heteropogon contortus (L.) Beauv.	Black speargrass
Sporobolus pyramidalis Beauv.	Giant rat's tail grass
*All plant species identified using Meredith (1955) and Coates Palgrave & Coates Palgrave (2002).	

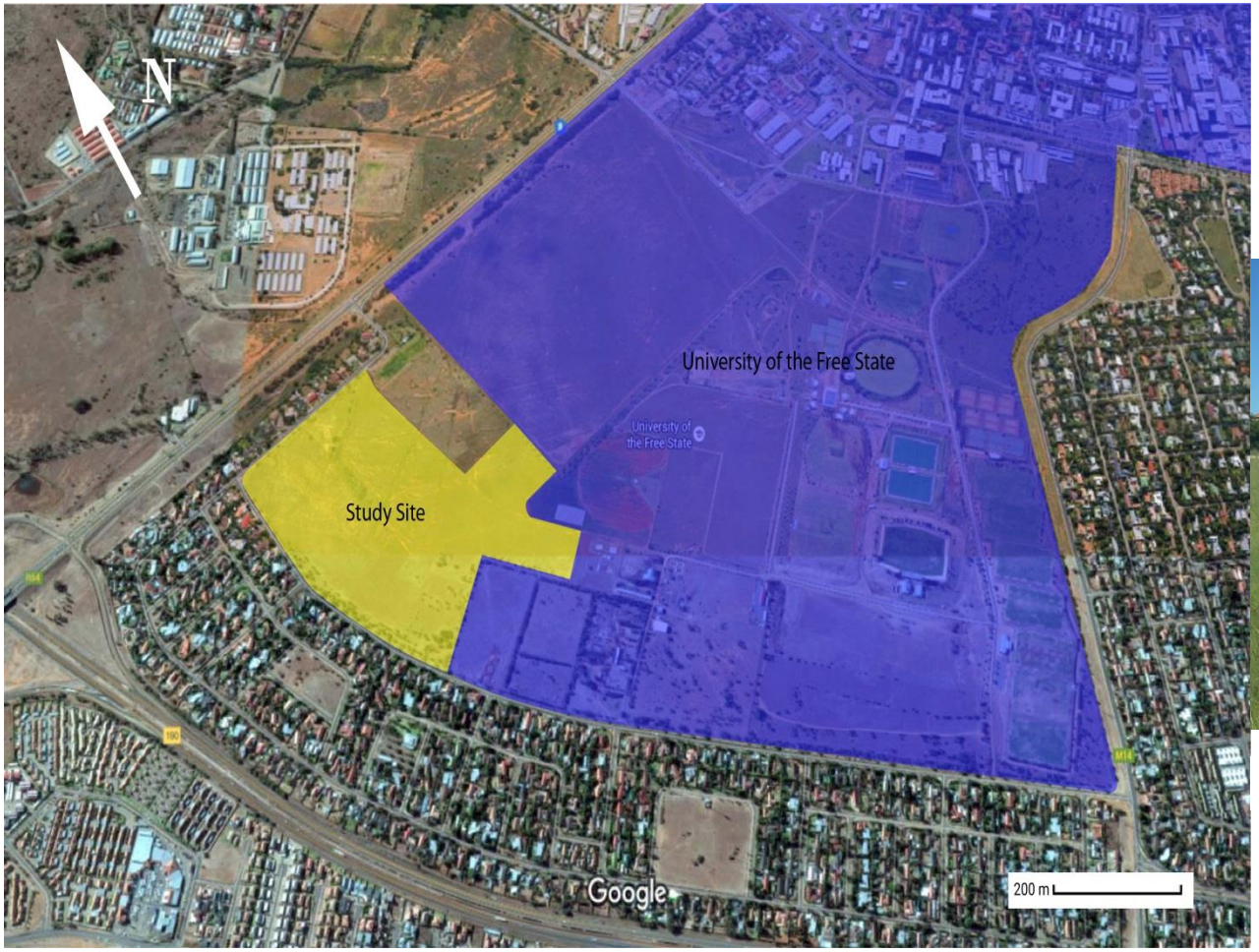


Figure 2.1.1: University of the Free State grounds, with the main university area highlighted in blue and the main study site (Experimentation Farm) highlighted in yellow (Image altered from Google Maps, accessed 15 September 2015).

Figure 2.1.2: Summer site showing horses grazing in the area.

Table 2.1.2: List of prominent vertebrate animal species within the study area, including common names of each species

Species name	Common name
Avian vertebrate species	
Bubulcus ibis (Linnaeus, 1758)	Cattle egret
Francolinus swainsonii (Smith, 1836)	Swainson's francolin
Guttera pucherani Hartlaub, 1861	Crested guineafowl
Hirundo cucullata Boddaert, 1783	Greater striped swallow
Lanius collaris Linnaeus, 1766	Common fiscal shrike
Mammalian vertebrate species	
Ovis aries Linnaeus, 1758	Sheep
Equus caballus Linnaeus, 1758	Domestic horse
Capra hircus Linnaeus, 1758	Domestic goat
Cynictis penicillata(Cuvier, 1829)	Yellow mongoose
Canis lupus familiaris Linnaeus, 1758	Domestic dog
*All taxonomic information sourced from ITIS report (www.itis.gov), accessed 23 July 2015.	

2.2. Carcass preparation, layout and placement

2.2.1. Carcass preparation

The aim of this study was to recreate the scenario of typical shallow burial cases in a grassland area in central South Africa. Pig carcasses (*Sus scrofa* Linnaeus 1758) were used in each trial. Pig carcasses used during the winter trial were obtained from the University of the Free State Animal Experimentation Unit. Carcasses were obtained after a prior study conducted by the Experimentation Unit and was obtained frozen before the commencement of the present study. Ethical application was not executed for the winter study as carcasses were received already euthanized by means of euthanasia injection (Euthapent), and approval was obtained as per protocol for the prior study done by the Experimental Unit. Pigs during the summer study were brought in and euthanised by single euthanasia injection (Euthapent) by the University of the

Free State Animal Experimentation unit. The carcasses were then placed in an industrial freezer until the placement date, designated as day 0. Ethical approval for the summer study, and the use of pig carcasses obtained as fresh pigs, was obtained from the University of the Free State Ethics committee (Appendix 1). Pigs are considered an internationally accepted substitute for human bodies (Catts & Goff 1992). A study by Bugajski *et al*. (2011) showed that there is no significant difference in decomposition between fresh and frozen pigs in burial studies. All carcasses chosen for use in each trial were selected within a weight range of 23 kg-40 kg, which is regarded to be an acceptable range for successional decomposition studies (Catts & Goff 1992).

Each carcass was wrapped in a sheet of 25 mm chicken wire (1.5 m x 1 m) and secured with plastic cable ties to hold the ends of the wire in place (Figure 2.2.1.1). This was to simplify weighing during the trials.

Figure 2.2.1.1: Euthanised pig carcass wrapped in poultry wire and secured with cable ties.

2.2.2. Control placement

The placement sites for the above ground carcasses were selected according to similar vegetation and ground level to the graves during the winter and summer trials (Figure 2.2.2.1-2.2.2.2). Above ground carcasses were placed at least 50 m away from the grave sites to avoid migration of insects from the above

ground carcasses to the graves (Figure 2.2.2.3-2.2.2.4). The above ground carcasses were placed in welded wire mesh cages (1.6 m x 0.9 m x 0.9 m) to prevent mammalian or avian predation on insects and scavenging of the carcass.

2.2.3. Burial placement

The grave positions were selected according to similar vegetation and ground level to ensure that the summer and winter trials took place within a similar environment. Grave sites were placed in a random scatterplot fashion at least 20 m away from each other to avoid migration of species from one grave to another. Graves of 60 cm depth were dug with dimensions 1.6 m x 1.2 m, using an industrial digger, one day before the carcasses were placed (Figure 2.2.3.1-2.2.3.2). Soil samples were taken from each grave during the winter and summer trials at 30 cm and 60 cm depths. Soil composition for each of the graves was then determined from these samples (Table 2.2.3.1).

A total of six pig carcasses were used for each seasonal burial trial. Grave carcasses were placed in pre-dug shallow graves with the head facing north and legs facing west (Figure 2.2.3.3-2.2.3.4). The carcasses were then covered with soil until the grave was level with the surrounding surface. No compaction of the soil was applied. Graves were then covered with an overturned welded wire mesh cage (1.6 m x 0.9 m x 0.9 m) to prevent mammals in the area from interfering with the grave sites (Figure 2.2.3.5).

Table 2.2.3.1: Soil composition of the graves during the winter and summer trials at 30 cm and 60 cm.

Season	Depth	Grave 1	Grave 2	Grave 3	Grave 4	Grave 5	Grave 6
Winter	30 cm	Sandy soil	Sandy-shale mix	Sandy-clay mix	Sandy-clay mix	Sandy-clay mix	Clay-loam mix
	60 cm	Sandy-shale mix	Clay-shale mix	Shale-sand mix	Clay-shale mix	Shale-sand mix	High clay-loam mix
Summer	30 cm	Clay-sand mix	Clay-loam mix	Clay-loam mix	Clay-sand mix	High clay-loam mix	Clay-loam mix
	60 cm	Clay-loam mix	High clay-loam mix	High clay-loam mix	Clay-loam mix	High clay-loam-shale mix	High clay-loam mix

Figure 2.2.2.1-2.2.2.2: Photographs taken on day 60 of each seasonal trial showing winter (top) and summer (bottom) field conditions.

Figure 2.2.2.3-2.2.2.4: Aerial photographs showing the grave positions during the winter trial (top) and summer trail (bottom). Control marked in blue and graves marked in red. (Images altered from Google Earth, accessed 09 November 2015).

Figure 2.2.3.1-2.2.3.2: (top) Industrial digger used to dig all graves (bottom) used in summer and winter trials.

Figure 2.2.3.3-2.2.3.5: Placement of each carcass during summer (top) and winter (middle). Cages were placed over each grave to prevent any animals in the area from disturbing the graves (bottom).

2.2.4. Trial period dates

Studies were planned according to the seasonal solstices (21 June 2014 for winter and 21 December 2014 for summer) to ensure that adequate seasonal temperatures had been reached for winter and summer. The trials for each season ran for 120 days, beginning one day after each solstice date. The winter trial ran from 22 June 2014 to 19 October 2014, running into spring months, and the summer trial took place from 22 December 2014 to 20 April 2015, running into autumn months. The overlap in seasons was unavoidable but necessary to accurately assess the long-term colonisation of Diptera on below ground carcasses.

2.2.5. Excavation and sampling dates

Excavation dates were modelled according to the article by Pastula & Merritt (2013) as a guideline. Each carcass was excavated once throughout each 60 day trial to avoid disturbance, with grave 6 exhumed again on day 120 (Table 2.2.5.1). This allowed for observations on the long-term effects of burial on dipteran succession, as well as to monitor colonisation in the absence of disturbances. Each excavation was performed by hand using conventional hand digging equipment, including shovels and gardening trowels, and soil was removed gradually to allow for careful analysis of each soil level and to avoid large levels of disturbance.

Table 2.2.5.1: Excavation date mapping during each season, indicating the excavation days of each grave as well as date of each excavation.

	Winter	Summer	Grave 1	Grave 2	Grave 3	Grave 4	Grave 5	Grave 6
Day 3	24/06/2014	24/12/2014	X					
Day 7	28/06/2014	28/12/2014		X				
Day 14	05/07/2014	04/01/2015			X			
Day 21	12/07/2014	11/01/2015				X		
Day 30	21/07/2014	20/01/2015					X	
Day 60	20/08/2014	19/02/2015						X
Day 120	19/10/2014	20/04/2015						X

2.3. Sampling and temperature recordings

2.3.1. Above ground temperatures and sampling from the above ground carcasses

The above ground carcass was observed each day to note the changes in dipteran species occurring on the body throughout each trial. Specimens were sampled every third day and recorded according to life cycle stage and classified to species level where possible. From these records, insects of potential forensic importance were listed and their numbers were estimated to indicate frequency of occurrence, as well as abundance of each species, throughout each trial period.

The internal body temperature of the above ground carcass was recorded on each day of excavation to provide comparative data with each grave. Ambient temperature was also recorded each day of the trial at midday using a probe thermometer to accurately measure field temperature.

2.3.2. Below ground temperatures and sampling from the grave carcasses

During excavations, a range of soil temperatures were recorded and noted to compare above ground and below ground temperatures (Figure 2.3.2.1, Table 2.3.2.1). Internal body temperature of each grave carcass was also taken during excavations (Table 2.3.2.1).

Table 2.3.2.1: List of temperatures taken during each trial.

Temperatures taken during each trial	
Above ground temperature points	***Grave temperature points***
Ambient temperature	Ambient temperature
Body temperature	Body temperature
	Ground surface temperature (2 cm depth)
	2 cm above body (30 cm depth)
	Left side of body
	Right side of body
	Directly below body (60 cm depth)

Sampling was done during each excavation. For the purpose of this study, only dipteran specimens were sampled from each carcass. Adult specimens were sampled using a small insect net and placed directly into separate 7 ml vials filled with a 70% ethanol solution for preservation and future analysis. Vials were carefully placed over scuttling adult specimens found in the in close vicinity to the body (i.e. in the surrounding soil) and live captured specimens were placed in empty ventilated containers. Larvae were sampled directly off the excavated carcass using a pair of soft tweezers and divided into two groups. Larvae of group 1 were killed by placing larvae in hot water (±90°C) and were then placed into separate vials filled with a 70% ethanol solution for future identification and analysis. Larvae of group 2 were placed into a 1 litre ventilated container containing 400 g pig liver covered with a 1 cm soil layer to allow development into the adult stage. Adults that emerged from the development of group 2 larvae were sampled and preserved in 7 ml vials filled with a 70% ethanol solution for species identification of larvae and analysis of forensically important flies.

Similar procedures were used for specimens collected from above ground carcasses. Adult specimens were sampled using a small insect net and placed directly into separate 7 ml vials filled with a 70% ethanol solution for preservation and future analysis. Larvae were sampled directly off of the above ground carcass using a pair of soft tweezers and divided into two groups. Larvae of group 1 were killed by placing larvae in hot water (±90°C) and were then placed into separate vials filled with a 70% ethanol solution for future identification and analysis. Larvae of group 2 were placed into a 1 litre ventilated container containing 400 g pig liver covered with a 1 cm soil layer to allow development into the adult stage. Adults were identified to verify identification of larvae from the carcass.

Pupal samples found in the soil around the buried carcasses were sampled using soft tweezers. Empty pupal cases were placed directly in 70% ethanol solution. Similar procedures were used for pupae found around above ground carcasses.

Extra sampling took place during the period 01/05/2015 – 05/06/2015 to collect more specimens for morphological analysis using bait traps and grave carcasses from the elapsed trial periods. Only specimens identified as forensically important during the seasonal studies were sampled during the extra sampling period.

2.4 Statistical analysis

Comparisons between grave data and above ground data were executed using a Sørenson's coefficient of similarity. These comparisons were done to numerically quantify the level of difference between each season as well as between above ground and below ground data for each season. The Sørenson's coefficient of similarity was calculated as follows:

$$S = \frac{2\mathrm{a}}{(2\mathrm{a} + \mathrm{b} + \mathrm{c})}$$

Where *S* represents the Sørenson's coefficient of similarity, *a* represents shared species between the two data sets, *b* represents species unique to data set one, and *c* represents species unique to data set two.

2.5 Parallel study

This study ran concurrently with another burial study conducted by Botham (2016 unpubl.), entitled: "Decomposition and arthropod succession on buried remains during winter and summer in central South Africa: Forensic implications and predictive analyses". This study utilised the same pig carcasses as the present study. Observations and results from this sister study was utilised as supporting material and is indicated in the text as such.

2.6 Breeding and preservation trials

Breeding and preservation experiments were conducted on sampled larvae specimens to test the success of conventional methods of breeding and preservation on burial species. Live adults sampled from the field were identified using identification keys by Zumpt (1961, 1965a, b, 1972), McAlpine *et al*. (1981), and Triplehorn & Johnson (2005). Dr. Henry Disney (corresponding scientist, Phoridae specialist) identified members of the Phoridae within the sampled specimens. Adult flies collected during excavations were separated into breeding cages according to species and bred using a breeding medium of 400 g pig liver covered with soil.

Mediums were placed in temperature controlled rooms at 25°C ± 1°C and a light cycle of 12 hours light and 12 hours dark photoperiod. Larvae sampled from successful breeding trials were placed in hot water (±90°C) to kill the larvae, and were separated by species for the preservation trials. The killing of larval specimens in hot water also ensures stretching of each specimen to its full length, allowing for ease of handling and identification. Two preservation solutions, namely 70% ethanol solution and a 10% buffered formaldehyde solution, identified as the simplest preservation methods to use during analysis of a crime scene (Catts & Haskel 1990, Byrd & Castner 2010), were tested on three groups of larvae of each species.

A secondary experiment testing a gradual ethanol dehydration of larval specimens was conducted using ethanol solutions at different concentrations. Table 2.6.1 shows the different concentration increases over each time interval per group. Nine groups of larvae from each species were divided into three cluster groups and each cluster group was tested using different time intervals and different incremental increases of the ethanol concentration. This was performed to monitor the effective diffusion of ethanol into larval specimens and record indications of failure of the dehydration solution. A total of three larval specimens were placed in each 10 ml vial during the dehydration process to avoid overcrowding within the solution.

2.7 Identification of dipteran species

Adult specimens collected during field sampling for identification purposes were placed directly into separate 7 ml vials filled with a 70% ethanol solution. Larval specimens sampled from the burial trials were reared to adulthood and then placed into 70% ethanol for identification. Adult specimens were divided according to species and half of each sample group was mounted using dissection pins. Various dissections of the remaining sample group were then conducted and mounted on non-permanent slides for observation and sketching.

Table 2.6.1: Ethanol dehydration test group design, showing each concentration increase over selected time periods as well as minimum and maximum range of concentration.

Ethanol dehydration test groups	
10% concentration increases over 2 hour intervals	Increase range starts at 30%, max at 70% (total 4 increases)
10% concentration increases over 6 hour intervals	Increase range starts at 30%, max at 70% (total 4 increases)
10% concentration increases over 12 hour intervals	Increase range starts at 30%, max at 70% (total 4 increases)
10% concentration increases over 2 hour intervals	Increase range starts at 10%, max at 70% (total 6 increases)
10% concentration increases over 6 hour intervals	Increase range starts at 10%, max at 70% (total 6 increases)
10% concentration increases over 12 hour intervals	Increase range starts at 10%, max at 70% (total 6 increases)
5% concentration increases over 2 hour intervals	Increase range starts at 10%, max at 70% (Total 12 increases)
5% concentration increases over 6 hour intervals	Increase range starts at 10%, max at 70% (Total 12 increases)
5% concentration increases over 12 hour intervals	Increase range starts at 10%, max at 70% (Total 12 increases)

Key identification points of adults were based on existing literature and identification keys based on morphology. These morphological characters include the analysis of wing venation and wing cells in accordance with the Comstock-Needham system for insect wings (Triplehorn & Johnson 2005; De Carvalho & De Mello-Patiu 2008; Disney *et al*. 2010), setal hairs of the mid legs and hind legs (Disney 1983; Disney *et al*. 2010), notopleural bristles of the thorax depending on family or species where applicable (Vairo *et al*. 2011), and external genitalia and tergites for more specific identification of Phoridae (Disney 1983; Disney *et al*. 2010). Morphological features for legs, wings and the thorax of Sphaeroceridae, Muscidae and Sarcophagidae were named using conventions used in Triplehorn & Johnson (2005). Morphological characteristics for tergites and external genitalia of Phoridae were named according

to conventions used in the literature (Disney 1983, 1989; Disney *et al*. 2010). Each insect part mounted on the slides was viewed under a dissection light microscope and photographed using a modified mounted camera designed for the specific analysis. Drawings for the simplified identification key were done using the photographs (according to scale) and rendered using Adobe™ Illustrator Artwork 15.0 to increase clarity and highlight key points of identification. Pictorial keys and a written identification key were drawn up according to the species identified.

Chapter 3: Preservation, breeding, morphology and identification

“Form ever follows function”

- Louis H. Sullivan (1856-1924)

3.1 Background

Basic guidelines for the collection of insect from a crime scene cadaver require investigators to sample two groups of insect samples: live samples and samples for preservation (Byrd & Castner 2010). Live larval samples are reared to adult stages to ensure accurate identification in cases where immature stages cannot be positively identified. Although identification of immature stages has been done extensively during recent years, breeding of crime scene samples are still done for positive identification of forensically important species (Byrd & Castner 2010; Tomberlin & Benbow 2015). The failure of preserving certain larval specimens can present a challenge in identification of larval samples as samples may not last long enough for accurate analysis. This points to the need to establish proper breeding methods and identification of adults in the case where certain larval members of Diptera cannot be preserved. However, breeding is species specific as each species has its own optimal condition preferences, which can alter the ability of species to develop and reproduce (Gennard 2007). It is therefore imperative that easy breeding techniques be established for use by forensic investigators, which may or may not have access to a fully controlled laboratory.

Identification of many forensically important species has been done in past studies with the main focus on above ground species as well as newly documented species. Many of these works encompass the identification of the immature stages of forensically important species due to the fact that larvae are mainly found on crime scene bodies. Although faunal compositions of forensic feeders and indicators have been documented extensively through the years, few studies have investigated the dipteran interactions on buried carrion as well as the identification of insects on buried carrion, especially in Southern Africa.

It is important to investigate simplified methods of identifying Diptera of the grave. The preservation and breeding of Diptera of the grave, the general morphology of adult Diptera of the grave, and a simplified pictorial key designed for use by investigators will be discussed in this section.

3.2. Section 1: Preservation and breeding of Diptera of the grave

3.2.1 Preservation of larvae of dipteran species of the grave

The effectiveness of preservation methods of larvae differ between the specimens of different species, and can prove to be a challenge if a preservative does not diffuse at a steady rate. Correct preservation for analysis is vital to obtain the natural shape of each sample and to prevent shrivelling and distortion. In a study by Adams & Hall (2003), it was found that certain preservative methods may cause expansion of larvae, sometimes causing distortion of the body and resulting in longer recorded larval lengths after long-term storage. This can cause problems in investigations as samples are often only examined days or even weeks after sampling and accurate results may not be possible if samples are distorted or lost through incorrect preservation (Adams & Hall 2003; Sanford *et al*. 2011). In light of this, it can be said that one method of preservation may not be effective for all species, and may call for alternative reagents or even different techniques.

The first step to preparing larvae collected on a crime scene for preservation is to kill larvae off in warm water (90°C) to avoid shrivelling of larvae before being placed directly into a preservative. This allows for ease of use for identification by forensic investigators. Byrd and Castner (2010) suggested that a 70% ethanol solution should be used as a preservation medium for all immature stages of Diptera larvae, pupae and adults. An alternative method of preservation commonly used is to use a 10% buffered formaldehyde solution. Each test group included 10 immature larvae of each instar for each trial. Solutions tested were then evaluated according to how soon larvae showed discolouration (first discolouration and blackening), if applicable, and success was noted only in the event of no discolouration.

The preservative methods used in the trial were not effective on immature stages of certain Diptera of the grave, especially on smaller species such as *Megaselia scalaris* (Loew 1866), and *C. tibialis*. Table 3.2.1.1 gives an overview of the results for each preservation medium used on larvae found on below ground carcasses during the present study. As can be deduced from the results of the preservation trial, Phoridae larvae became progressively discoloured over time when stored in 70% ethanol after being killed in hot water, indicating that diffusion was not taking

place at an effective rate. These larvae started shrivelling and rotting. A similar result was observed in the formaldehyde trials, with blackening of larvae occurring within 70 hours of administering the chemical solution to the sampled larvae. Although larvae of *Muscina stabulans* (Fallén 1817), showed a delay in the time before discolouration had occurred, all larvae tested with both preservation agents showed signs of rotting and failure in preservation over a seven day period. *Sarcophaga* Meigen 1826 sp. larvae displayed no blackening of larvae in both solutions and was successfully preserved using methods commonly used for crime scene sampling.

Table 3.2.1.1: Preservation agents tested on larval instars of Diptera of the grave, showing average times of noted discolouration in the present study of each instar for each species (n = 10 p/group). First discolouration values were characterised by the first sightings of larvae turning yellow (denoted as discolour) and blackening was considered as the observed time for 50% of the larvae to turn black (denoted as blacken).

Preservation of larval instars in 70% EtOH solution								
	C. tibialis		*M. scalaris*		*M. stabulans*		*Sarcophaga* sp.	
	Discolour	Blacken	Discolour	Blacken	Discolour	Blacken	Discolour	Blacken
First instar	24 hrs	48 hrs	24 hrs	48 hrs	30 hrs	168 hrs	N/A	N/A
Second instar	24 hrs	48 hrs	24 hrs	48 hrs	30 hrs	168 hrs	N/A	N/A
Third instar	36 hrs	96 hrs	36 hrs	96 hrs	33 hrs	144 hrs	N/A	N/A
Preservation of larval instars in 10% formaldehyde solution								
	C. tibialis		*M. scalaris*		*M. stabulans*		*Sarcophaga* sp.	
	Discolour	Blacken	Discolour	Blacken	Discolour	Blacken	Discolour	Blacken
First instar	18 hrs	56 hrs	16 hrs	N/A	24 hrs	168 hrs	N/A	N/A
Second instar	18 hrs	56 hrs	16 hrs	N/A	24 hrs	168 hrs	N/A	N/A
Third instar	24 hrs	96 hrs	18 hrs	72 hrs	36 hrs	168 hrs	N/A	N/A

A gradual ethanol dehydration method using an incremental method of increase in concentration of ethanol solution was also tested on larvae to test the effects of gradual dehydration over time. A total of nine groups of larvae (n=15 p/group) were subjected to gradual increases in ethanol concentration over time. Table 3.2.1.2

shows the effect of different increases in ethanol concentration on larvae of each species. Larvae of *M. scalaris*, *C. tibialis* and *M. stabulans* were noted to show full discolouration in all larvae groups over a period of seven days. Larvae of *M. scalaris* and *C. tibialis* showed the most rapid discolouration, specifically with the 5% concentration increase over 12 hour intervals trial. *Muscina stabulans* larvae displayed a less rapid rate of discolouration. However, larvae were still seen to discolour over time and showed signs of shrivelling. Larval test groups of *Sarcophaga* sp. showed success in all 10% ethanol increases starting from 10% as well as 30% ethanol concentration. Generally slower dehydration, however, proved to be problematic over longer time intervals, with discolouration seen after 48 hours of each trial. This may have been due to the elongated time in which larvae were exposed to low concentrations of ethanol which resulted in the rotting of larval tissue.

Little success was observed during each preservation trial, with three out of the four tested species showing preservation failure and only *Sarcophaga* species larvae displaying successful preservation using both formaldehyde as well as ethanol solutions.

It is important to note that larval members of *Leptocera* were not tested due to the lack of larval specimens sampled off of the carcass. It is possible that members of *Leptocera* may not oviposit on the body and are present for reasons other than breeding on a below ground carcass.

The results obtained from the present study showed that presently preferred methods of preservation for field cases may not be suitable for burial species. However, the investigated preservation mediums could prove more successful if used in conjunction with a fixative solution.

Table 3.2.1.2: Results of dehydration experiment of 9 groups (n=15 p/group) of larval instars of each species of Diptera of the grave using incremental increases in ethanol concentration over time. This indicates average time of first discolouration (in hours), where applicable, as well as concentration level of first discolouration during dehydration process (in percentage values).

EtOH concentration increase and concentration range used	Species			
	M. scalaris	*C. tibialis*	*Muscina stabulans*	*Sarcophaga* sp.
10% increase, 2 hour intervals (30%-70%)	4 hrs; 50% conc.	4 hrs; 50% conc.	N/A	N/A
10% increase, 6 hour intervals (30%-70%)	12 hrs; 50% conc.	12 hrs; 50% conc.	N/A	N/A
10% increase, 12 hour intervals (30%-70%)	12 hrs; 40% conc.	12 hrs; 40% conc.	48 hrs; 70% conc.	N/A
10% increase, 2 hour intervals (10%-70%)	8hrs; 50% conc.	8 hrs; 50% conc.	12 hrs; 70% conc.	8 hrs; 50% conc.
10% increase, 6 hour intervals (10%-70%)	18 hrs; 40% conc.	18 hrs; 40% conc.	30 hrs; 60% conc.	N/A
10% increase, 12 hour intervals (10%-70%)	24 hrs; 30% conc.	12 hrs; 20% conc.	48 hrs; 50% conc.	N/A
5% increase, 2 hour intervals (10%-70%)	16 hrs; 50% conc.	18 hrs; 55% conc.	20 hrs; 60% conc.	N/A
5% increase, 6 hour intervals (10%-70%)	18 hrs; 25% conc.	36 hrs; 40% conc.	48 hrs; 50% conc.	48 hrs; 50% conc.
5% increase, 12 hour intervals (10%-70%)	24 hrs; 20% conc.	36 hrs; 25% conc.	72 hrs; 40% conc.	48 hrs; 30% conc.

Other methods that could be used for preservation may not be suitable for field use. For example, gluteraldehyde or glutaric dialdehyde is an excellent fixative as it penetrates tissue more speedily than formaldehyde (Bozzola & Russell 1992). However, the process of fixing with the specific chemical requires temperature control within a low narrow range and may prove difficult to execute outside of a laboratory environment. Gluteraldehyde requires a constant 4°C for fixing, washing in the buffer and dehydration for effective fixing of tissue. Gluteraldehyde also poses certain health risks as overexposure to fumes and direct skin contact may cause irritation and long-term health problems as the chemical is a known irritant, and is volatile and carcinogenic (Eltoum *et al*. 2001). Gluteraldehyde often requires secondary fixation in osmium tetroxide, especially in cases where electron microscopy analysis needs to be done (Bozzola & Russell 1992). However, osmium tetroxide may be used on its own for fixing specimens for long-term preservation.

Osmium tetraoxide is a highly volatile chemical that fixes specimens upon contact. Because of its volatility, it should be used with great care in a fume hood because the fumes will easily fix the conjunctiva of the eye as well as the nasal mucosa on contact. It is therefore not preferable for use in field cases as the chemical is toxic, volatile and carcinogenic, making it dangerous to use outside of laboratory environments (Eltoum *et al*. 2001).

3.2.2 Breeding of dipteran species of the grave

The difficulty in preservation discussed earlier in the chapter may present a problem in identifying larvae collected from a crime scene cases as larvae may degrade before analysis can take place.

An alternative method of identifying these larvae found on a crime scene cadaver is to keep sampled specimens alive and to breed the immature samples to adulthood. In contrast to the difficulty in preserving larval instars, larvae sampled from grave carcasses in the present study were successfully reared to adulthood using a breeding medium of 400 g of raw pig liver spread over a surface with a diameter of 30 cm lined with a 3 cm layer of soil from the study area. These breeding mediums were contained in a ventilated container and placed in a temperature controlled insectarium of 25°C (±1°C) with a relative humidity of 50%. The room was subjected to a 12 hour light and 12 hour dark photoperiod cycle during breeding trials. Soil was sprayed lightly with water every three days to prevent soil from drying out. Breeding medium bait was replaced with fresh bait when depletion of old bait was noted.

Table 3.2.2.1 shows breeding results using the breeding mediums over two generations. Larvae sampled from grave carcasses effectively represented the F_0 generation. Larvae of *M. scalaris, C. tibialis* and *M. stabulans* obtained during sampling were successfully bred to adult stage. According to results, potential forensically important species, *M. scalaris, C. tibialis* and *M. stabulans,* showed successful breeding to the F_1 generation. This points out the effective use of a pig liver breeding medium on rearing Diptera of the grave. Although members of *Leptocera* are shown, these specimens were only sampled as adults and no larvae of this species were found on grave carcasses.

Table 3.2.2.1: List of species used in the breeding test, showing the species which were successfully bred through to the second generation (F_2) marked by X.

Species name	F_0	F_1	F_2
Megaselia scalaris	X	X	X
Conicera tibialis	X	X	-
Leptocera sp.	X	-	-
Muscina stabulans	X	X	X
Sarcophaga sp.	X	X	X

Members of *M. scalaris* were observed displaying high levels of intraspecific competition once the colony had reached the F_1 generation. Flies reproduced from the F_0 generation and a larger number of individuals were seen by the adult F_1 generation, resulting in more adult individuals occupying the breeding net. The increased competition for breeding space, food source and water source among the higher number of individuals was seen to cause a higher mortality in adult specimens within the cage. The increased level of competition may also have driven the expansion of territory by *M. scalaris*. Adult *M. scalaris* individuals were seen to easily take over other colonies of Diptera of the grave when these specimens managed to escape through accidental holes in the breeding cage. This caused contamination of the *C. tibialis* breeding cage, which subsequently resulted in the outcompeting of *C. tibialis* individuals. No F_2 generation of *C. tibialis* was seen during the breeding trial, and may have been the result of interspecific competition with *M. scalaris*.

The main purpose of the breeding analysis was to test the viability of breeding below ground Diptera on a meat based breeding medium for the purpose of morphological analysis. As four out of the five species were successfully bred from the F_0 generation to adulthood on a meat based breeding medium, it can be deduced that these flies can be successfully bred under mild laboratory conditions (i.e. humidity: 50%; temperature: 25°C (±1°C)) on a carrion based breeding medium. It is, however, still unclear as to what the optimal conditions are for each species and further

investigation is necessary to clarify preferences. Larval mortality in general was noted for the purpose of this study, but the rate of mortality over time was not quantified as this was not the purpose of the investigation. Male and female ratios were not recorded. However, the success of breeding throughout the study is indicative of the fact that the male and female ratio was sufficient for breeding to the F_2 generation. Although breeding to the F_1 generation would be sufficient for identification, the F_2 generation was bred to prove the viability of breeding Diptera of the grave under laboratory conditions for the purpose of laboratory studies, as well as to prove the breeding capability of these flies on meat based breeding mediums over a longer time period. As many factors influence the on-going health of a colony (Gennard 2007; Byrd & Castner 2010), this can only be viewed as a preliminary result and further investigation will be needed to optimise methods of laboratory breeding of Diptera of the grave.

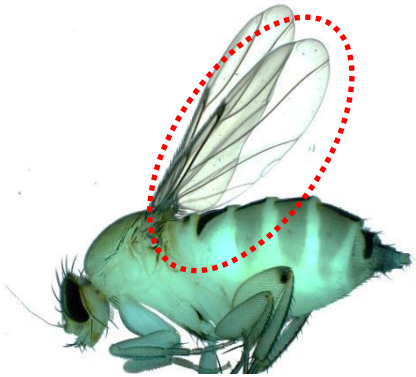
3.3 Section 2: Morphology of adult Diptera of the grave

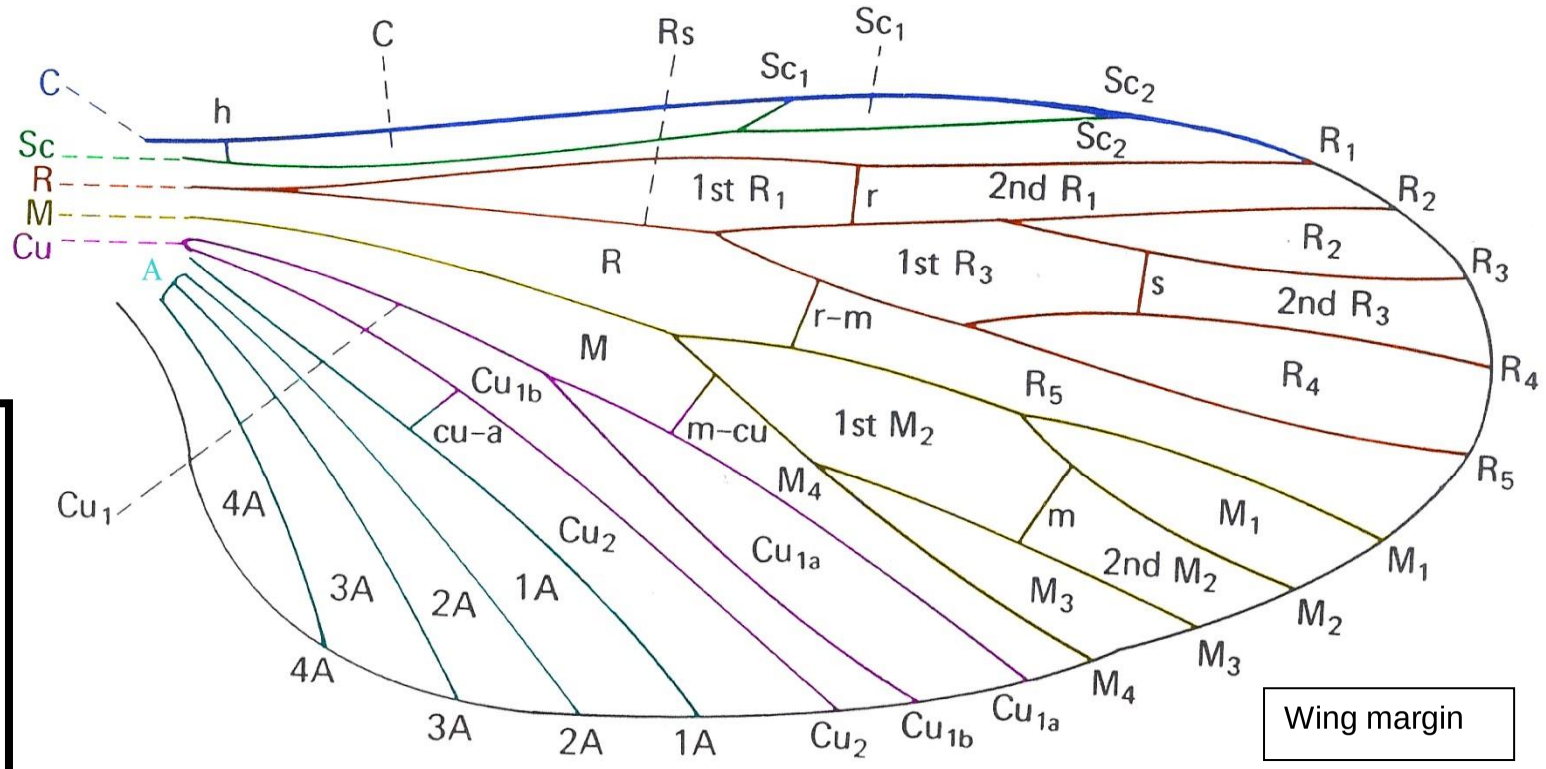
3.3.1 Basic morphology

Wing venation is one of the most common traits used to separate dipteran species down to family level (Triplehorn & Johnson 2005). In certain cases, wing venation can be used to separate genera within a family. Figure 3.3.1.1 shows a photomicrograph of the basic venation pattern of a dipteran wing from a dipteran species sampled during the study, as well as an interpretation of wing venation applicable to Diptera in general taken from the literature by Triplehorn & Johnson (2005), according to the Comstock-Needham naming system. This diagram shows all possible venation which can be present, or absent, in members of Diptera. The system works on the basis of recognising a series of six longitudinal wing veins. These are the costal vein (*C*) at the leading edge of the wing, followed by the subcostal vein (*Sc*), the radial vein (*R*), the medial vein (*M*), the cubital vein (*Cu*) and the anal vein (*A*). Each of these veins may be branched, with the exception of the costal vein. Veins that connect the major longitudinal veins are called crossveins, and are usually named as a combination of each vein system. For example, the crossvein m-cu is named the medio-cubital vein as it is a connection between the

medial and the cubital branch systems. Some crossveins have special names according to their position, such as in the case of the humeral crossvein (*h*) and the sectorial crossvein (*s*). Furthermore, each vein branch seen at the wing margin is named according to its primary longitudinal vein originating from the base of the wing. For example, vein R_2 is a branch of the main radial vein (*R*). These vein branches are usually named according to their position on the wing. For example, the second vein in the radial system is called the second radial vein or R_2.

Spaces between the veins, called cells, may be open (meaning that they extend to the wing margin), or closed (cells that are completely surrounded by veins). The cells are named according to the longitudinal vein on the anterior margin of the cell. For example, the open cell between the veins M_1 and M_2 is called the m_1 cell. When two cells that would ordinarily have the same name are separated by a crossvein, the two cells are designated by numbers. For example, the medial crossvein (*m*) connects veins M_2 and M_3 and divides the m_2 cells. The basal cell is then recognised as the first m_2 cell and the distal one is designated as the second m_2 cell. In cases where a cell is bordered anteriorly by a fused vein (e.g. vein R_{2+3}), it is named after the posterior component of that fused vein (vein R_{2+3} – cell r_3).



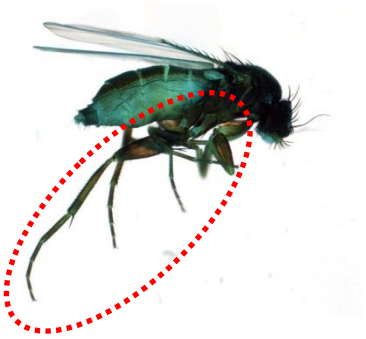


Vein system colours

Blue	-	Costal
Green	-	Subcostal
Red	-	Radial
Yellow	-	Medial
Magenta	-	Cubital
Cyan	-	Anal

Figure 3.3.1.1.: Female *Megaselia scalaris* (Loew 1866), showing the basic position and structure of a dipteran wing (top); A colour coded diagram of generalised wing venation according to the Comstock-Needham system (adjusted from Triplehorn & Johnson 2005) (centre). Colours denote each longitudinal vein.

Structures on the legs are also used to identify members of Diptera (Triplehorn & Johnson 2005). Figure 3.3.1.2 shows a diagram of a typical dipteran leg, adjusted from Triplehorn & Johnson (2005). The thoracic legs of Diptera are typically divided into six segments: the coxa (cx), which is the basal segment of the leg; the trochanter, shown as trc, which is the small segment following the coxa; the femur (fem), which is usually the first long segment of the leg; the tibia (tb), recognised as the second long segment; the tarsus (ts), which is usually a series of small subdivisions beyond the tibia; and the pretarsus (ptar), consisting of the claws and padlike structures (pulvilli) at the apex of the tarsus. The subdivisions of the tarsus are commonly called tarsal segments or tarsomeres.



Abbreviations:

cx	-	coxa
trc	-	trochanter
fem	-	femur
tb	-	tibia
ts	-	tarsus
ptar	-	pretarsus

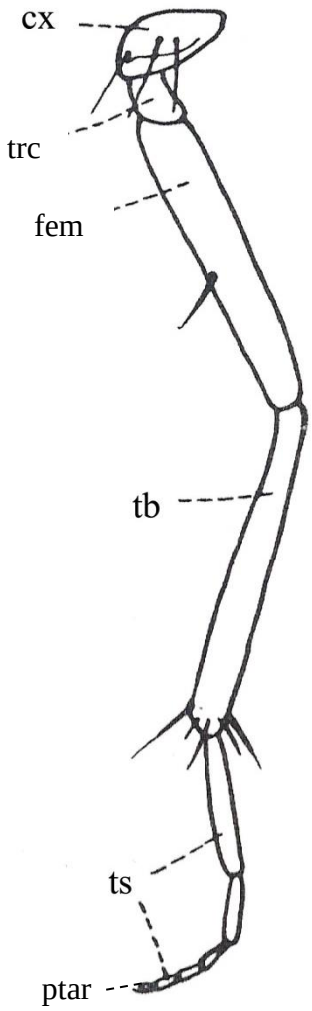
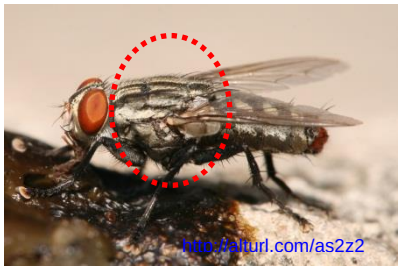


Figure 3.3.1.2: Photomicrograph of *Conicera tibialis* Schmitz 1925, showing the position of thoracic legs (left); diagram of the general morphology of a dipteran leg (adjusted from Triplehorn & Johnson 2005) (right).

Figure 3.3.1.3 shows the general chaetotaxy of a dipteran thorax, as viewed from a lateral angle (adjusted from Triplehorn & Johnson 2005). The thorax is characterised by several sclerotised plates and the corresponding bristles on certain plates are often used in identifying some members of Diptera. General areas used for their characteristic bristles are the notopleuron (npl) situated between the mesonotal plates, the pteropleuron (pt) found directly below the wing attachment point, and the hypopleuron (hypl) situated above the mid and hind coxae. Bristles are named after the section it is found on. For example, bristles found on the hypopleuron are called the hypopleural bristles.



Abbreviations:

n_2	-	mesonotum
npl	-	notopleuron
scl_2	-	mesoscutellum
pl_2	-	mesopleuron
pt	-	pteropleuron
epm_2	-	mesipimeron
pscl	-	postscutellum
stpl	-	sternopleuron
hypl	-	hypopleuron
cx_1	-	front coxa
cx_2	-	mid coxa
cx_3	-	hind coxa

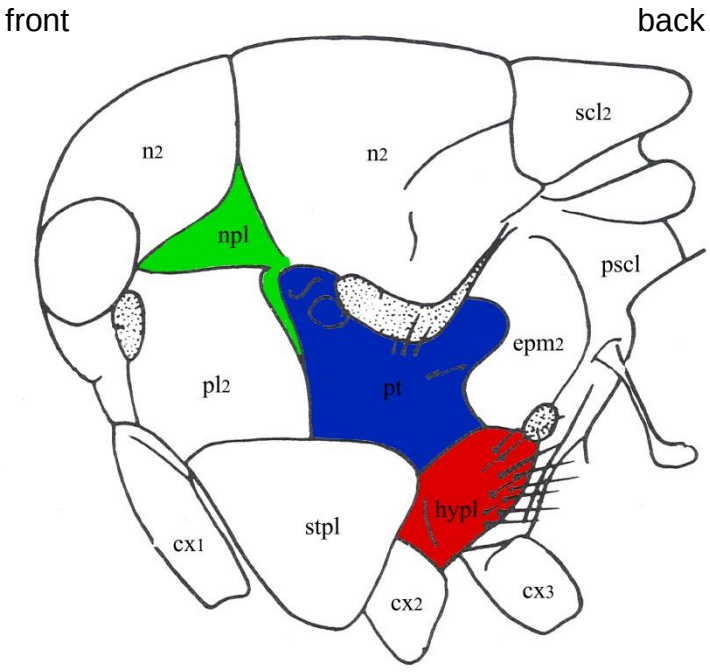


Figure 3.3.1.3: Photograph of *Sarcophaga* Meigen 1826 species, showing the position of the thorax (http://alturl.com/as2z2, accessed 22 December 2015)(top); a general diagram of a dipteran thorax, indicating the positions of the notopleuron (npl, green), the pteropleuron (pt, blue), and the hypopleuron (hypl, red) (Adjusted from Triplehorn & Johnson 2005) (bottom).

In specific cases, the tergites and external genitalia is used to separate members of Diptera. Characterisation of Phoridae species rely on structures such as the abdominal tergites, as well as external genitalia of male specimens (Disney 1983; Disney 1989; Disney *et al*. 2010). Figure 3.3.1.4 shows a typical diagram of a dipteran abdomen (adjusted from Disney 1989). The abdomen is subdivided into segments and the dorsal plates are called tergites. Each tergite is named and numbered according to its position away from the thorax. For example, the tergite closest to the thorax is called tergite 1. In many cases, the tergites are denoted by a number only in diagrams.

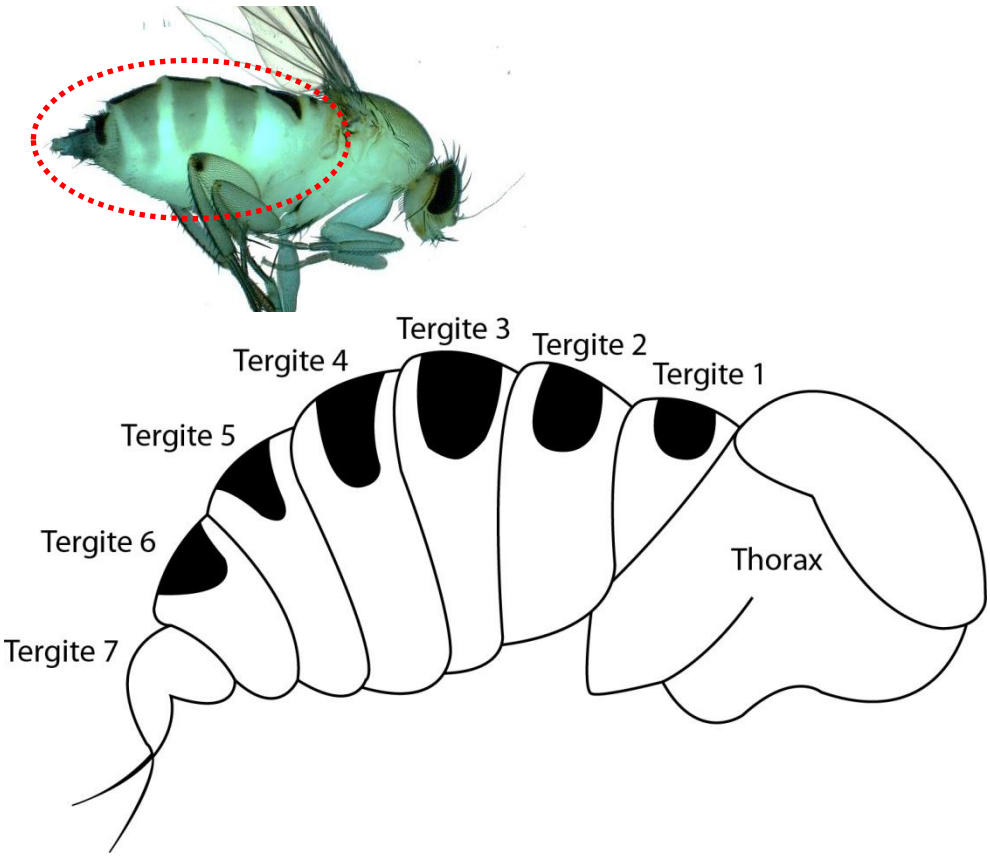
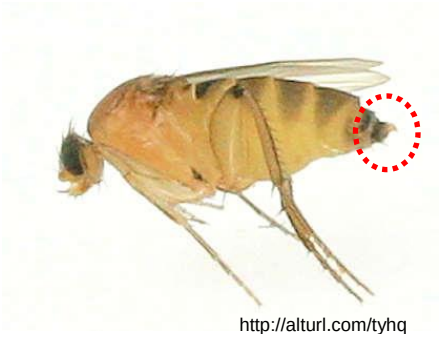


Figure 3.3.1.4: Female *Megaselia scalaris* (Loew 1866), showing the lateral view of the abdomen (top); simplified diagram of a typical Phoridae thorax and abdomen (adjusted from Disney 1989) (bottom).

In some cases, tergite 7 may form part of the external genitalia structures, which is commonly seen in Phoridae males. Figure 3.3.1.5 shows the general structure of the male hypopygium of a typical Phoridae male (adjusted from Disney 1983). The male hypopygium may vary in complexity from species to species. The anal tube (or claspers) can be shortened or elongated in certain species of Phoridae. Characters

of the anal tube and the epandrium are commonly used in the identification of Phoridae males (Disney 1983; Disney *et al*. 2010).



http://alturl.com/tyhq

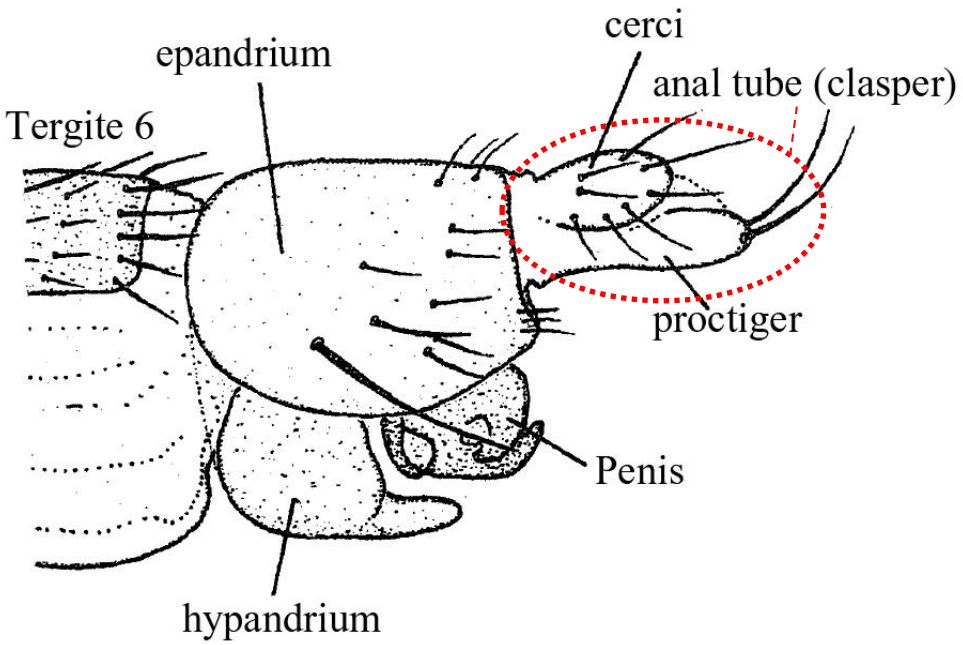


Figure 3.3.1.5: Male *Megaselia scalaris* (Loew 1866), showing the position of the hypopygium (www.opsu.edu, accessed 22 December 2015) (top); diagram showing the lateral view of the Phoridae male hypopygium, indicating the anal tube circled in red (adjusted from Disney 1983) (bottom).

3.3.2: Morphology of adult Diptera of the grave

3.3.2.1: Morphology of sampled Sphaeroceridae specimens

Sphaeroceridae, also known as lesser-dung flies, are minute, black flies often found around manure and excretions (Pitkin 1988). The larvae are saprophagous and feed upon a variety of decomposing plant and animal matter. The family itself is cosmopolitan with several subfamilies and species occurring world-wide due to the shipping of cattle in the past (Pitkin 1988). There are over 1 300 species and about 125 genera with more still being discovered (Roháček *et al*. 2001). Some species within this family, especially those within the genus *Leptocera*, are active during winter (Narchuk 1989).

The genus *Leptocera,* belonging to the family Sphaeroceridae and subfamily Limosininae, was first erected by Olivier as a monotypic genus for the species *Leptocera nigra* (Roháček *et al*. 2001). Olivier's paper, however, was unfortunately overlooked by many entomologists until 1888 when Mik re-examined the taxonomic position of the species and incorrectly synonymised the genus with *Limosina* Macquart 1835 (Roháček *et al*. 2001).

Having a worldwide distribution and currently containing 64 species, the genus *Leptocera* is commonly collected by dipterologists (Dong & Yang 2015). Papp (2012) found that the Afrotropical region contained a moderately diverse *Leptocera* genus, with approximately 14 species recorded.

Sampled male and female *Leptocera* sp. adults possessed an incomplete subcostal vein (*Sc)* not reaching the costal vein (*C*), with longitudinal veins shortened and not reaching the wing margin (Figure 3.3.2.1.1). The lower-calypter was also noted to be absent. The basal segment of the first hind tarsi was short, swollen and was noted to be shorter than the second segment (Figure 3.3.2.1.2). Such characteristics are descriptive of the family Sphaeroceridae (De Carvalho & De Mello-Patiu 2008). A distinct apical bristle on the first tarsomere of the mid leg was also present along with the distinct ventral preapical seta on the mid tibia (Figure 3.3.2.1.3). These characteristics are indicative of the genus *Leptocera* (Dong & Yang 2015). In the present study, wing and body measurements averaged 4 mm and 5.5 mm in length respectively (n=5) . Due to the required knowledge and skill necessary to accurately

dissect genitalia to identify *Leptocera* species, specimens were not identified down to species level.

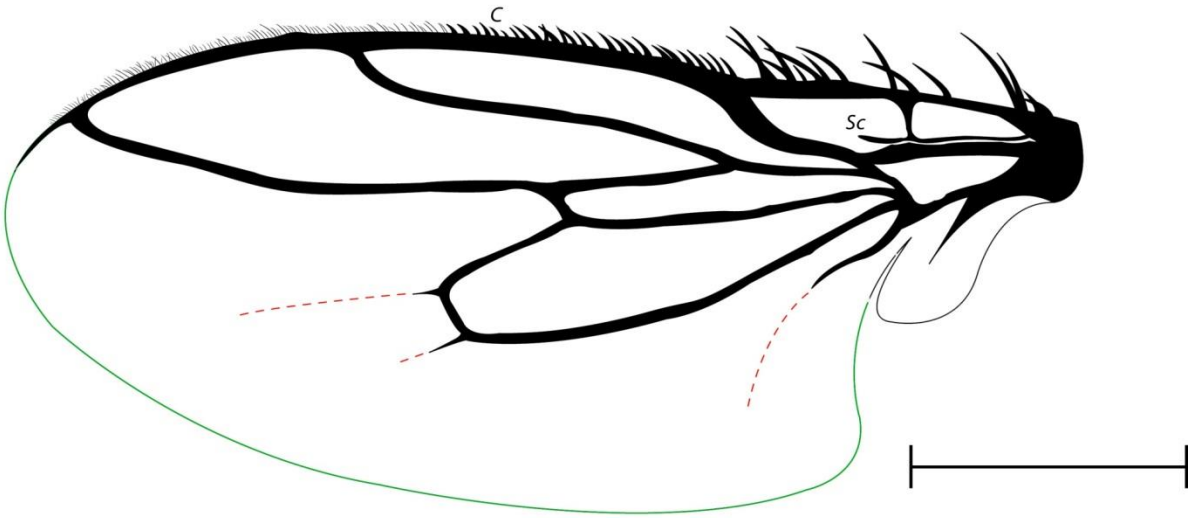


Figure 3.3.2.1.1: Wing venation of sampled *Leptocera* Olivier 1813 sp. specimens, with the subcostal vein (*Sc*) incomplete and not reaching the costal vein (*C*). The anal veins and medial veins (red dotted lines) do not reach the wing margin (green) (Scale = 1 mm).

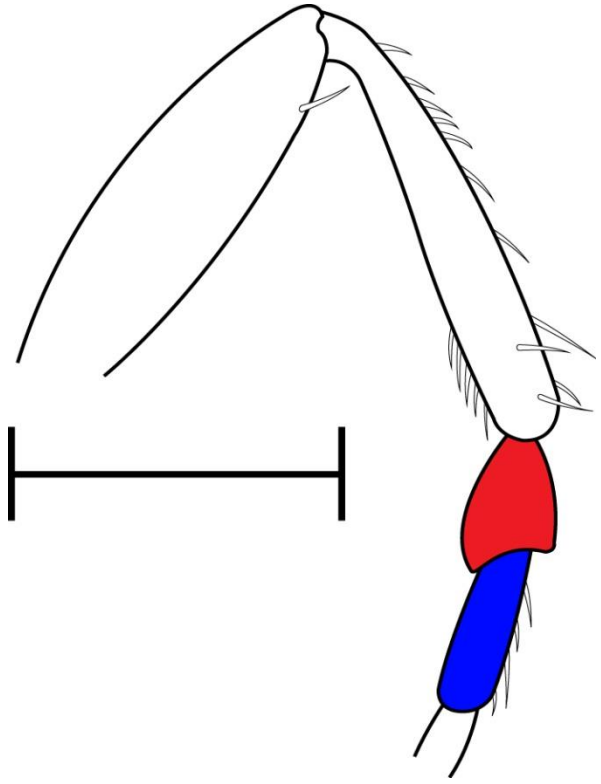


Figure 3.3.2.1.2: Hind leg of sampled *Leptocera* Olivier 1813 sp., showing basal segment of the first hind tarsomere (red) as short and swollen. Basal segment is also seen to be shorter than the second segment (blue) (Scale = 1 mm).

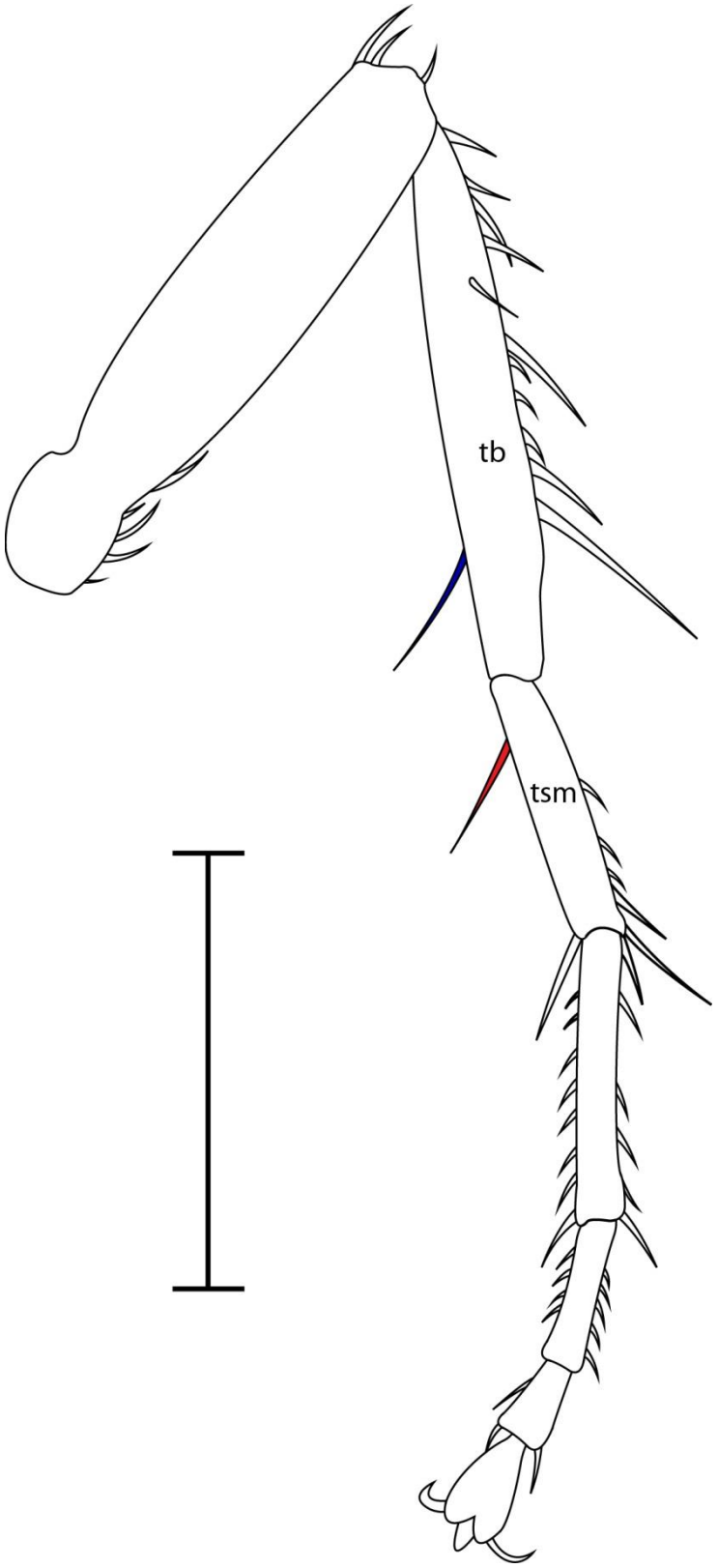


Figure 3.3.2.1.3: Mid leg of sampled *Leptocera* Olivier 1813 sp., with a single apical seta (red) on the first tarsomere (tsm), and a distinct ventral preapical seta (blue) on the tibia (tb) (Scale = 1 mm).

3.3.2.2: Morphology of sampled Sarcophagidae specimens

Sampled male and female Sarcophagidae specimens were identified as belonging to the subfamily Sarcophaginae and the genus *Sarcophaga*. The family Sarcophagidae, commonly known as flesh flies, is widely distributed and contains approximately 2 510 extant species, most of which occur in warm climates (Vairo *et al*. 2011). Members of the subfamily Sarcophaginae frequent decaying matter and excrement, and are often utilised in forensic investigations of murder cases (Byrd & Castner 2010). Certain species are also known vectors of pathogens and have been found to cause myiasis in vertebrates (Zumpt 1965). Their role in the decomposition process of carcasses has made them important in forensic sciences (Byrd & Castner 2010).

Sampled *Sarcophaga* sp. specimens showed presence of crossveins on wings along with an enlarged lower-calypter. Radial cell 5 (r_5) was noted to be narrowed distally and the combined medial veins 1 and 2 (M_{1+2}) were seen to angle sharply towards the combined radial veins 4 and 5 (R_{4+5}). Radial vein 1 (R_1) was noted to be independent of the subcostal vein (*Sc*) (Figure 3.3.2.2.1). Another defining characteristic of this family (Triplehorn & Johnson 2005) is also the presence of three black stripes along the dorsal side of the thorax (Figure 3.3.2.2.2). Defining characteristics for the subfamily Sarcophaginae (McAlpine *et al.* 1981) include the notopleural region of the thorax possessing 3-4 bristles (2 primary and 2 subprimary) as well as bristles present on the pteropleuron (Figure 3.3.2.2.3) along with multiple elongated setae on the anterodorsal side of the hind tibia (Figure 3.3.2.2.4).

Length of the sampled specimens averaged 15 mm, wing length measured during analysis averaged 9.5 mm, while thorax length averaged 5 mm (n=6). Due to the required knowledge and skill necessary to accurately dissect genitalia to identify adult members of the *Sarcophaga* species, specimens were not identified down to species level. *Sarcophaga* specimens were identified to genus level using the larval morphological characteristics described by Zumpt (1972).

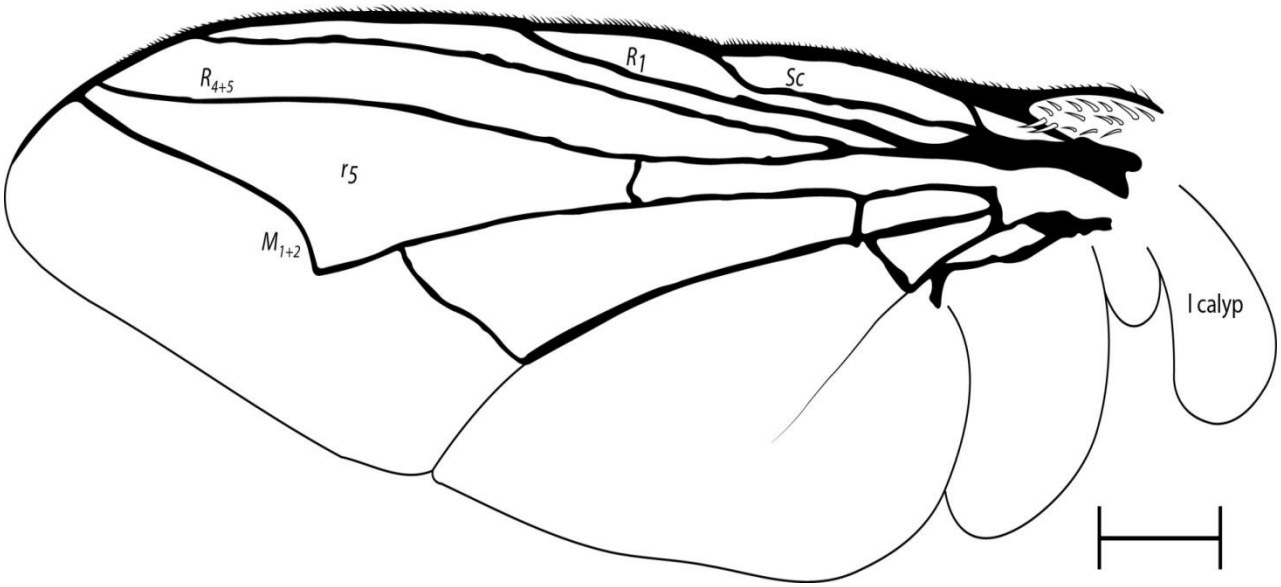


Figure 3.3.2.2.1: Wing venation of sampled *Sarcophaga* Meigen 1826 sp., with the radial cell 5 (r_5) narrowed distally. The combined medial veins 1 and 2 (M_{1+2}) are angled sharply towards the combined radial veins 4 and 5 (R_{4+5}). Radial vein 1 (R_1) is independent from the subcostal vein (*Sc*) and the lower-calypter (l calyp) is enlarged (Scale = 1 mm).

Figure 3.3.2.2.2: Dorsal view of the thorax of sampled *Sarcophaga* Meigen 1826 sp., showing three black stripes across the top of the thorax (Scale = 1 mm).

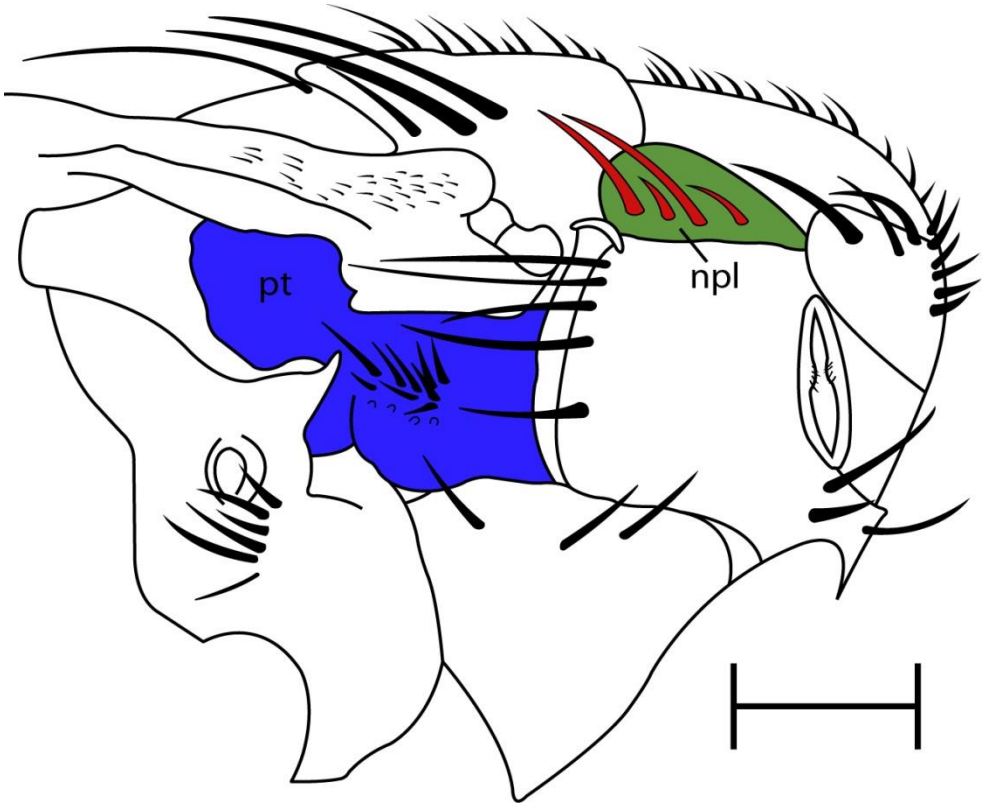


Figure 3.3.2.2.3: Lateral thoracic view of the thorax of sampled *Sarcophaga* Meigen 1826 sp., showing the notopleural region (green, npl) with two primary and two subprimary bristles (red). The pteropleural region (blue, pt) shows distinct bristles (Scale = 1 mm).

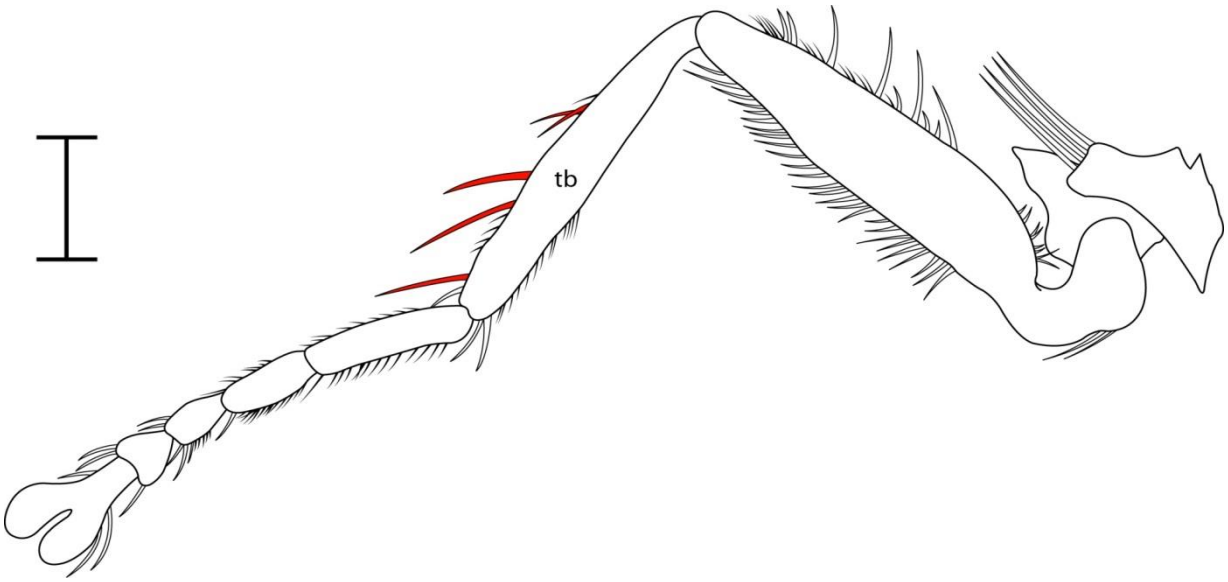


Figure 3.3.2.2.4: Hind leg of sampled *Sarcophaga* Meigen 1826 sp., showing multiple elongated setae (red) on the anterodorsal side of the hind tibia (tb) (Scale = 1 mm).

3.3.2.3: Morphology of sampled Muscidae specimens

The family Muscidae is a large group with several species occurring worldwide (Service 2012). The family Muscidae has been recorded as possessing roughly 58 Afrotropical genera (Couri 2007). All muscid specimens sampled were identified belonging to the subfamily Azeliinae and the genus *Muscina* Robineau-Desvoidy 1830. Specimens that were sampled during the trial were identified as the false stablefly *M. stabulans*. The false stablefly is distributed worldwide, often found around animal and poultry housing and excreta, and is commonly mistaken for the cosmopolitan house fly *Musca domestica* Linnaeus 1758 (Service 2012). Occurrences of intestinal myiasis in mammals by this species have been reported (Snyder 1955; Shivekar *et al*. 2008).

Analysis of sampled male and female specimens showed that the radial cell 5 (R_5) of the wing was not narrowed distally. The lower-calypter was seen to be enlarged and radial vein 1 (R_1) was noted to branch from the subcostal vein (*Sc*) (Figure 3.3.2.3.1). It was noted that the joint second cubitus vein and second anal vein ($Cu_2 + A_2$), as well as the third anal vein (A_3) were short and were observed to fade out before reaching the wing margin (Figure 3.3.2.3.1). The third anal vein (A_3) is also not curved forward on a trajectory that would otherwise intersect with the combined second cubitus vein and second anal vein ($Cu_2 + A_2$) if extended (Figure 3.3.2.3.1). Unlike Sarcophagidae, the pteropleuron located on the thorax of *M. stabulans* specimens was bare of all bristles (Figure 3.3.2.3.2). A single elongated seta was present on the anterodorsal side of the hind tibia (Figure 3.3.2.3.3). These characteristics coincide with those of the family Muscidae (Couri *et al.* 2012).

Characteristics for *M. stabulans* in particular saw that the joint first and second medial vein (M_{1+2}) was not angled, but curved slightly. Although only a slight curve was observed, the joint first and second medial vein was noted to bend distinctly upwards towards joint radial vein four and five (R_{4+5}) (Figure 3.3.2.3.1). This characteristic in particular separates this genus from the other Muscidae genera, *Musca* Linnaeus 1758 and *Fannia* Robineau-Desvoidy 1830 (Service 2012). The thorax possesses four dark stripes, unlike Sarcophagidae that was found to typically have three dark stripes along the abdomen. Such characteristics are indicative of the species *M. stabulans* (D'Assis Fonseca 1968; Service 2012).

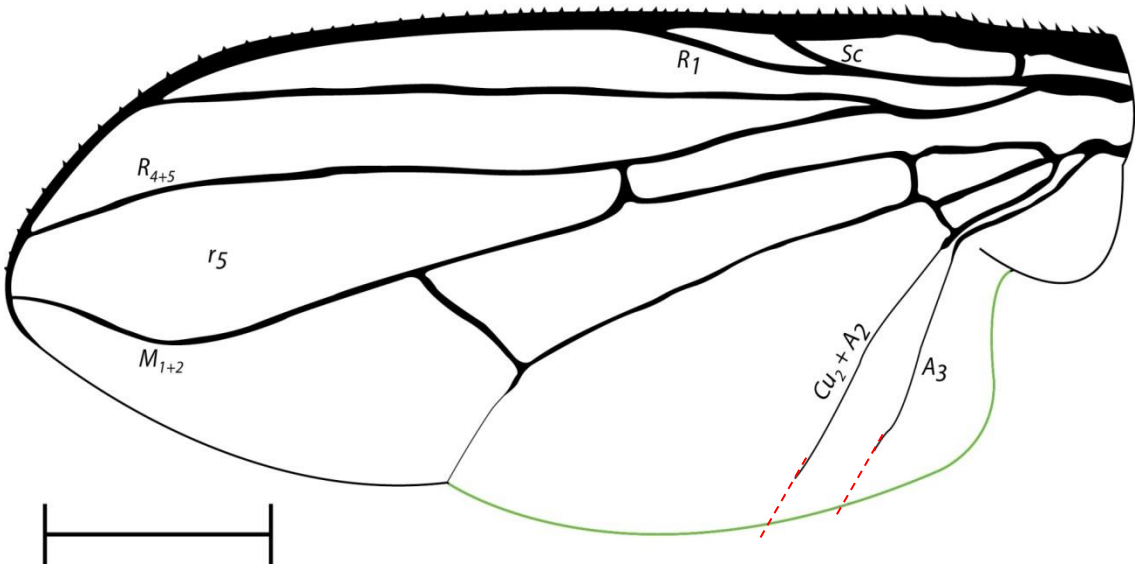


Figure 3.3.2.3.1: Wing venation of sampled *Muscina stabulans* (Fallén 1817) specimens with radial vein 1 (R_1) branching from the subcostal vein (*Sc*). Joint second cubitus vein and second anal vein ($Cu_2 + A_2$), as well as third anal vein (A_3) are both short and fade before the wing margin (green). The third anal vein (A_3) does not curve on a trajectory that would intercept previous vein if extended (extension shown by red dotted line) (Scale = 1 mm).

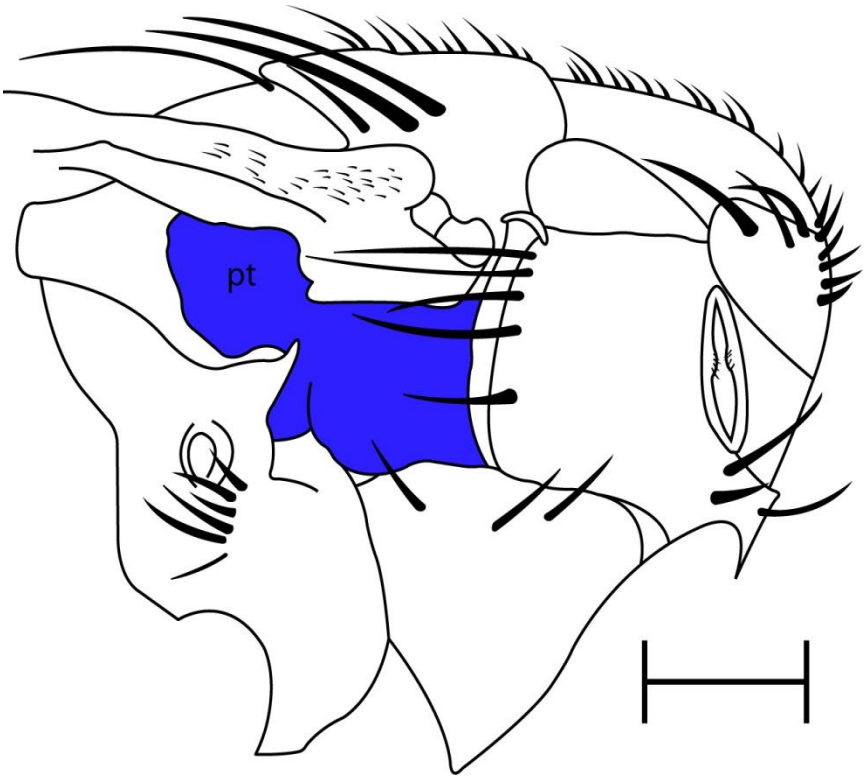


Figure 3.3.2.3.2: Lateral thoracic view of the thorax of sampled *Muscina stabulans* (Fallén 1817), showing no bristles on pteropleural region (blue, pt) (Scale = 1 mm).

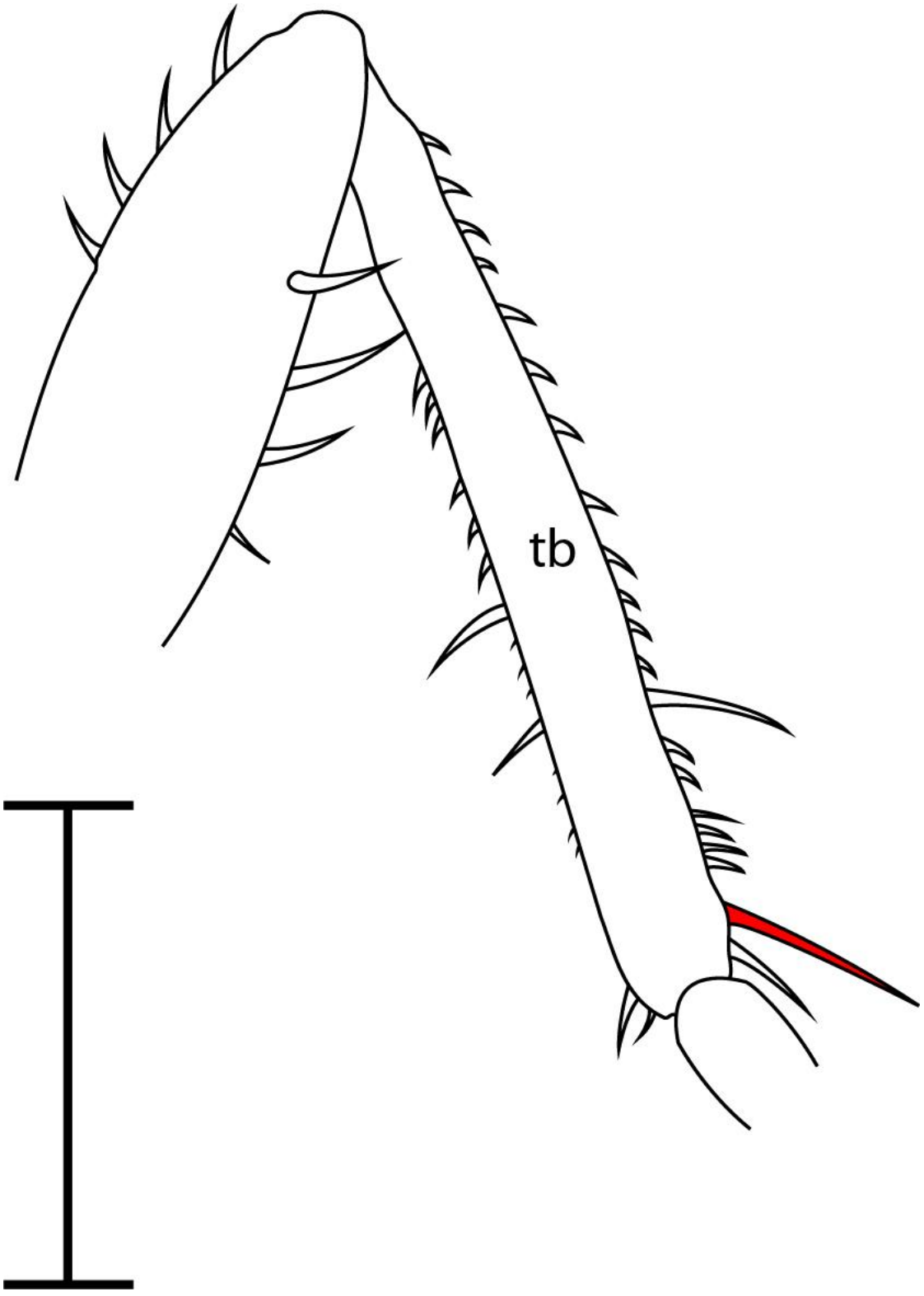


Figure 3.3.2.3.3: Hind leg of sampled *Muscina stabulans* (Fallén 1817), showing a single elongated seta (red) present on the anterodorsal side of the tibia (tb) (Scale = 1 mm).

3.3.2.4: Morphology of sampled Phoridae specimens

Phoridae, more commonly known as scuttle flies, are minute flies often associated with decaying material and have a worldwide distribution (Disney 2008). While a few thousand species have been identified from this family alone, incorporating almost all forms of feeding habits and habitats, it is estimated that over 30 000 species remain unidentified (Marshall 2012). With such a large biodiversity for one family, morphological differences become more diverse. Despite such a large taxonomic record only two species were identified interacting with buried carrion during this study. The two species were identified as *M. scalaris* and *C. tibialis*.

The genus *Megaselia* Rondani 1856 alone currently possesses over 1 500 species and is the most common within the family Phoridae, it has been hypothesises that this family may contain between 5 000 and 20 000 species (Disney 1989). *Megaselia scalaris* is a cosmopolitan species and is the most commonly sampled and recorded species of Phoridae. Cases of myiasis by this species have been reported due to its opportunistic lifestyle (Disney 2008).

The genus *Conicera* contains more than 20 species (Disney 1983). The species most associated with burial cases is *C. tibialis*. Its ability to colonise buried carrion in a short amount of time and at a depth of six feet has earned it the appropriate name of coffin fly (Martín-Vega *et al*. 2011). While a number of Phoridae species are associated with buried carrion, *C. tibialis* is considered as the true coffin fly and is widely distributed (Marshall 2012).

The family Phoridae possesses simplified wing venation with no crossveins present. Branches of the radial vein (*R*) are strongly thickened and crowded into the anterior base of the wing. Three to four weak veins with no crossveins were noted to run in parallel to the radial vein (*R*) beyond the base of the wing (Figure 3.3.2.4.1). The hind legs were noted to be long in comparison to the rest of the body, with femora flattened laterally. Adult flies are generally seen to have a large thorax and can be considered to have a humped-back appearance.

The genus *Megaselia* possesses a radial sector vein (*Rs*) that is forked, allowing radial vein 1 (R_1) to be present (Figure 3.3.2.4.1). The anterodorsal side of the hind tibia was noted to have no bristle-like hairs (Figure 3.3.2.4.2). In comparison, the

genus *Conicera* does not possess a branching-off radial vein 1 (R_1) due to the radial sector vein (*Rs*) remaining unforked (Figure 3.3.2.4.3). The hind tibia of *Conicera* was seen to have two true dorsal bristles and one anterodorsal bristle near the upper one (Figure 3.3.2.4.4).

The species *M. scalaris* possesses unique attributes which separate males and females. Males were observed to possess a pointed anal tube on the hypopygium (Figure 3.3.2.4.5). Females were noted to have an elongated and flattened tergite 6 on the abdomen (Figure 3.3.2.4.6). These are considered unique to *M. scalaris* (Disney 1989, Disney *et al*. 2010).

The species *C. tibialis* possesses unique attributes which separate males and females. The anal tube of the hypopygium of males was seen to form an irregular lobe which is rounded and not tapered (Figure 3.3.2.4.7). The mid femur of male specimens was also seen to possess a sense organ with a pit which is large and has a long apical process (Figure 3.3.2.4.8). Females were noted to have a distinct upper dorsal bristle on the hind tibia which is set below the anterodorsal bristle that is usually longer than bristle just below middle quarter (Figure 3.3.2.4.9). Females were also seen to have a distally pointed dorsal tergite 6 (Figure 3.3.2.4.10). These are considered unique to *C. tibialis*. (Disney 1983; Disney *et al*. 2010).

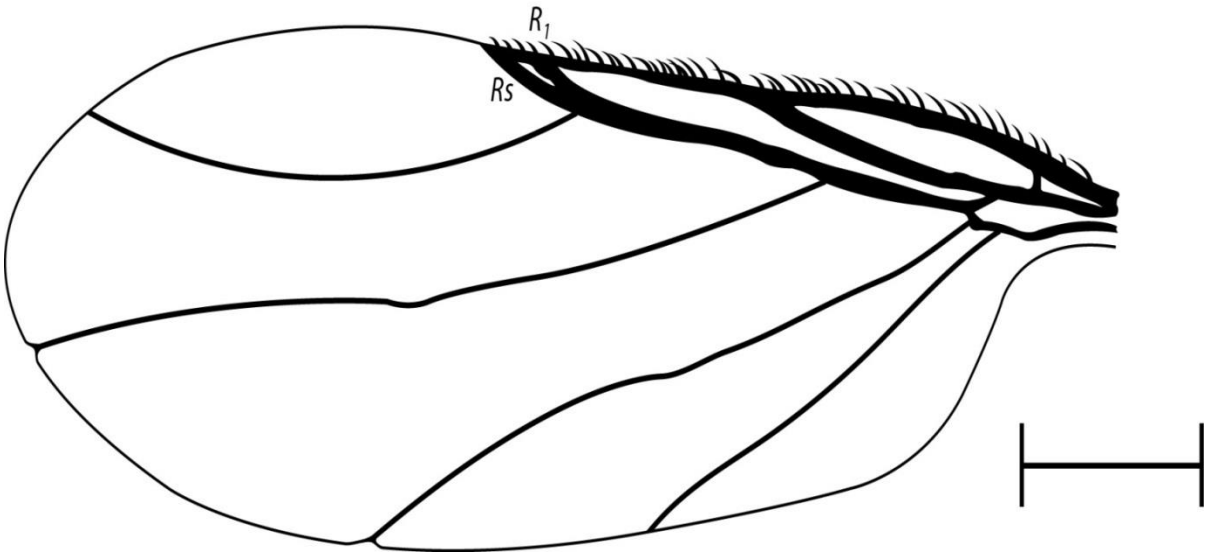


Figure 3.3.2.4.1: Wing venation of sampled *Megaselia scalaris* (Loew 1866) specimens, showing the radial sector vein (*Rs*) with the first radial vein (R_1) branching from *Rs*, forming a fork. All veins below *Rs* run in parallel to *Rs* (Scale = 1 mm).

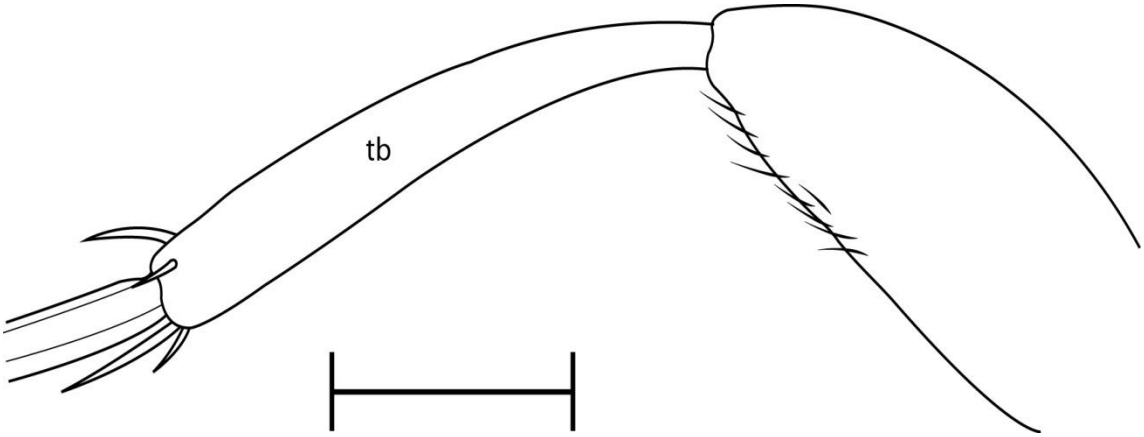


Figure 3.3.2.4.2: Hind leg of sampled *Megaselia scalaris* (Loew 1866) specimens, showing the anterodorsal side of the tibia (tb) being absent of bristle like hairs (Scale = 1 mm).

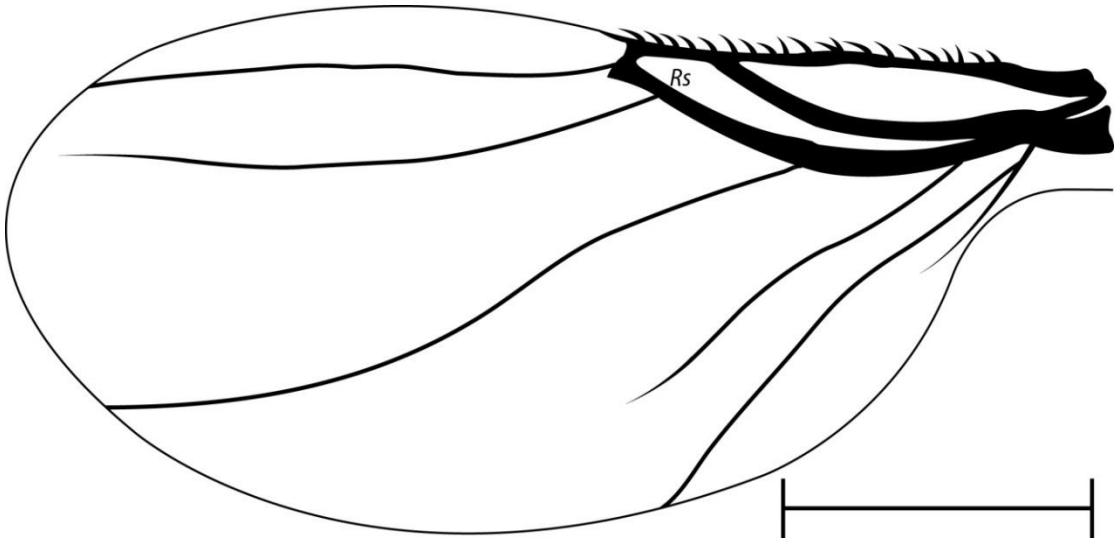


Figure 3.3.2.4.3: Wing venation of sampled *Conicera tibialis* Schmitz 1925 specimens, showing the radial sector vein (*Rs)* unforked, i.e. no veins are seen branching from *Rs* (Scale = 1 mm).

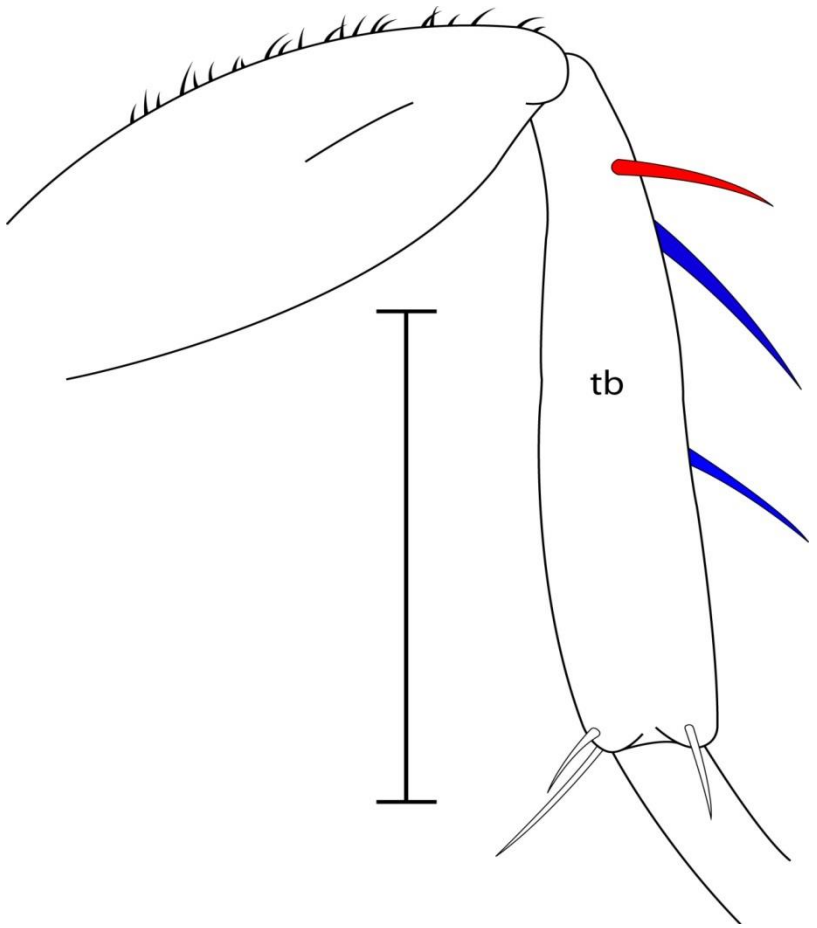


Figure 3.3.2.4.4: Hind leg of sampled *Conicera tibialis* Schmitz 1925 specimens, with two true dorsal bristles (blue) and one anterodorsal bristle (red) near upper one on tibia (tb) (Scale = 1 mm).

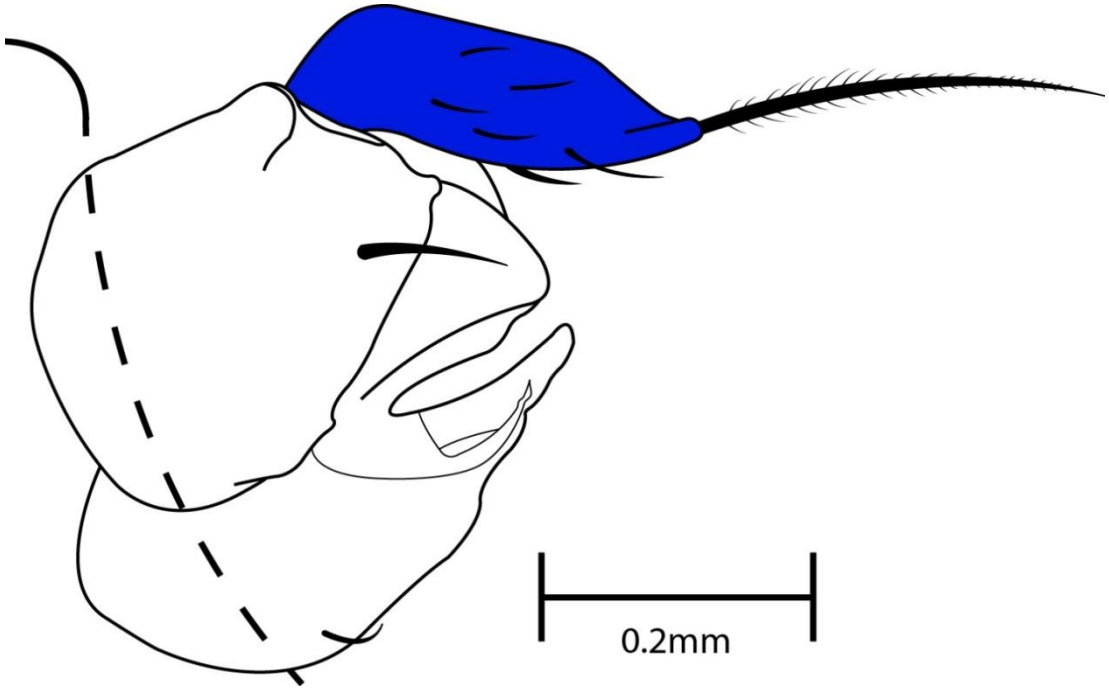


Figure 3.3.2.4.5: Lateral view of the external side of the hypopygium of sampled *Megaselia scalaris* (Loew 1866) male specimens, showing a pointed anal tube (blue) (Scale = 0.2 mm).

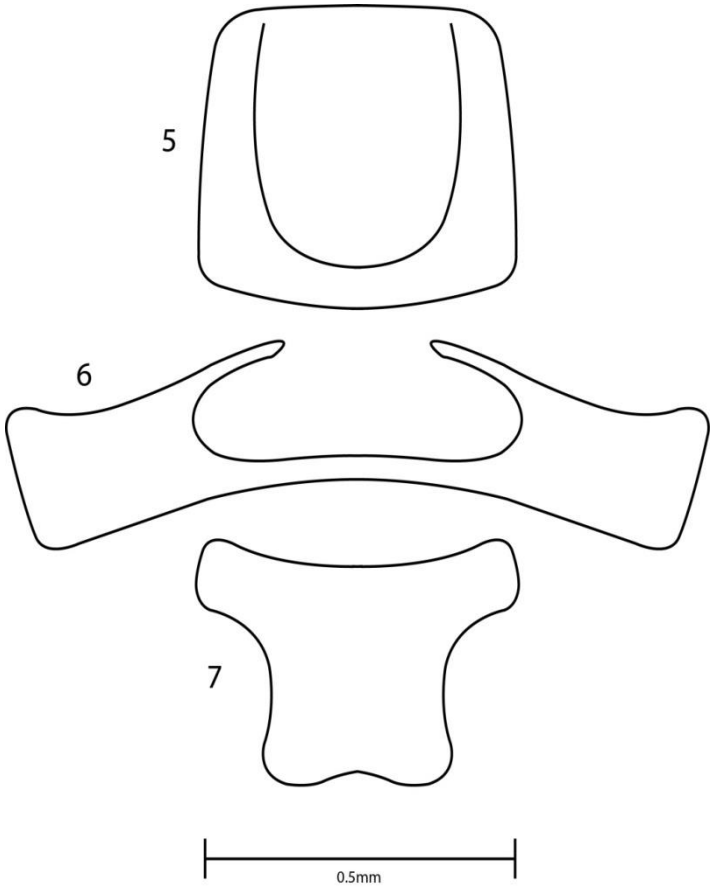


Figure 3.3.2.4.6: Dorsal view of the abdominal tergites of sampled *Megaselia scalaris* (Loew 1866) female specimens, showing tergite 6 (6) as elongated and flattened compared to tergite 5 (5) and 7 (7) (Scale = 0.5 mm).

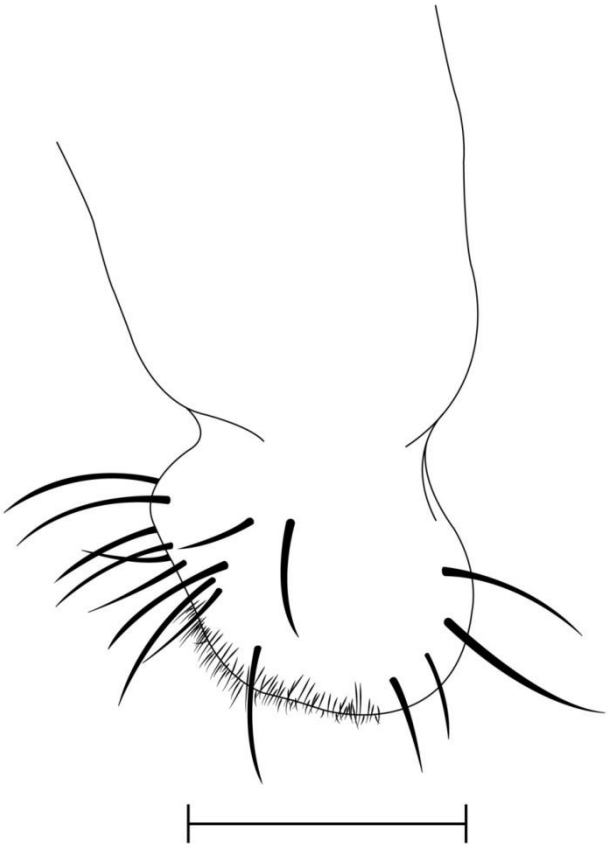


Figure 3.3.2.4.7: Anal tube of the hypopygium of sampled *Conicera tibialis* Schmitz 1925 male specimens showing an irregular and rounded lobe (Scale = 0.1 mm).

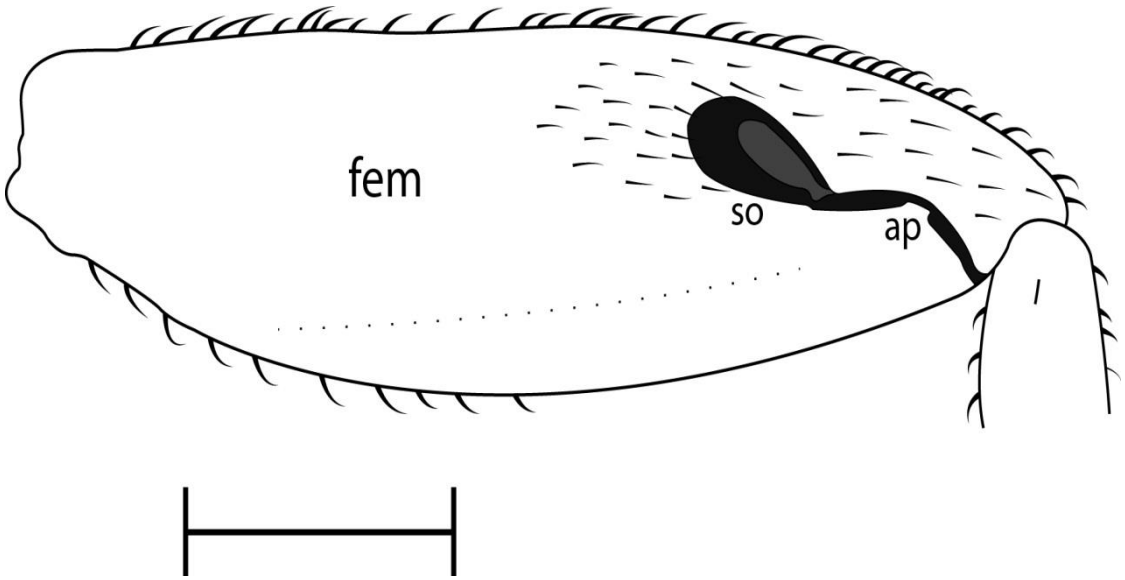


Figure 3.3.2.4.8: Mid femur (fem) of sampled *Conicera tibialis* Schmitz 1925 male specimens possessing sense organ (so) with large pit and long apical process (ap) (Scale = 0.3 mm).

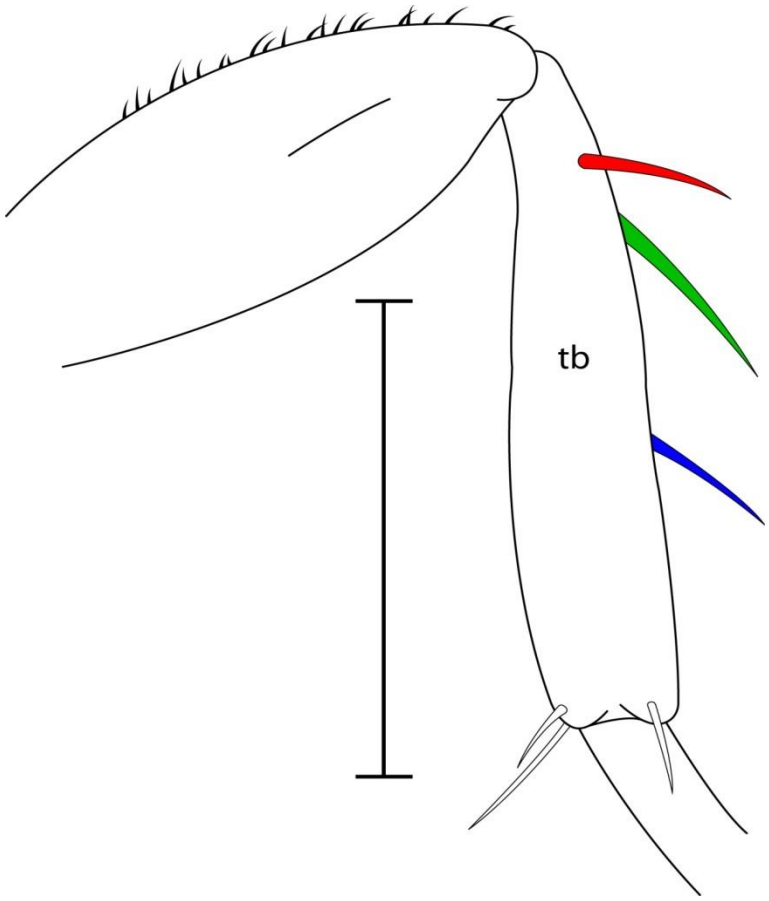


Figure 3.3.2.4.9: Hind leg of sampled Conicera tibialis Schmitz 1925 female specimens, showing the distinct upper dorsal bristle (green) on the hind tibia (tb) which is set below the anterodorsal bristle (red) that is usually longer than bristle just below middle quarter (blue) (Scale = 1 mm).

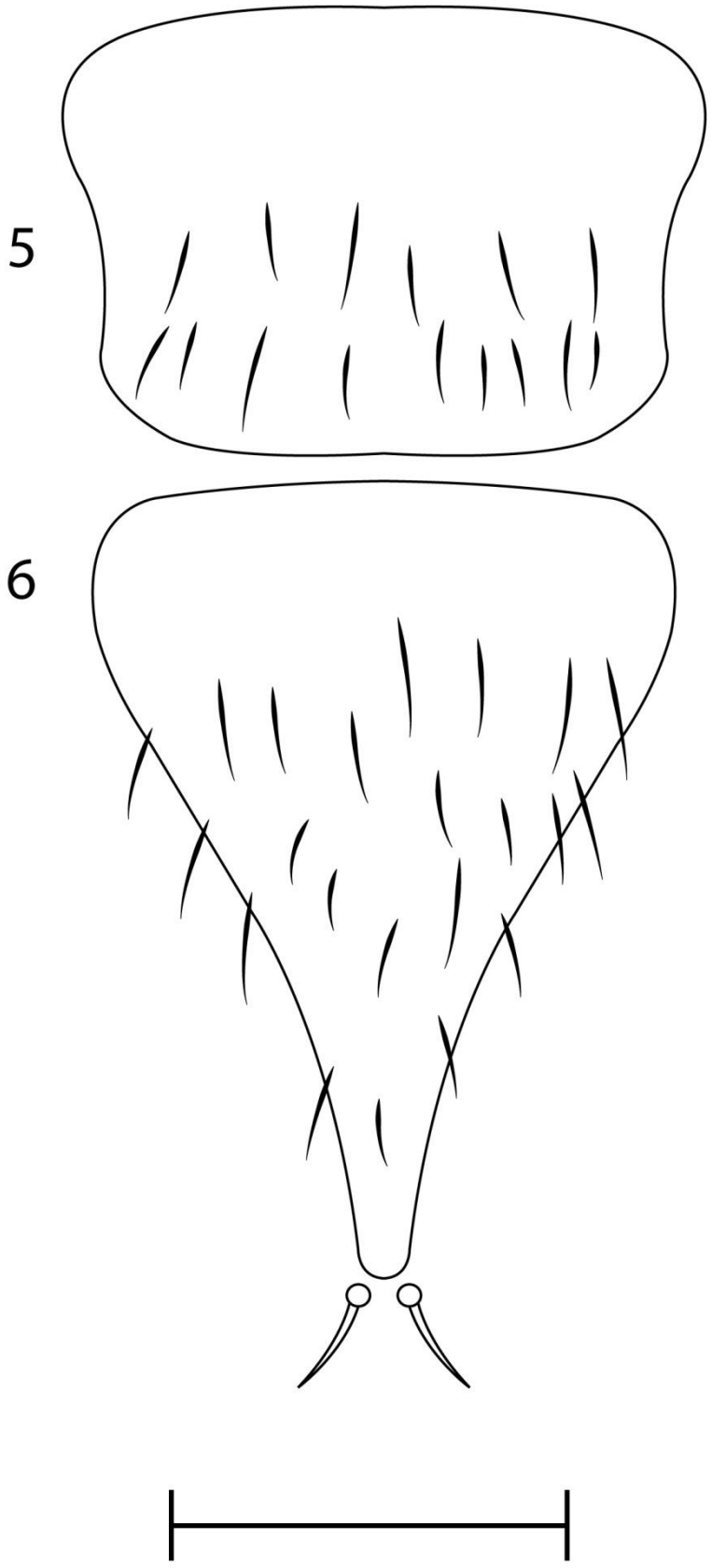


Figure 3.3.2.4.10: Dorsal tergites of sampled *Conicera tibialis* Schmitz 1925 female specimens showing tergite 6 (6) pointed distally (Scale = 0.2 mm).

3.4 Section 3: Pictorial key for use by investigators

One of the major objectives of identifying species of forensic importance is to establish ways to accurately identify these species when they are encountered during a forensic case. Many investigators that may encounter Diptera of the grave in a forensic case may have not had experience with these species. It is therefore imperative that a guided key is compiled.

For the purpose of practical use during crime scene cases, a simplified pictorial-guided key was compiled applicable to adult Diptera of the grave. This can be used to easily identify adult fly samples with the use of a light microscope and relatively simple dissection techniques. The key comprises of three sections, with section 3 divided into two sections, which are linked by distinct morphological characteristics, as was discussed in the previous section (Section 2) of this chapter.

It is important to note that this key is only applicable to Diptera of the grave found on clay soil buried carcasses in the Free State area, South Africa.

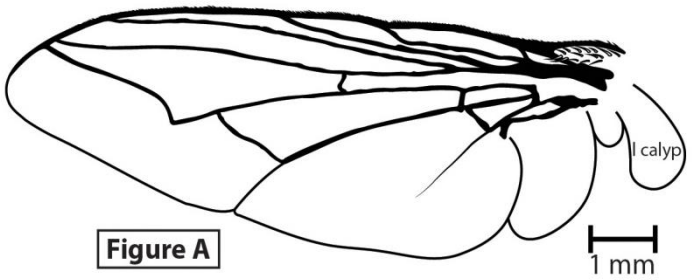


Figure A

DIPTERA OF THE GRAVE

Section 1

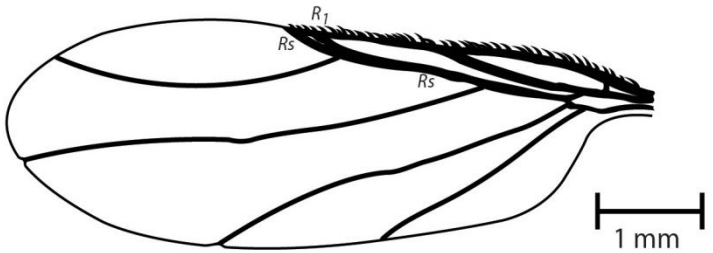


Figure B

- Wing venation not simplified, crossveins present (Figure A)
  - Incomplete venation, longitudinal veins (red) do not reach distal portion of wing (green), Lower-calypter absent (Figure C)
    - *Sc* vein of wing incomplete, not reaching *C* (Figure C); basal segment of hind tarsi (red) short, swollen, shorter than second segment (blue) (Figure D)
      - Family: **Sphaeroceridae**
        - Distinct apical bristle (red) on first tarsomere (tsm) of mid leg, distinct preapical seta (blue) on mid tibia (tb) (Figure E)
          - Genus: ***Leptocera***
  - Complete venation, veins reaching distal portion of wing, Lower-calypter (l calyp) enlarged (Figure A)
    - **Section 2**
- Simplified wing venation, no crossveins present, branches of *Rs* (*Rs* and R_1) strongly thickened and crowded into anterior base of wing (Figure B); thorax possesses humpbacked appearance
  - **Section 3**

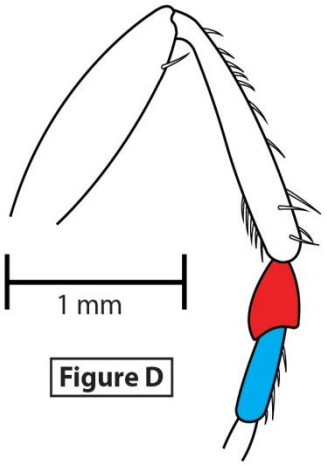


Figure D

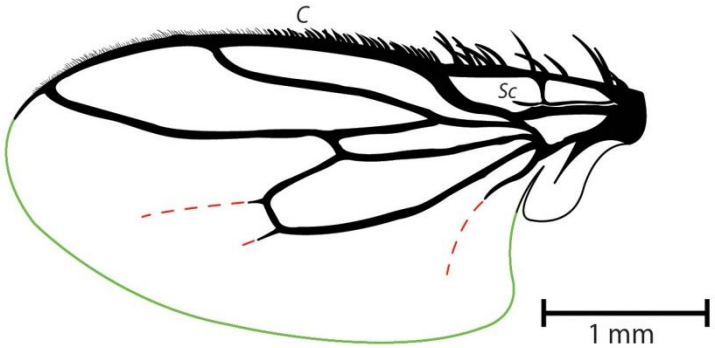


Figure C

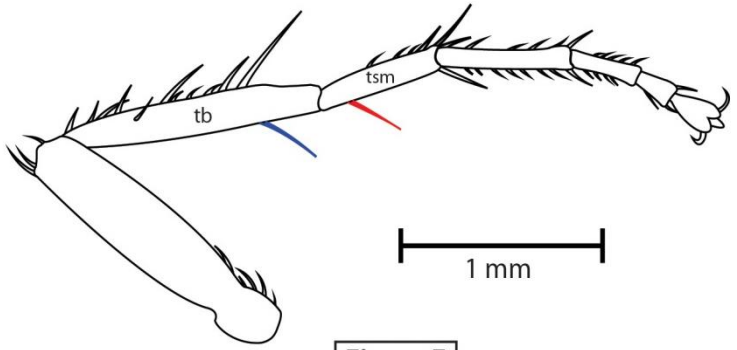


Figure E

DIPTERA OF THE GRAVE
Section 2

Calyptrate Muscoids

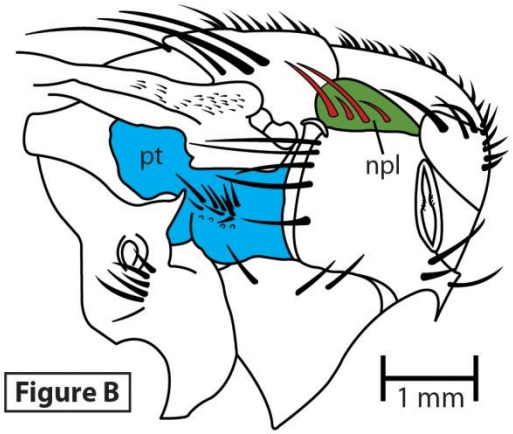


Figure B

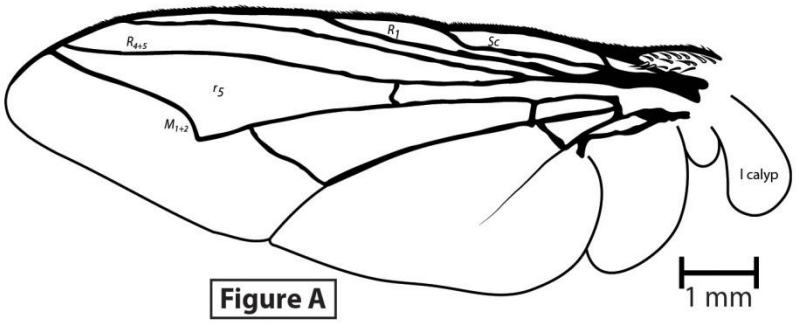


Figure A

R_1 vein branching from *Sc*, r_5 cell not narrowed distally, $Cu_2 + A_2$ and A_3 vein short and fading out before wing margin (green), vein *3A* not curved forward on a trajectory that could intersect $Cu_2 + A_2$ if extended (red) (Figure D); pteropleuron (pt) (blue) bare (Figure B); single elongated seta (red) on anterodorsal side of hind tibia (tb) (Figure E)

Family: **Muscidae**

M_{1+2} vein not angled sharply but curves slightly, but distinctly, towards R_{4+5} vein (Figure D)

Muscina stabulans

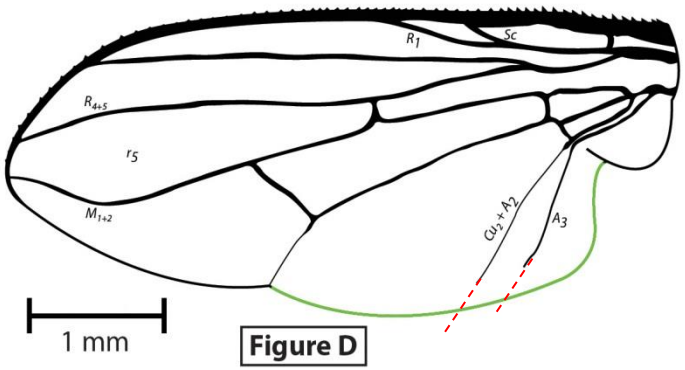


Figure D

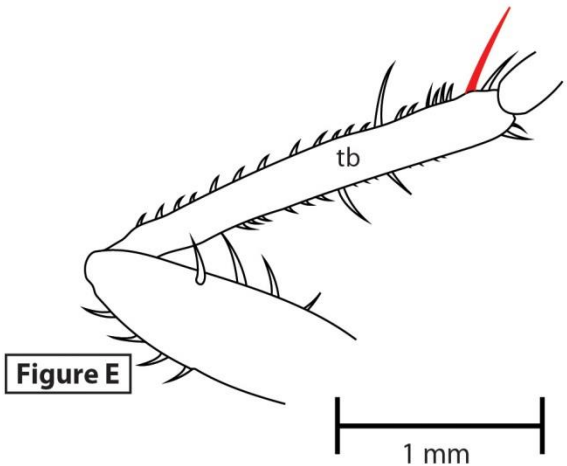


Figure E

R_1 vein independent of *Sc* vein, r_5 cell narrowed distally, M_{1+2} vein angled sharply towards R_{4+5} vein, (Figure A); three black stripes on dorsal side of thorax

Family: **Sarcophagidae**

3-4 notopleural (npl) bristles (2 primary, 2 subprimary), pteropleuron (pt) (blue) bristles present (Figure B); multiple elongate seta (red) on anterodorsal side of hind tibia (tb) (Figure C)

Subfamily: **Sarcophaginae**

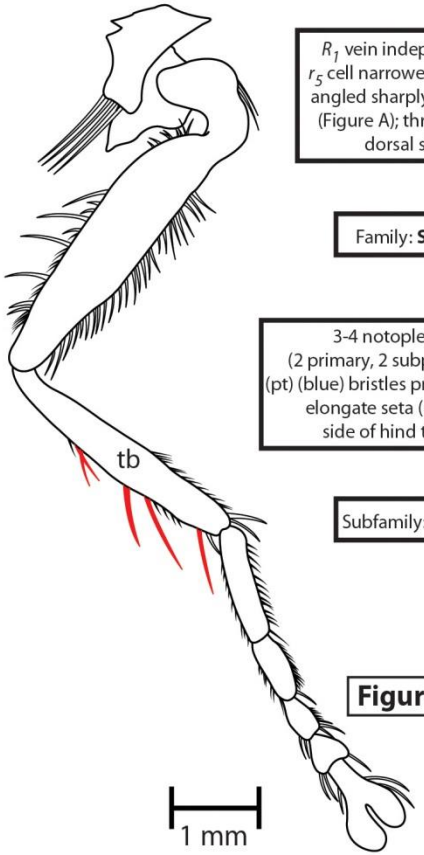


Figure C

DIPTERA OF THE GRAVE
Section 3

Family: **Phoridae**

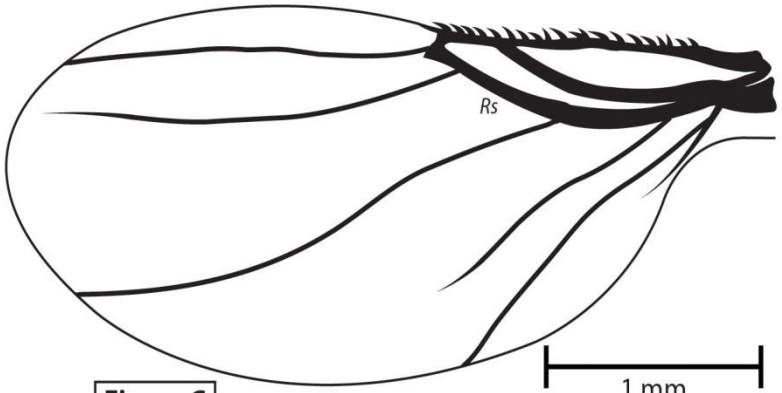


Figure C

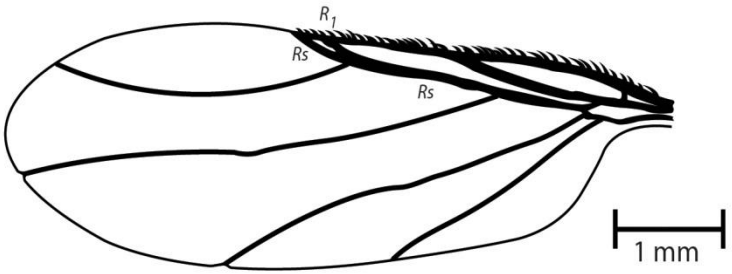


Figure A

Vein *Rs* unforked (Figure C); hind tibia (tb) with two true-dorsal bristles (blue) and one anterodorsal bristle (red) near upper one (Figure D)

Section 3A

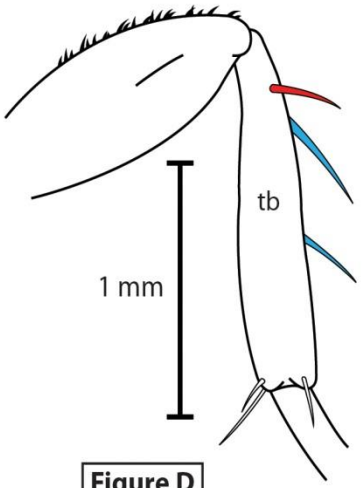


Figure D

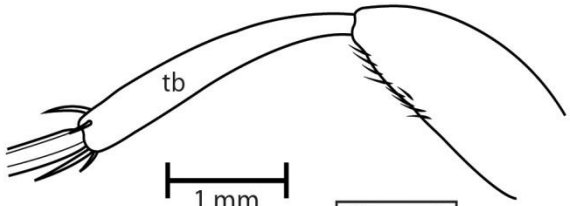


Figure B

Vein *Rs* forked to form veins *Rs* and R_1 (Figure A); anterodorsal side of hind tibia (tb) absent of bristle-like hairs (Figure B)

Megaselia scalaris

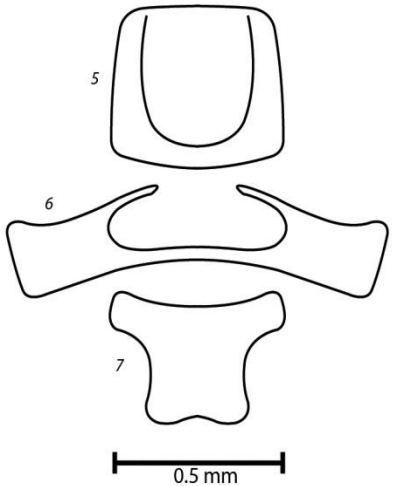


Figure F

Anal tube of hypopygium (at) pointed (Figure E)

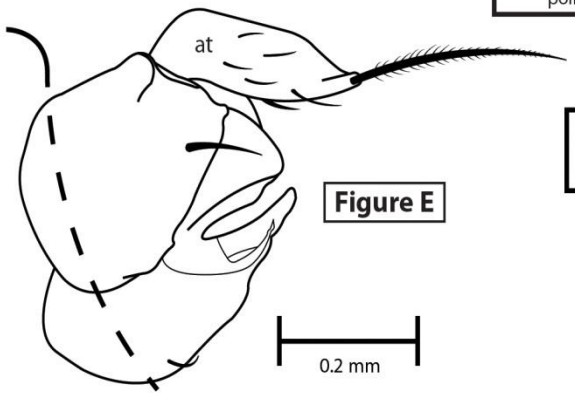


Figure E

Dorsal tergite 6 (*6*) elongated horizontally and flattened (Figure F)

DIPTERA OF THE GRAVE

Section 3A

Conicera tibialis

- Anal tube of hypopygium with irregular lobe, rounded and not tapered (Figure A); pit of sense-organ (so) on mid femur (fem) large and apical process (ap) long (Figure B) — ♂
- Upper dorsal bristle (green) of hind tibia (tb) below upper anterodorsal bristle (red) and usually longer than bristle just below middle quarter (blue) (Figure C); dorsal tergite 6 (*6*) pointed distally (Figure D) — ♀

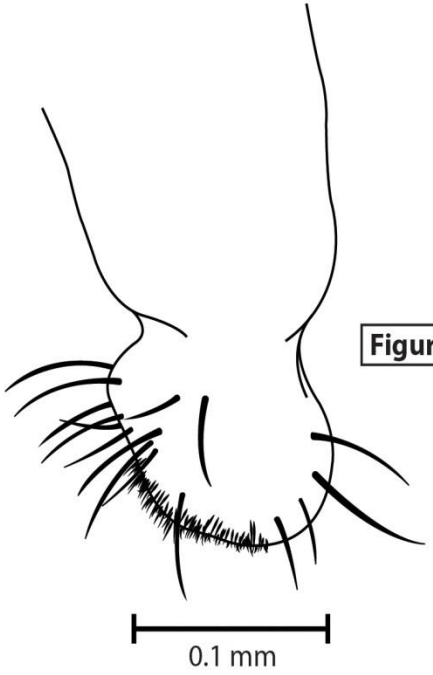


Figure A

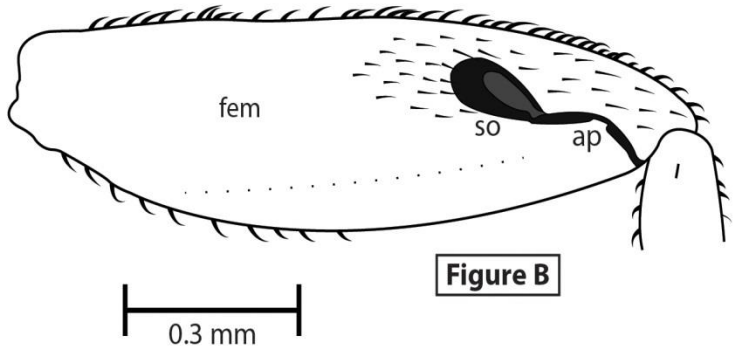


Figure B

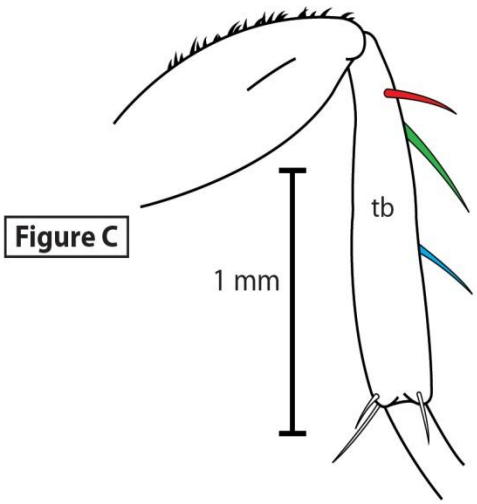


Figure C

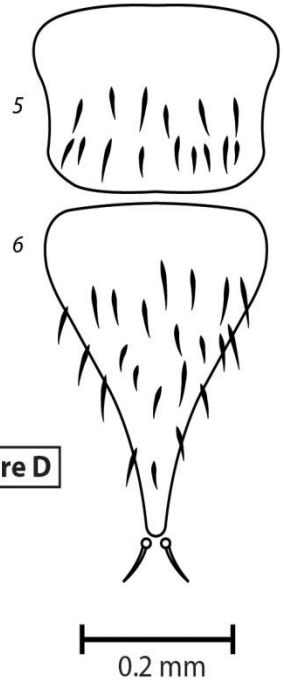


Figure D

Chapter 4: Seasonal Diptera succession patterns above and below ground

"Catch, then, oh catch the transient hour;
Improve each moment as it flies!
Life's a short summer, man a flower;
He dies - alas! how soon he dies!"
- Samuel Johnson. 1709-1784, Winter. An Ode

4.1 Background

The bulk of the studies done in forensic entomology have dealt with above ground decomposition and insect succession during summer months. This may be due to the prominence of forensic cases involving above ground cadavers. Many studies in the northern hemisphere concentrate on summer data as winter periods have been described as being too cold for insect activity. However, this may not be true for below ground environments. In an above ground study conducted in Tennessee, USA, by Miller (2002 unpubl.), insect activity was noted to be completely absent on human cadavers during winter experimentation, whereas, in a burial study in France conducted by Bourel *et al*. (2004), species such as *Leptocera caenosa* (Rondani 1880) (Diptera: Sphaeroceridae), *Ophyra capensis* (Wiedemann 1818) (Diptera: Muscidae) and *C. tibialis* (Diptera: Phoridae) was noted on buried bodies during winter trials. The species involved in the succession on a cadaver may change according to factors such as region, environment and season. However, many succession studies concentrate on analysing uncovered bodies and certain environmental factors have not yet been fully investigated (Bourel *et al.* 2004).

Summer usually shows an increased level of insect activity above ground as temperature increases and rainfall becomes more constant in the Free State area. Rainfall provides sufficient moisture for larval growth and development on a carcass (Byrd & Castner 2010). A fluctuation in number of individuals is commonly observed in the presence of a suitable food source (Turchetto & Vanin 2004; Kelly 2006 unpubl.; Byrd & Castner 2010), and often leads to the subsequent increase in predators and scavengers. Opportunistic predation of forensically important insects can have a substantial impact on insect succession of cadavers, affecting forensic analysis (Byrd & Castner 2010). An example of this is the effects of predation by *Solenopsis* Westwood 1840 spp. (Hymenoptera: Formicidae) on blowfly eggs and larvae, noted in a study by Stoker *et al*. (1995). The presence of predators on buried carrion is highly dependent on temperature, soil type and depth of the carcass (Anderson 1995; Turner *et al*. 2013). In literature by Byrd & Castner (2010), it was noted that opportunistic predators may alter the numbers of Diptera considerably on a cadaver. In contrast, the absence of predators may result in a larger number of Diptera surviving on a body and colonising a cadaver.

In order to understand the importance of species on a cadaver, it is important to document the interactions of species over time. Individuals within a species may change their behaviour according to their surroundings in order to survive under different circumstances. Variations in the nature of interactions are often noted as behavioural patterns shift to adjust to their surroundings (Wajnberg *et al*. 2008). Below ground environments can be considered as a highly variable environment as changes in soil type, pH, moisture levels and depth of the soil can affect the chances and even the rate of colonisation by insects (Carter *et al*. 2007; Byrd & Castner 2010; Carter *et al*. 2010). Due to this variation, Diptera that are commonly found on carrion may either adapt considerably to survive in the relatively anaerobic environment such as soil, or will not colonise carrion at all due to the inability to adapt to the below ground conditions (Pokines & Symes 2013). Many studies on forensically important Diptera have focussed on their above ground carrion interactions as well as the behaviour of above ground species (Greenberg 1990; Greenberg 1991; Mullen & Durden 2009; Benbow *et al*. 2015; Tomberlin & Benbow 2015). A few studies have looked into insects associated with buried carrion. In a study conducted by Pastula (2012 unpubl.) in Michigan, members of the families Sarcophagidae and Muscidae were found to colonise carrion at a soil depth of 30 cm after 5 days of burial, whereas representatives of *Hydrotaea* Linnaeus 1758 and *Megaselia* were found at 60 cm soil depth after 7 days after burial. However, soil environments vary between the northern and the southern hemisphere and between different biomes (Chesworth 2007), and interactions of forensically important Diptera of the grave, including the time it takes to colonise a body and interactions between species on buried bodies, may change according to the variation in soil environments (Byrd & Castner 2010). Although separate studies have been done for burial with concentration on one season, no studies have been done that contrast the effects of seasons on arthropod succession in burial cases.

In the summer pilot study conducted from 09/03/2013 to 29/03/2013 in the Free State area, four species of Diptera were found on carrion buried at 30 cm. These species included two representatives of the Muscidae, *M. stabulans* and *Hydrotaea capensis* (Wiedemann 1818), one representative of Calliphoridae, *C. vicina*, and one Sarcophagidae species, *Sarcophaga cruentata* Meigen 1826. The finding of these species on below ground carrion may point to the possibility of Diptera playing a

significant role in the succession of below ground carrion. However, this may change as soil depth increases and further investigation was needed to clarify the difference at a larger depth.

In this chapter, environmental conditions within the study area, dipteran succession on the above ground carcass, and the dipteran succession on buried cadavers over 120 days will be dealt with for the winter and summer trials, as well as a comparison between each season. This chapter will also encompass the below ground interactions, incidences of predation, the influence of soil conditions on species behaviour and reproductive strategies of burial Diptera in regards to factors that may affect dipteran succession.

Weather data results between day 60 and day 120 are not supplied due to the transition of seasons between this time and the fact that this transition may not be considered as a winter or summer trend. Environmental conditions noted on day 120, as well as successional data on day 120 for both above ground and below ground will be discussed to clarify the long-term dipteran activity on carcasses.

Grass and plant species within the area were not seen to affect the results of the below ground study as the bodies were buried beyond the rooting systems of these grasses and away from tree lines. Although certain bird species seen in the field are known to feed on insects and sometimes dipteran larvae, these birds were not observed to feed or linger around the graves during times in which field observations were made. Very few birds were seen around the above ground control carcass and were never observed to feed on any of the emerging insects. These factors were excluded from analysis during the winter and summer trials.

4.2 Section 1: The influence of shallow burial on dipteran succession during winter months

4.2.1 Environmental conditions during the winter trial

To fully understand the impact of environmental conditions during winter on dipteran succession, an overview of temperatures and rainfall data was necessary to see the full picture. Figure 4.2.1.1 gives an overview of midday ambient temperatures as well as rainfall recorded over the course of the 60 day trial period. Ambient temperatures fluctuated over the 60 day period, generally ranging between 11°C and 28°C. Only five rainfall days were observed during this period, with the most rainfall recorded at just less than 3 mm on a single day (Figure 4.2.1.1). Despite rainfall, it was not enough to penetrate the soil, thus leaving soil dry throughout the trial period (Figure 4.2.1.2). Temperature fluctuations could be attributed to the fact that the study was conducted in an open area and was exposed to direct environmental conditions without any enclosed areas, such as concrete buildings or thick vegetation, to conserve heat within the area over a longer period of time. This allowed for changes to happen quickly in response to wind and solar changes.

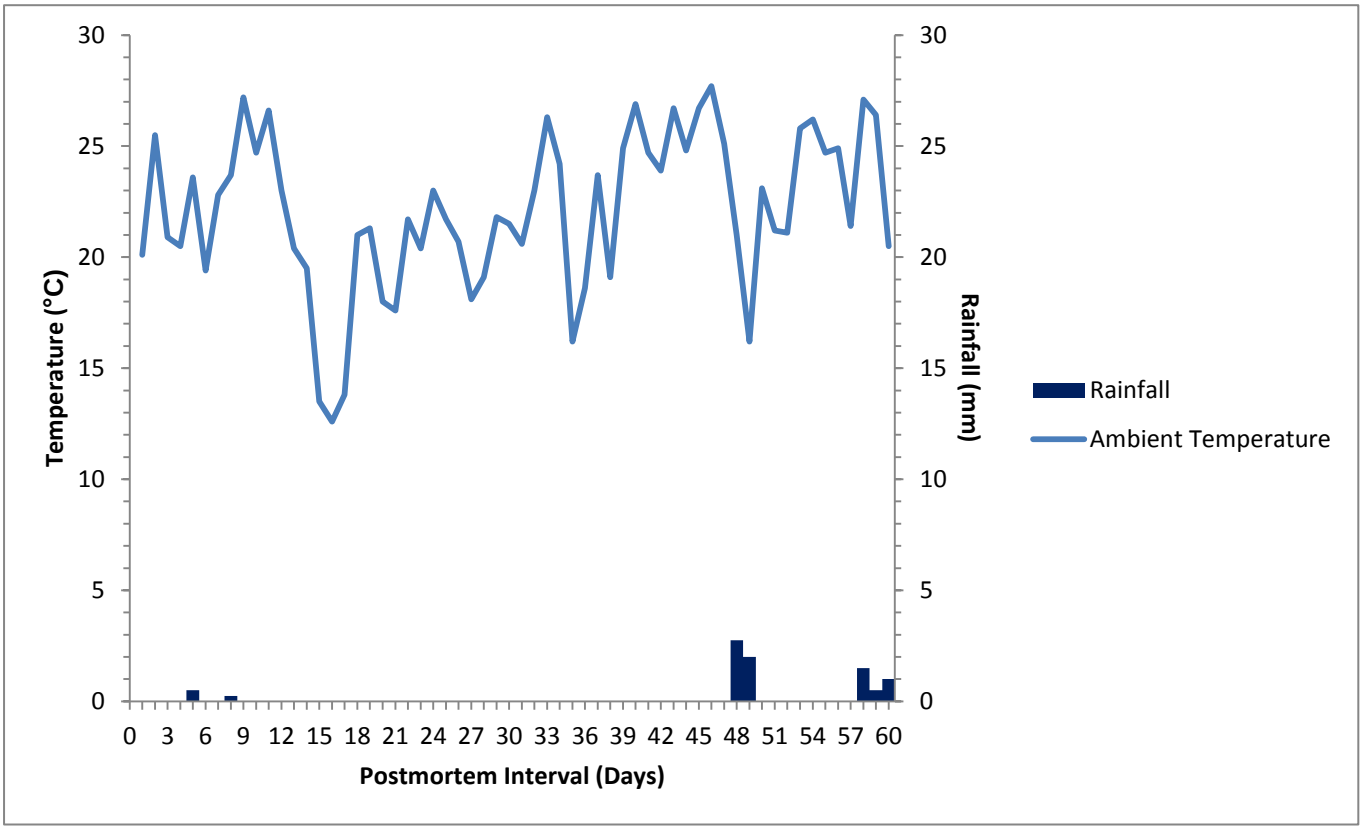


Figure 4.2.1.1: Recorded temperature and rainfall data taken at the winter study site over a period of 60 days.

Figure 4.2.1.2: Grave excavated to 30 cm deep, showing dry soil and surface of buried carcass.

Ambient temperature taken on day 120 was recorded at 36.7°C and was 14.6°C higher than the average winter temperatures previously recorded. Only one rainfall event was noted during the last 60 days of the trial, with a total of 3 mm recorded overall. No rain was noted on day 120 of the winter trial.

During the 60 day winter trial, average surface temperature was, on most days, 4.3°C higher compared to average below ground temperatures (Figure 4.2.1.3). The two occasions where this was not the case, namely day 7 (grave 2) and day 21 (grave 4), will be discussed consequently. Temperatures for grave 2 (day 7) showed a fairly constant temperature (average 14.4°C) in the soil around the body. An important observation is the increase in the body temperature (15.1°C-21.5°C) of the carcass in grave 2 (day 7) from grave 1 (day 3), which may be attributed to the decomposition process taking place and microbial action in the body resulting in the production of heat. This was the first occasion where the body temperature of the buried carcass was higher than the soil surface temperature. Excavation temperatures taken on day 14 (grave 3) displayed a gradual increase in soil temperatures (13.8°C-15.6°C) from the area above the body to below the body (Figure 4.2.1.3). Grave 4 (day 21) and grave 5 (day 30) showed similar results in regards to temperature around the body (Figure 4.2.1.3), with soil temperature increasing (average 12.1°C-16.4°C) closer to the body in comparison to temperatures taken at 30 cm depth (i.e. above the body). Body temperature taken on day 21 of the winter trial was observed to be 1.8°C higher than soil surface

temperature, being the second occasion where carcass temperature was higher than soil surface temperature. Body temperatures between the two graves show a slight 2.6°C increase over the seven day interval between the excavation days (Figure 4.2.1.3). An interesting observation was the increase in deep soil temperatures as well as body temperatures over time from day 30 (grave 5) onwards. Excavation temperatures taken on day 30 (grave 5) showed a 3.1°C increase in body temperature. This was seen to result in an average 2.5°C increase in soil temperature around the body, specifically below the body (Figure 4.2.1.3). Temperatures taken on day 60 (grave 6) showed similar results in regards to increased body temperature (16.9°C-20.0°C) and displayed slightly higher soil temperatures (average increase of 1.2°C) than that recorded on day 30 (grave 5). The final excavation on day 120 (grave 6) showed a further increase in body temperature (20°C-21.2°C) and slightly higher temperatures (average increase of 0.6°C) recorded in the soil around the body (Figure 4.2.1.3). Body temperatures of buried carcasses, with the exception of day 21 (grave 4) and day 30 (grave 5), were, on average, 3.2°C higher than that of the surrounding soil temperatures. The heat recorded from the buried carcasses may be the conserved heat produced by the decomposition process of the carcass and the gradual cooling of surrounding soil may explain the slightly lower temperatures recorded during the excavations.

Ambient temperatures taken during each excavation day was seen to coincide with surface temperatures taken from the grave on each excavation day. The drop in surface temperature from day 14 (grave 3) to day 21 (grave 4) (19.2°C-12.5°C) coincided with the decrease in ambient temperature (19.5°C-17.6°C) (Figure 4.2.1.3). Similarly, surface temperature on day 120 coincided with the 16.2°C increase in ambient temperature from day 60 to day 120. It can be deduced that ambient temperature influenced ground surface temperature during the winter trial.

On day 3 (grave 1), the grave carcass body temperature was 6.4°C lower than that of the above ground control carcass (Figure 4.2.1.3). Temperature of the grave carcass on day 7 (grave 2) showed a large increase in temperature (15.1°C-21.5°C) from day 3 (grave 1), whereas the above ground carcass body temperature showed a decrease in temperature (21.5°C-16.6°C) from day 3 to day 7. The above ground carcass body temperature was noted to drop from day 14 to day 21 (17.0°C-10.7°C), which coincided with the decrease of the ambient temperature (19.5°C-17.6°C) as

well as the soil surface temperature of grave 4 (day 21) (19.2°C-12.5°C) (Figure 4.2.1.3). However, it was noted that grave carcass body temperature on day 21 (grave 4) was 3.6°C higher than the above ground control carcass body temperature (Figure 4.2.1.3). An increase in the above ground carcass body temperature (10.7°C-21.1°C) was noted from day 21 to day 30, coinciding with the increase in ambient temperature (17.6°C-21.5°C) as well as soil surface temperature of grave 5 (day 30) (12.5°C-22.1°C).

A similar observation was made on the above ground carcass body temperature where a slight decrease in temperature (21.1°C-20.7°C) was observed from day 30 to day 60, which also coincided with the decrease in ambient temperature (21.5°C-20.5°C), but soil surface temperature of grave 6 (day 60) showed a very small increase of 0.2°C (Figure 4.2.1.3). Temperature of the grave cadaver on day 60 (grave 6) did not show a decrease in temperature from day 30 to 60 and rather showed an increase in temperature from day 30 to day 60 (16.9°C-20.0°C). Temperature taken from the above ground control carcass on day 120 shows an increase from day 60 to day 120 (20.7°C-32.1°C), which corresponded to the increase in ambient temperature (20.5°C-36.7°C) and soil surface temperature of grave 6 (day 120) (22.3°C-34.2°C) (Figure 4.2.1.3). Although the carcass of grave 6 showed a 1.2°C increase in temperature on day 120, the slight increase in temperature did not coincide with the relatively large increase of above ground ambient temperature, as well as the temperature taken from the above ground control carcass. Ambient temperature strongly influenced soil surface temperatures ($r=0.917$) and body temperatures of the above ground carcass ($r=0.884$). However, no specific relation pattern was noted between deeper soil temperatures and above ground ambient temperatures ($r=0.576$). Soil nearest to the grave carcasses showed a closer relation ($r=0.695$) to the temperatures taken from the grave carcasses on each excavation day compared to ambient temperatures ($r=0.621$). It can be deduced that ambient temperature did not influence the deeper grave temperatures but did influence soil surface temperatures. Body temperature taken from the above ground control carcass was not seen to coincide with the grave carcass body temperatures and no pattern was noted between the above ground control carcass and the below ground grave carcasses. Recorded surface temperatures for all graves show variation to below ground soil temperatures (Figure 4.2.1.3), thus

suggesting that solar radiation only affected the shallow levels of soil in the grave area.

Heat levels recorded around the below ground body may have been attributed to increased microbial activity and, in later stages of the study, may have been due to insect activity around the body. Moisture below the body may have also aided in conservation of heat as this section was not exposed to the initial low temperatures experienced above ground and little heat was transferred away from the body (Turner & Wiltshire 1999). However, some graves showed an increase in temperature nearest to the carcass, as can be seen during all excavation days (Figure 4.2.1.3). This shows a possible conservation of heat emitted by the decomposition process, and may be attributed to soil composition and compaction leading to less air circulating in the soil as was found by Turner and Wiltshire (1999). Moisture below the carcass, resulting from the seepage of fluid from the carcass, also caused mud to harden around the body, further sealing the carcass away and preventing heat transferral (Figure 4.2.1.4). Soil was generally seen to be “glued” to the body and had to be peeled away to reveal the body surface. The compact soil around the body may have aided in the conservation of heat. However, it is unclear at present as to the factors playing a role in the conservation of heat in an enclosed environment such as a grave.

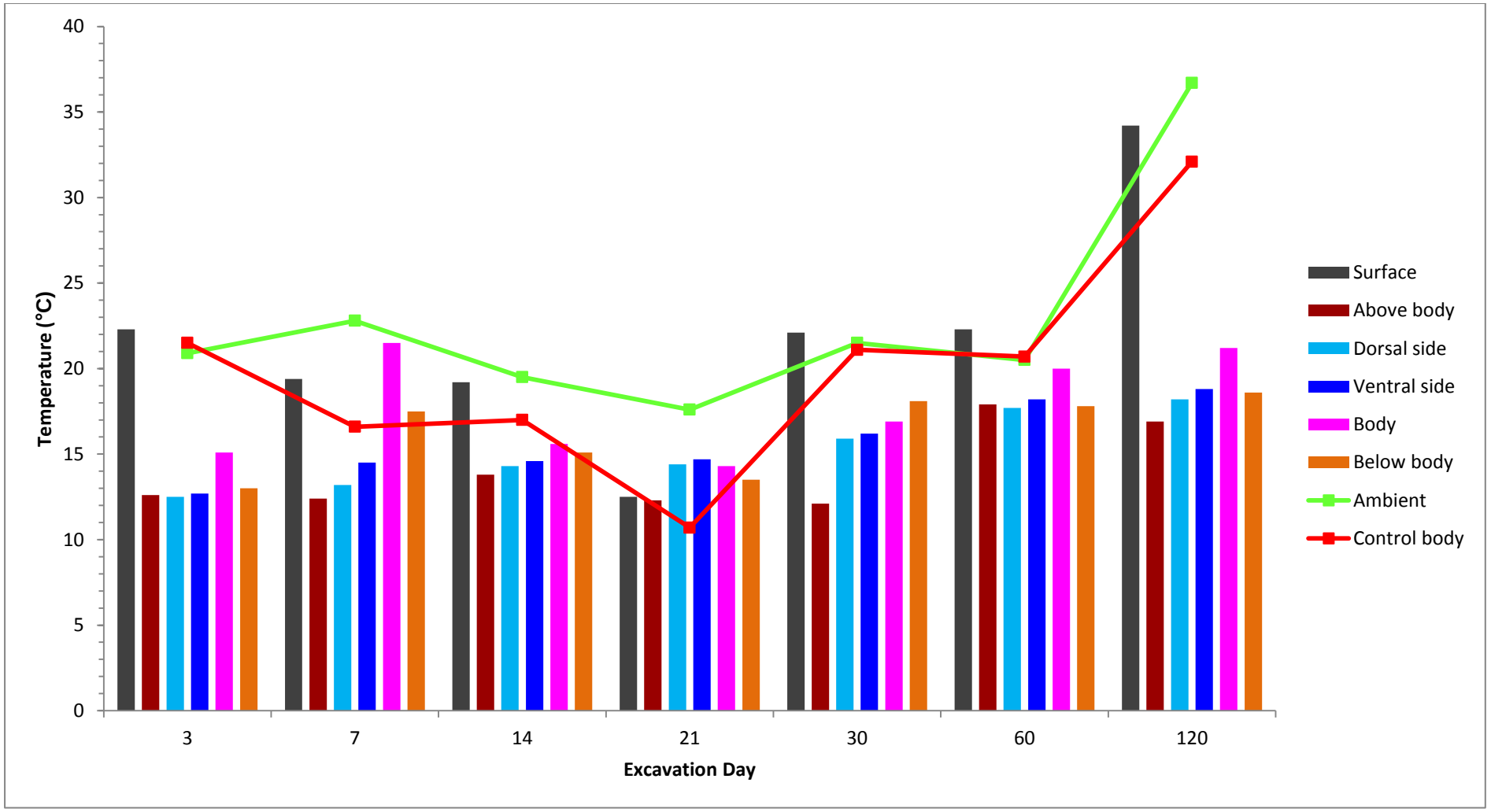


Figure 4.2.1.3: Grave temperatures taken during each excavation day of the winter trial, showing temperature points from above ground to below the body.

Figure 4.2.1.4: (top left) Clump of wet soil removed from the body surface, containing Phoridae larvae. Body fluid clearly seen pooled in the grave; (top right) head of grave five carcass caked with soil; (bottom) carcass covered in soil.

4.2.2 Dipteran succession on above ground carcass

Succession on carcasses above ground during winter months has been well-documented over the years in various studies (Arnaldos *et al.* 2001; De Carvalho & Linhares 2001; Tabor *et al.* 2004; Kelly 2006 unpubl.; Corrêa *et al.* 2014), showing constant patterns within a region regarding species composition. Figure 4.2.2.1 shows quantitative data of dipteran species observed on the above ground carcass during winter months observed over a course of 120 days. Cold temperatures during the trial period as well as little rainfall caused a delay in dipteran colonisation. Hardly any odour was noted during the first 10 days, with minimal decomposition and insect activity seen during this time.

Members of Calliphoridae were first prominently noted after the carcass transitioned from the fresh stage to bloat. This may be attributed to the steady accumulation of gasses within the body cavity due to decomposition and the emission of olfactory cues, in turn increasing the chance of attracting carrion feeders (Campobasso *et al.* 2001). Common sightings of *Lucilia* Robineau-Desvoidy 1830 spp., *C. albiceps* and *C. chloropyga* were noted throughout the rest of the study period (6-20 individuals per day) (Figure 4.2.2.1). Fluctuations in numbers of *C. chloropyga* adults were observed throughout the later days (between days 30 and 60) (6-20 individuals per day), and were mainly sighted on the cage surrounding the body. Isolated sightings of *C. marginalis* and *C. vicina* were noted during the last few days of the first 60 days (<5 individuals per day) (Figure 4.2.2.1).

Although a slight fluctuation in the number of adult flies was seen during the trial, no emergence from oviposition occurred at this time and adult flies sighted during this period quickly disappeared afterwards (Figure 4.2.2.1). The reason for no emergence occurring was due to active feeding by the coleopteran species *Dermestes maculatus* De Geer 1774 on oviposited egg clusters. Mass emergence by new species individuals is usually seen in cases of summer decomposition above ground where oviposition had taken place during early stages of decomposition and postfeeding larvae had pupated close to the carcass (Gennard 2007). A surge in numbers of new adults is then observed in the later stages and these adults typically stay around the body before migrating to a new food source.

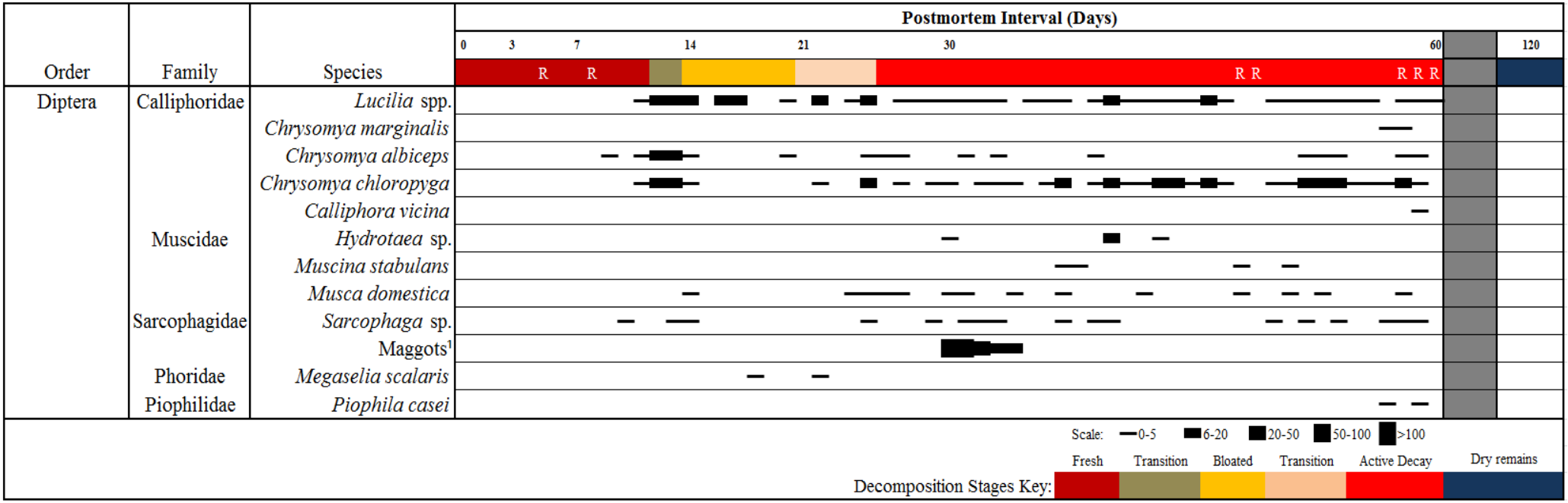


Figure 4.2.2.1: Diptera species recorded on the above ground carcass during a 60 day period during the winter trial, also showing species noted on day 120. Maggots were recorded as one number as only representatives of *Sarcophaga Meigen* 1826 sp. were identified during the trial. The symbol R shown above the species abundance represents rainfall occurring on the day. Observed decomposition stages are shown above species abundance and recorded per day.

However, no new emergence was observed during the later stages of decay of the winter above ground carcass due to increased levels of predation by *D. maculatus*. For more information on the impact of *D. maculatus*, please refer to Botham (2016 unpubl.).

Very few adult members of *Sarcophaga* sp. were observed on the body during winter months (<5 individuals per day). However, larvae of this species were found on the body (50-100 individuals) (Figure 4.2.2.1). This could be explained by the capability of *Sarcophaga* adults to thrive in harsh conditions and an adaptation in their breeding that allows members of the species to colonise otherwise inaccessible areas. Adult *Sarcophaga* females are known to larviposit (Coupland & Baker 1994; Singh & Bharti 2008). Due to this adaptation, larvae are able to move away and burrow into the body soon after larviposition, thus escaping from predators. Furthermore, *Sarcophaga* larvae are generally large and possess sunken spiracular plates, making the instars capable of reserving the warmer areas and deeper cavities of a carcass for their own growth and development (Gennard 2007; Byrd & Castner 2010). The fact that these larvae were found burrowing into the lower armpit of the carcass may support this assumption, and explains why other species were not able to oviposit successfully under winter conditions.

Two isolated appearances of *M. scalaris* adults was noted during the earlier stages of the trial (<5 individuals per day) (Figure 4.2.2.1), but the few specimens sighted were not seen to compete with any other species for the food source. *Megaselia scalaris* has been observed to prefer a more enclosed food source due to their small stature and tend to thrive in the absence of representatives of Calliphoridae and Sarcophagidae (Ives 1988; Vasconcelos & Araujo 2012). Adult members of *Piophila casei* (Linnaeus 1758) were noted during the last few days of the first 60 days (<5 individuals per day) (Figure 4.2.2.1).

Adult members of the Muscidae (*Hydrotaea* sp., *M. stabulans* and *M. domestica*) were recorded on isolated occasions throughout the first 60 days of the study period (1-20 individuals per day) (Figure 4.2.2.1). Although *M. domestica* was observed most frequently (Figure 4.2.2.1), no individuals were seen competing with larger flies for the food source or oviposition site. The frequent sighting of representatives of the Muscidae during winter has been recorded on several occasions in past studies

(Centeno *et al.* 2002; Kelly 2006 unpubl.; Barros de Souza *et al.* 2008; Joseph *et al.* 2011; Prado e Castro *et al.* 2012). However, many of these species have been dismissed as only indicators in the past, as they may be attracted to olfactory cues but have not been seen to oviposit and colonise a carcass, and thus do not contribute to succession overall (Greenberg & Kunich 2002; Gennard 2007).

The above ground carcass had only reached the starting stage of advanced decay on day 120, with no fly species observed during this time. This may be due to the rapid drying of the body, rendering the carcass unfavourable as a source of food and breeding to members of Calliphoridae and Sarcophagidae, as well as the dominance of predatory beetle species during this stage of decay (Figure 4.2.2.2). *Sarcophaga* species were seen within the vicinity of the carcass, but were not observed near the body, thus assuming that these flies did not utilise the carcass at this time. Species classified as typical winter species, namely *C. vicina*, have been recorded to play a pivotal role in winter succession in the past due to their tolerance of cold temperatures (Donovan *et al*. 2006). However, only a small number of individuals of the species *C. vicina* were recorded during the full 120 day trial period (<5 individuals) (Figure 4.2.2.1). These findings coincide with the results obtained in past studies by Kelly (2006 unpubl.), Kolver (2009 unpubl.) and Hoffman (2014 unpubl.), where only a few *C. vicina* individuals (<10 individuals) were observed on above ground control carcasses throughout the winter trails within the Bloemfontein area. In the present study, other species such as *Sarcophaga* species and *C. chloropyga* were present on the above ground carcass throughout the dry and cold weather. *Chrysomya chloropyga* adults are typically found during spring and summer months with a temperature preference of above 14°C, and are seen to be intolerant of colder conditions (Richards *et al.* 2009). Earlier descriptions of these flies include observations such as their niche preference of sunlit areas and behavioural reactions in regards to short-term migratory patterns (Zumpt 1965). This brings into question whether the older studies in forensic entomology of species by time of appearance could still be used. Variability in seasonal temperature shifts with climate from year to year, and inconsistencies in weather patterns could change the viability of previous research on outdoor decomposition and succession.

Figure 4.2.2.2: (top) Adult *Dermestes maculatus* De Geer 1774 beetles aggregating on the winter above ground carcass; (bottom) mass of *D. maculatus* beetle adults and immatures seen below the body.

4.2.3 Dipteran succession on winter grave carcasses

Due to the difficulty of monitoring buried carcasses in a natural environment, species records were made only during excavation periods. Only members of Diptera that were found on the body during excavations were sampled as these were determined to have some direct association with the carcass. Species that were observed at all life stages in the duration of the study were considered as most significant in succession due to the possibility of these species completing their life cycles (or part of the life cycle) on and around the carcass.

A full species list was compiled in conjunction with the concurrent study by Botham (2016 unpubl.), showing all arthropods that occurred on the buried carcasses during the summer trial and can be seen in Appendix 2.

Figure 4.2.3.1 shows the recorded dipteran species found during each excavation for the winter trial, including quantitative data of these species obtained. The excavation done on day 3 (grave 1) yielded no dipteran specimens. The carcass was still seen to be in the fresh stage of decomposition, showing no signs of decay as of yet. Day 7 (grave 2) showed similar results to grave one, yielding no dipteran species. However, progression of decomposition was noted as the body had entered the bloat stage of decay.

Although no dipteran species were noted on the carcass on day 14 (grave 3) (Figure 4.2.3.1), the carcass was in active decay. This may be due to the microbial action of fungi and bacteria on the body as well as inside the body that had started decomposing the body. It is important to note that although no dipteran specimens

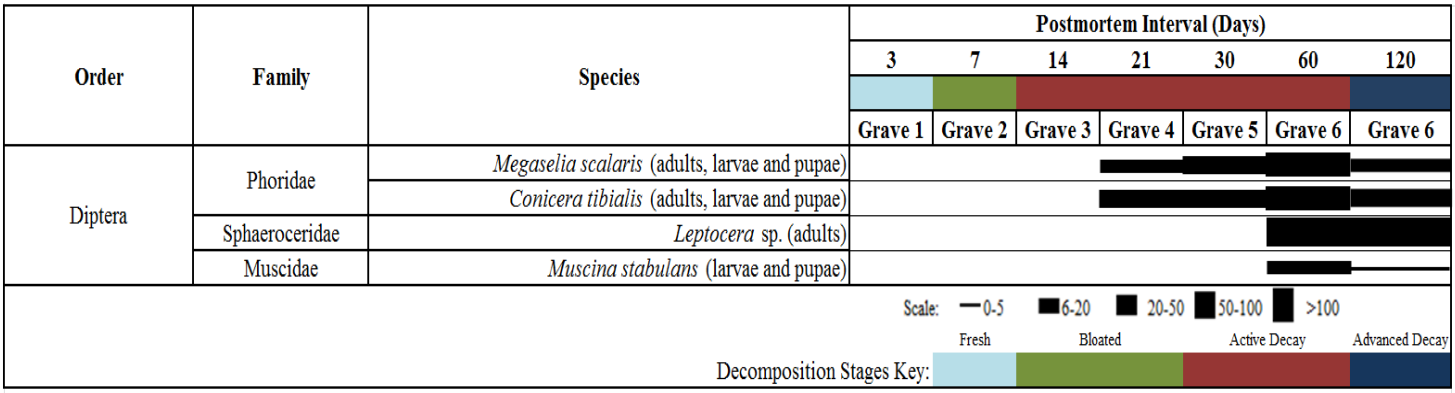


Figure 4.2.3.1: Recorded dipteran results obtained during each excavation day for all seven excavations of the winter study, including decomposition stages and quantitative data with scale.

were recovered from the graves during the first three excavations, fly species were seen on the surface during isolated occasions. In the concurrent study, it was reported that carcasses still decomposed at this stage despite the absence of Diptera individuals. Carcasses were also observed to develop fungal and bacterial growth concentrated below the body during the early stages of the trial, which may have contributed to decomposition even in the absence of Diptera.

The first prominent dipteran activity was seen in grave 4 (day 21), with *M. scalaris* (6-20 individuals) and *C. tibialis* (20-50 individuals) adults and larvae being the only members of Diptera present (Figure 4.2.3.1). This is possibly an indication of colonisation by adult flies and mating and oviposition on the carcass between day 14 and day 21. Adult members of these species were observed to aggregate below the body where the most moisture and seepage was seen. Although a large number of these flies were found around the carcass, no flies were seen burrowing from the carcass to the soil surface to disperse. Larvae of *C. tibialis* and *M. scalaris* were noted to aggregate mainly under the body (Figure 4.2.3.4), showing preference to the ventral side of the body as well as the legs of the carcass. Furthermore, the larvae of each species showed preference to certain sites, with *C. tibialis* larvae showing preference to the posterior end of the carcass and larvae of *M. scalaris* showing preference to the leg areas as well as the abdomen.

Grave 5, excavated on day 30, yielded similar results to grave 4 as can be seen in Figure 4.2.3.1. A larger number of *M. scalaris* adults (20-50 individuals) were observed in comparison to grave 4, suggesting either further colonisation or a cycle of breeding between days 21 and 30. The steady increase in the number of the representatives of Phoridae, as well as the presence of larvae, pupae and adults at this stage, together with the lack of observed dispersal, may point to the possibility of these insects remaining on the carcass after colonisation. A study by Martín-Vega *et al.* (2011) also reported the prolonged colonisation by several generations of *C. tibialis* on an 18-year buried carcass, further supporting the possibility that these species may survive on a single carcass without migrating.

The largest colonisation was observed in grave 6 (day 60), which showed the most species found on a carcass throughout the winter trial (Figure 4.2.3.1). By day 60, pupal cases of both *M. scalaris* and *C. tibialis* were noted on various parts below the

body (Figure 4.2.3.4). Adults of both phorid species were still noted by this stage (50-100 individuals). However, dead *M. scalaris* and *C. tibialis* adults were noted from day 60 at 60 cm in grave 6. This may have been an indication of these species staying near the carcass without leaving the grave. The representative of *Leptocera* (Family: Sphaeroceridae) made its first appearance on day 60 and was found in very large numbers as adults during excavation (>100 individuals) (Figure 4.2.3.1). Adult *Leptocera* individuals were found to aggregate below the body where most moisture and seepage was seen. This points to a possibility of late colonisation by these lesser corpse flies. Two publications suggest that these flies are attracted by late olfactory or sensory cues emitted by the carcass (Buck 1997; Byrd & Castner 2010). The carcass was in the active decay stage on day 60. *Leptocera* adults were observed in close association with the fungal growth on the body (Figure 4.2.3.3). Furthermore, some species of Sphaeroceridae are attracted by fungal growth and readily feed on certain species of fungi (Buck 1997). Increased fungal growth was seen below the body as time progressed (Figure 4.2.3.2). Adult *Leptocera* individuals were not seen to actively feed on the carcass and was possibility feeding on the fungal growth on the carcass instead of the carcass itself. No eggs, larvae or pupae of the *Leptocera* species were found on the buried carcass. This may be an indication that *Leptocera* did not actively feed or breed on the carcass and did not play the role as forensic feeders. It is unclear as to the precise cues these flies used to colonise the body, and what direct role these flies play in succession of the carcass. *Muscina stabulans* larvae were also observed on the grave carcass at this stage (6-20 individuals) (Figure 4.2.3.1). Representatives of the Muscidae are generally not thought of as forensically important due to their variable feeding habits and the lack of above ground evidence of colonisation has dismissed many Muscidae as indicators only (Greenberg & Kunich 2002; Gennard 2007).

Figure 4.2.3.2: Grave 6 carcass flipped over to expose underside of the body, showing fungal growth (circled in red) on the posterior side and the middle of the body.

Figure 4.2.3.3: Adult *Leptocera* Olivier 1813 around the fungal growth on the grave 4 carcass on day 60 of the winter study (left), photomicrograph of *Leptocera* sp. adult found on grave 4 (right).

Figure 4.2.3.4: Members of Phoridae observed during the winter trial, showing larvae found on day 21 (top left), an adult *Conicera tibialis* Schmitz 1925 seen on day 21 (top right), and pupae found on day 60 of the trial (bottom).

Day 120 yielded the highest abundance of Diptera, specifically members of Phoridae as well as Sphaeroceridae (Figure 4.2.3.1). A greater number of *C. tibialis* (20-50 individuals) adults in comparison to *M. scalaris* (6-20 individuals) adults were observed during excavation. This may point to either the possibility of competition below ground or the end of the natural lifespan of *M. scalaris* adults therefore suggesting their prolonged stay on the carcass. The dead adult *M. scalaris* and *C. tibialis* individuals were noted in the soil around the body, concentrated below the carcass. Phorid larvae were noted in greater abundance compared to the few live adult individuals. A large increase in representatives of *Leptocera* adults (>100

individuals) was noted after 120 days of burial (Figure 4.2.3.1), indicating either further colonisation by migrating adults or breeding that may have occurred below ground.

Throughout the 120 day winter study, no adult *M. scalaris* and *C. tibialis* individuals were seen leaving the graves prior to excavation. Larvae, pupae and adults of *M. scalaris* and *C. tibialis* were noted over the course of the 120 day trial (Figure 4.2.3.4) The sightings of all life stages during the entirety of the trial as well as the observation of dead adults, possibly due to the completion of their life cycles, may point to the possibility of these Diptera staying around a buried carcass for several generations without leaving the carcass. After colonisation, phorid individuals were noted to stay within 5 cm of the carrion and were not noted to leave the body area.

It has been hypothesised that the depth of burial of a carcass could affect the time it takes for insects to colonise a carcass since the time taken for species to reach below ground carrion would be longer as depth of soil increases (Pokines & Symes 2013). This time frame can further be elongated by soil composition, moisture levels in the soil, and temperature fluctuation (Byrd & Castner 2001; Panagiotakopulu & Buckland 2012). Some species are more resilient in winter conditions and studies have shown that species such as *M. scalaris* and *C. tibialis* are able to thrive in colder temperatures (Martín-Vega *et al.* 2011; Zuha *et al.* 2012). The presence of larvae, pupae and adults of the representatives of Phoridae during the winter trial suggest their forensic importance in burial cases. However, the extent of their interactions is still unclear as well as the attractant factors that helps the adults find the carcass from above the soil.

4.3 Section 2: The influence of shallow burial on dipteran succession during summer months

4.3.1 Environmental conditions during the summer trial

Summer temperatures were recorded and mapped with rainfall to show the relation between rainfall and ambient temperature fluctuations. Figure 4.3.1.1 shows an overview of midday ambient temperatures over the course of the 60 day period. Ambient temperatures were generally high, ranging between 22°C and 46°C throughout the 60 days. The lowest temperature was recorded at 22°C, coinciding with heavy rainfall. Rainfall periods were observed to be sporadic and infrequent as periods of no rainfall were commonly seen during the 60 day period. High levels of rainfall were recorded during the summer trial, with the most rainfall seen at 44 mm on day 6, and a total of 140 mm observed over the full 60 day period. Rain caused muddy graves (Figure 4.3.1.2), effectively sealing the grave surfaces due to their clay-soil composition.

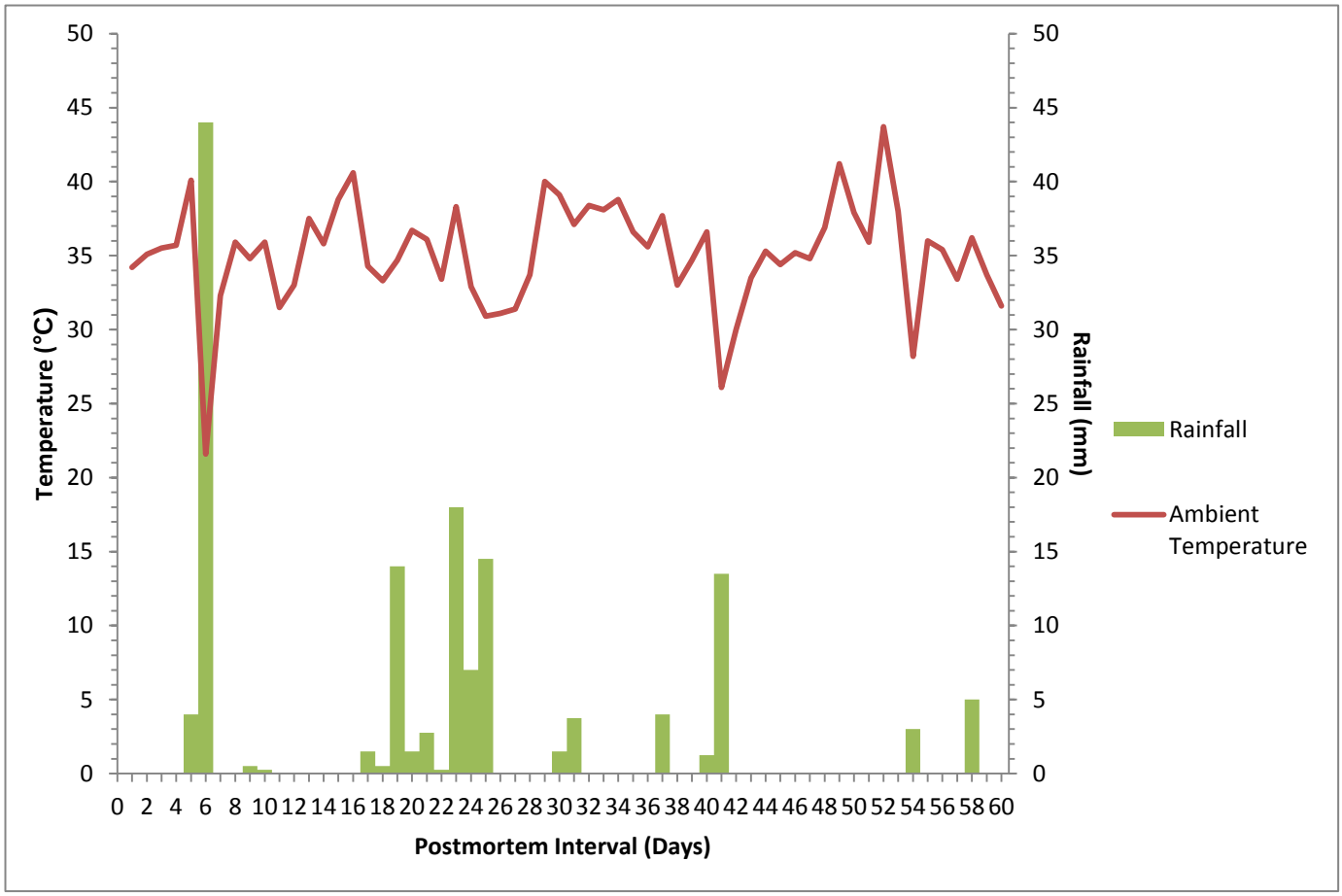


Figure 4.3.1.1: Recorded temperature and rainfall data taken at the summer study site over a period of 60 days.

Figure 4.3.1.2: Grave 2 surface showing water seepage due to heavy rainfall (left); excavation taking place on day 7, showing wet soil at 20 cm depth (right).

Ambient temperatures were seen to drop during days of rainfall and fluctuations in temperature were commonly seen over the course of the 60 day trial.

Ambient temperature taken on day 120 was recorded at 22.4°C and was noted to be lower than most recorded summer temperatures. Several isolated rainfall occurrences were noted between day 60 and 120, with a total of 72 mm recorded overall. No rain was noted on day 120 of the summer trial.

During the 60 day summer trial, grave temperatures showed a marked difference in surface temperature in comparison to below ground temperatures (Figure 4.3.1.3). Deeper soil temperatures taken on day 3 (grave 1) were 15.0°C lower than the recorded surface temperature. Day 7 (grave 2) displayed the lowest surface temperature (20.5°C) recorded over the first 60 days at this occasion, coinciding with rainfall during this time. Grave temperatures taken on day 7 were noted to be 5.9°C higher on average than the soil surface temperature. Deeper grave temperatures around the body taken on day 14 (grave 3) were 4.3°C higher on average in comparison to that of day 7 (grave 2). Grave 4 (day 21) shows a similar increasing trend (Figure 4.3.1.3); the only day that the body temperature (34.0°C) was higher than the surrounding soil temperatures (20.7°C-32.5°C). The gradual increase in body temperature from day 7 to day 21 (24.8°C-34.0°C) may be attributed to the decomposition process taking place and microbial action on the body resulting in the production of heat. This may also be explained by the increased insect activity on the

body by day 21. Grave 5 (day 30) displayed an unusual pattern in comparison to other graves, which showed similar trends to that of grave 2 (day 7) (Figure 4.3.1.3). The body temperature of the carcass (25.3°C) was noted to be slightly lower than surrounding soil temperatures (26.3°C-28.3°C). This may be due to the shale deposits in the grave. In the present study, shale was seen to form a hard rocky layer above the body that was difficult to penetrate during excavation. The packed layer of shale rock prevented looser soil from the top layers of the grave compacting around the body and aided in forming an air cavity around the body. This may have facilitated heat transferral to the surrounding soil due to loose clay soil not becoming caked onto the carcass that could have resulted in the conservation of heat by the body. However, the compact soil above the shale, together with the rocky layer of shale, formed a barrier preventing heat from escaping the closed-system grave environment, preventing complete cooling of the carcass and the surrounding soil.

Below ground temperatures taken on day 60 (grave 6) showed a decrease in body temperature as well as the surrounding soil temperatures in comparison to temperatures taken on day 21 (average decrease 32.3°C-29.5°C) (Figure 4.3.1.3). Temperatures taken from the lower levels of soil of day 120 (grave 6) showed a further decrease in temperature from day 60 to day 120 (average decrease 29.5°C-24.0°C), indicating gradual cooling of the body over time.

Surface temperatures on each grave represented long exposure to solar radiation levels on each day, showing the high temperatures that graves were subjected to during the study. Day 7 (grave 2) had a lower surface temperature (20.5°C) compared to other graves (average 38.4°C) due to the grave being subjected to heavy rainfall on previous days (Figure 4.3.1.3). Ambient temperature dropped between days 30 and 120 (39.1°C-22.4°C). However, soil surface temperatures of the grave did not follow this trend. Soil surface temperature taken on day 7 was 11.8°C lower than the ambient temperature. As can be seen in Figure 4.3.1.3, temperatures taken from the above ground carcass showed a relation pattern to ambient temperature ($r=0.953$), although the carcass temperatures were seen to be higher than ambient temperatures. No specific relation pattern was noted between deeper soil temperatures and above ground ambient temperatures ($r=0.289$). It can be deduced that ambient temperature did not influence the grave temperatures, with the exception of soil surface temperatures ($r=0.522$).

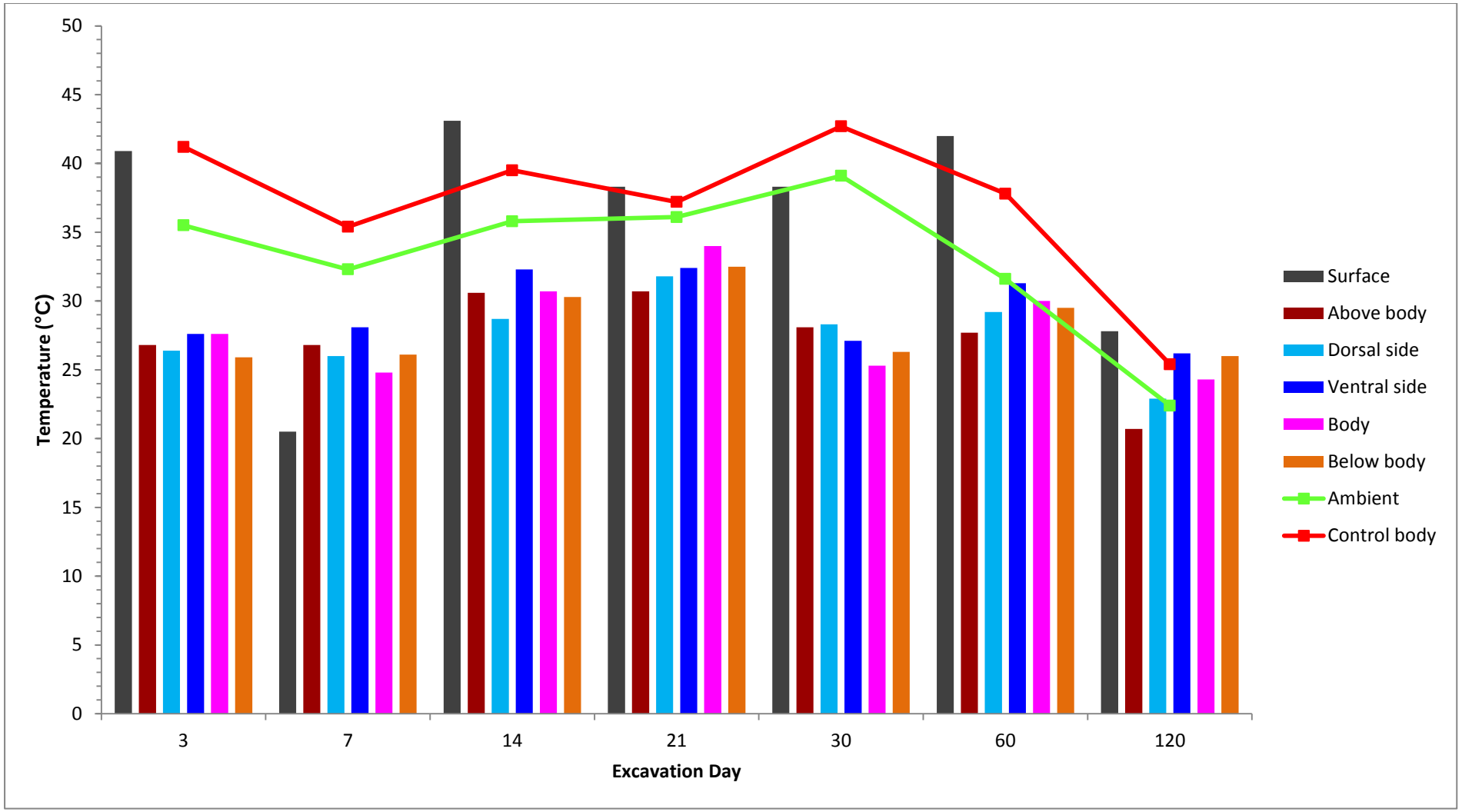


Figure 4.3.1.3: Grave temperatures taken during each excavation day of the summer trial, showing temperature points from above ground to below the body.

The above ground carcass temperatures (average 37.0°C) were higher than body temperatures of the grave carcasses (average 28.1°C) during the full 120 days of the summer trial. Temperature of the above ground carcass (25.4°C) and the excavated buried carcass (24.3°C) on day 120 showed a small difference of 1.1°C (Figure 4.3.1.3). However, temperature results taken on day 21 showed an increase of 3.3°C in grave carcass temperature and a decrease of 2.3°C in the above ground control carcass temperature from the results taken on day 14. Body temperature fluctuations between the above ground carcass and the grave carcasses were seen to coincide from day 3 to day 14 (Figure 4.3.1.3). However, fluctuations in body temperature on following study days did not coincide with each other.

Similarly, temperatures taken from the above ground carcass showed a relation pattern to soil surface temperatures in most cases. A deviation from this was seen on day 60 where soil surface temperatures were seen to increase from day 30 to day 60 (38.3°C-42.0°C), whereas the ambient temperature (39.1°C-31.6°C) and the above ground carcass body temperature (42.7°C-37.8°C) was noted to decrease from day 30 to day 60 (Figure 4.3.1.3). Soil surface temperatures and above ground carcass body temperatures were generally found to be considerably higher than ambient temperatures (Figure 4.3.1.3). This may be due to the grave surfaces and the above ground carcass being exposed to the high levels of solar radiation over a longer period of time during the summer study. Prominent cloud cover was noted on day 7, resulting in the lower soil surface temperature (20.5°C) seen on grave 2 (day 7) (Figure 4.3.1.3).

Body temperature taken from the above ground control carcass was not seen to coincide with the grave carcass body temperatures in most cases and no similar pattern was noted between the above ground control carcass and the below ground grave carcasses (Figure 4.3.1.3). It can be deduced that temperature patterns of the carcass observed above ground was not similar to that observed in the below ground carcasses.

Graves can be considered as disturbed areas as these are often pre-dug and exposed to the external environment for a period of time. Aeration of the soil as well as loosening of soil particles during pre-digging may affect the constant conditions usually observed in such an enclosed environment, and can explain the variation in

temperature recorded during excavations. Rainfall after filling of graves were seen to seal the surface of the clay-soil graves and, once dry, were noted to become hard and compact. The compaction and sealing of bodies in the graves may have attributed to heat distribution variance around the body being conserved below ground and may explain the temperatures observed over the 120 day period.

4.3.2 Dipteran succession on above ground carcass

Generally, succession studies have concentrated on summer months as species activity is significantly higher in warmer periods (Oliva 2002; Carter *et al*. 2007; Castro *et al*. 2011), making species involved in summer succession more well-documented than those of other seasons. Figure 4.3.2.1 shows quantitative data of dipteran species observed on the above ground carcass during the summer months of the present study trial period over the course of 120 days.

Members of Calliphoridae were found to be the first Diptera to colonise the carcass. Adult *Lucilia* spp. (6-20 individuals per day), *C. marginalis* (20-50 individuals per day) and *C. albiceps* (6-20 individuals per day) were noted from day 1 of the study, with *C. marginalis* being the most dominant species sighted on the body in regards to the number of individuals (Figure 4.3.2.1). Calliphorid maggots were first noted from day 2 (6-20 individuals), indicating oviposition occurred between day 0 and day 1. A decrease in maggot numbers during days 8 through 11 (from >100 to 20-50 individuals per day) was seen to precede a large increase in newly emerged adults (>100 individuals) seen around the body (Figure 4.3.2.1). This could be considered as a basic pattern of emergence of the F_1 generation usually seen after initial colonisation, and is a typical representation of succession under summer conditions (Kelly 2006 unpubl.; Eberhardt & Elliot 2008; Voss *et al.* 2009). Although adults may not be seen around the body upon discovery, the presence of eggs is usually the first indication of colonisation of a carcass. Due to the fact that dipteran eggs are affected greatly by their environment, eggs are found on sufficient food sources and in the presence of adequate cover. Food sources, such as a large carcass, can also serve as refugia for developing larvae. This explains why, depending on the environmental conditions around a carcass, larvae of certain dipteran species will pupate very close to the body and emergence would typically be seen shortly afterwards in the vicinity

of the body. This lead to a surge in species numbers after initial colonisation, similar to what was seen during the current 120 day trial.

During the present study, it was observed that newly emerged calliphorid adults stayed around the cage for a short length of time before dispersing. Although rainfall was noted between days 17 and 23, which coincided with observed emergence of new adults, dispersal of newly emerged adults was still seen. This may be attributed to the intensity and frequency of rainfall during these days, and may show that although rainfall may have occurred it may have not been heavy enough to inhibit the dispersal of newly emerged adults.

According to Gennard (2007), temperatures above 30°C may inhibit activity of members of Calliphoridae. This was not observed during the summer trial period, when temperatures above 30°C were experienced on most days. This did not seem to inhibit calliphorid activity and, due to regular rainfall, slight temperature drops may have kept the environment conducive enough for dipteran activity to occur. However, this emphasises the importance of considering environmental factors into dipteran succession of a corpse in any type of environment as it can affect the rate of colonisation as well as the extent of activity.

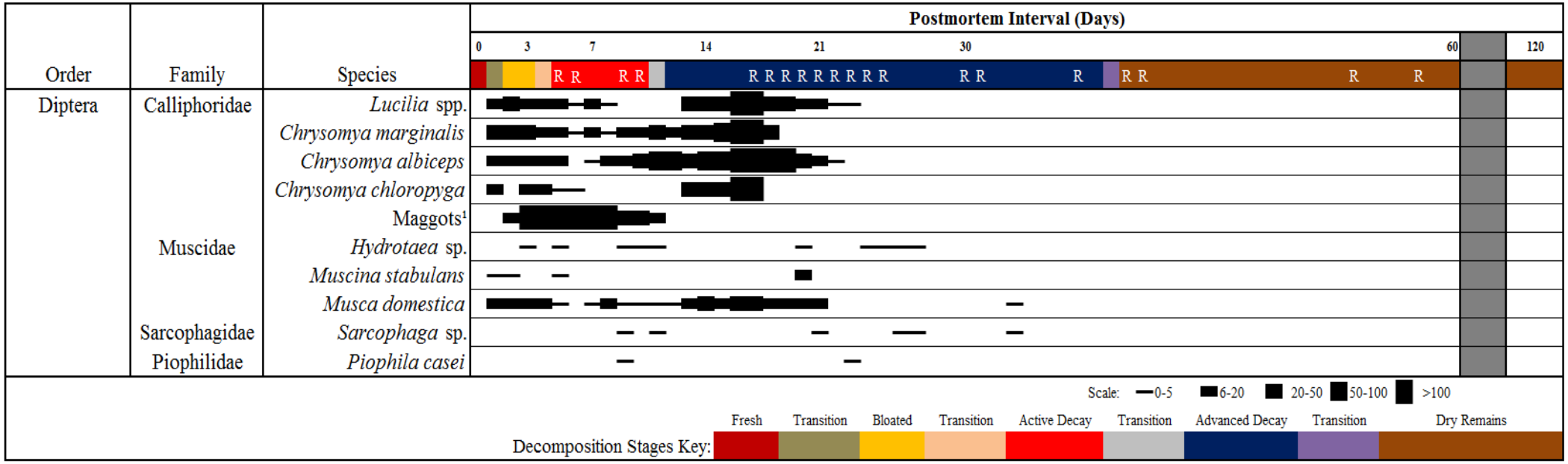


Figure 4.3.2.1: Diptera species recorded on the above ground carcass during a 60 day period during the summer trial, also showing species noted on day 120. Maggots[1] – Maggot mass included species *Lucilia* Robineau Desvoidy 1830 spp., *Chrysomya marginalis* (Weidemann 1830) and *Chrysomya albiceps* (Weidemann 1819). The symbol R shown above the species abundance represents rainfall occurring on the day. Observed decomposition stages are shown above species abundance and recorded per day.

Only a few members of Sarcophagidae were noted around the above ground carcass (<5 individuals per day) throughout the 120 day trial during isolated occasions (Figure 4.3.2.1). Although adults were seen on the body, no Sarcophagidae larvae were identified among the maggots sampled. Two isolated appearances of adult *P. casei* individuals were recorded during the first 60 days of the trial (<5 individuals per day) (Figure 4.3.2.1).

Members of the family Muscidae, *Hydrotaea* sp. (<5 individuals), *M. stabulans* (6-20 individuals) and *M. domestica* (20-50 individuals), were recorded throughout the first 60 days of the summer trial period, with *M. domestica* observed in larger numbers (Figure 4.3.2.1). Despite the observed numbers, adults of *M. domestica* were not seen competing with members of Calliphoridae for the food source or breeding space. Isolated sightings of *M. stabulans* and *Hydrotaea* sp. were recorded. Olfactory cues provided by gasses from the decomposing carcass as well as putrefying muscle and organ tissue could explain the presence of members of the Muscidae during summer months, especially in environments with air movement that can carry these cues across a larger area (Dekeirsschieter *et* al. 2009; Byrd & Castner 2010). Muscids are known to be attracted to decomposing matter and have been seen to feed on blood droplets as a protein meal for oviposition (Gennard 2007). However, many species of Muscidae are not known to be prominent carrion feeders and their exact role on a carcass is still unclear (Battán Horenstein *et al*. 2010; Chen *et al.* 2010).

The above ground carcass had reached the beginning stages of skeletonisation by day 120 of analysis, with only small amounts of dry skin left and exposure of dry bone around the head as well as the upper torso area noted at this stage. Exposure to high temperatures and increased levels of insect activity attributed to the rapid decomposition of the above ground carcass. No dipteran individuals were noted at this stage (Figure 4.3.2.1). However, members of the Coleoptera were observed to feed on the dry skin of the carcass on day 120 of the summer trial. Patterns seen overall in the summer study can be considered as typical in comparison to patterns of succession observed in past studies, with fly species being absent during late advanced stages of decay (Byrd & Castner 2010).

4.3.3 Dipteran succession on summer grave carcasses

Only members of Diptera that were found on the body during excavations were sampled as these were determined to have some direct association with the carcass. Dipteran species that were observed at all life stages in the duration of the study were considered as most significant in succession due to the possibility of these species completing their life cycles on and around the carcass.

A full species list was compiled in conjunction with the concurrent study by Botham (2016 unpubl.), showing all arthropods that occurred on the buried carcasses during the summer trial and can be seen in Appendix 2.

Figure 4.3.3.1 shows the recorded dipteran species during each excavation for the summer trial, including quantitative data obtained over a period of 120 days. The excavation done on day 3 (grave 1) yielded no dipteran specimens. The carcass was still seen to be in fresh stages of decomposition, showing no signs of decay as of yet. Day 7 (grave 2) showed similar results to grave 1, yielding no dipteran specimens (Figure 4.3.3.1). However, progression of decomposition was noted as the body had entered active stages of decay. Excavation on day 14 (grave 3) yielded similar results to grave 2.

Even though no dipteran species individuals were found during the first three excavations on the grave carcasses, several flies were seen around each grave. Bodies were seen to develop rapid fungal growth even in the absence of dipteran activity and carcasses excavated had entered advanced decay by day 7. This could be due to increased microbial activity brought about by optimal conditions, as was noted in the parallel study conducted by Botham (2016 unpubl.).

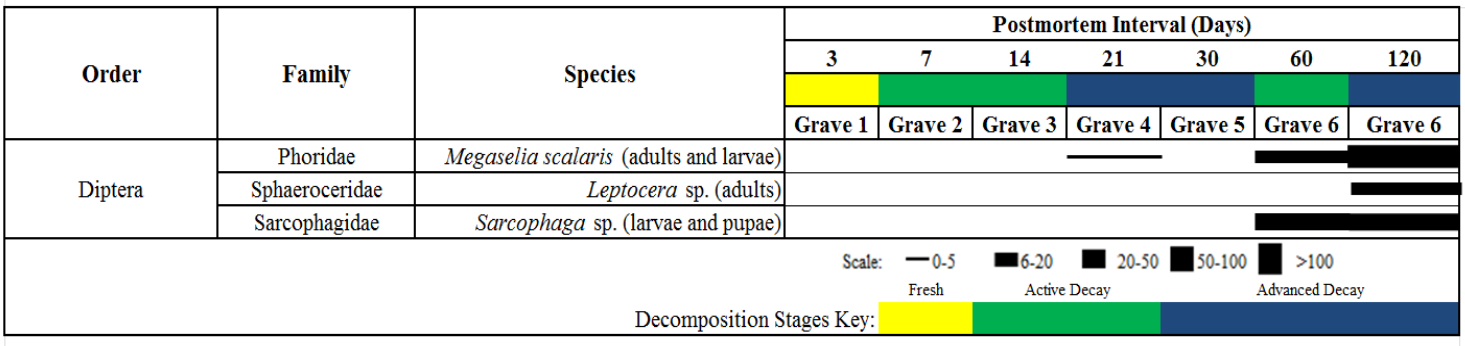


Figure 4.3.3.1: Recorded members of Diptera obtained during each excavation day for all seven excavations of the summer study, including decomposition stages and quantitative data with scale.

The first dipteran evidence was found on day 21 (grave 4), with only a few *M. scalaris* individuals found on the carcass (<5 individuals) (Figure 4.3.3.1). Adult flies were observed to aggregate below the body where most moisture and seepage was seen. Although these flies were found around the carcass, no flies were seen burrowing from the carcass to the soil surface to disperse. It is possible that adult *M. scalaris* individuals had oviposited on the carcass between day 14 and 21, but no phorid larvae were found on the body. A large number of predatory *Aleochara* Gravenhorst 1802 larvae and adults were found on the body on day 21, indicating colonisation of these predators between day 14 and 21. Mass predation of *M. scalaris* individuals may have resulted in the absence of *M. scalaris* larvae at this stage during the summer trial.

A peculiar observation was the absence of dipteran individuals on day 30 (grave 5), despite the carcass being in advanced stages of decay (Figure 4.3.3.1). Large patches of fungal and bacterial growth was noted on both the top side of the body as well as below the body, which may have continued the decomposition process in the absence of arthropod activity (Figure 4.3.3.2). In this specific grave, shale was seen to form a hard rocky layer above the body that was difficult to penetrate during excavation. This may have also formed a barrier that members of Diptera could not penetrate to reach the body. Rainfall caused the clay-based soil above the carcass to compact and, due to the high temperatures discussed in section 4.3.1, soil above the body dried to form a hardened barrier. This, in turn, sealed moisture into the lower levels of the grave, keeping the clay soil waterlogged. Compaction of the wet clay soil on top of the shale could have also prevented odours from leaving the grave, thus resulting in no flies detecting the body.

Figure 4.3.3.2: Grave 5 carcass excavated on day 30, showing rapid decay even in absence of necrophagous Diptera activity. Fungal growth is visible on the posterior end as well as head area.

Day 60 (grave 6) showed an increase in dipteran presence compared to day 21 (grave 4), with a larger number of *M. scalaris* adults observed (6-20 individuals) (Figure 4.3.3.1). At this stage the carcass had reached active decay. This could point to the possibility of *M. scalaris* typically colonising a buried body during active decay. No *M. scalaris* larvae were noted on the body at this stage. *Megaselia scalaris* adults were noted to show no niche area preference since predators were spread over the entire carcass surface, and adults were often observed scattered across the body surface. Literature commonly discusses predation of beetles on larvae (Slansky & Rodriguez 1987; Kočarek 2003; Gennard 2007), and the observation was made of the active feeding of *Aleochara* adults on *M. scalaris* adults. Predation by *Aleochara* adults may have resulted in the absence of *M. scalaris* larvae at this stage during the present summer trial. No phorid pupae were noted throughout the trial, indicating that no F_1 generation was present on the body. This may mean that all adult Phoridae seen during day 60 were adults that burrowed into the grave from above ground to attempt colonisation of the carcass. Pupae of representatives of *Sarcophaga* species ,found within 10 cm of the body, was also found around the body in high numbers (20-50 pupal cases) (Figure 4.3.3.1), suggesting earlier larviposition between day 30 and day 60 of the study. Soil in the graves formed

clumps as the ground dried after days of summer rainfall. These hardened clumps may have formed temporary micro-niches for dipteran species, providing adequate cover due to hardened clay, and provided an incubation area for larviposition and pupation (Pastula 2012 unpubl.). *Sarcophaga* sp. females have been seen to larviposit into the surface layers of the soil near a food source, where after larvae will burrow towards a carcass and colonise the food source (Rodriguez & Bass 1985). It may be possible that, although larvae are able to colonise a below ground food source, adult *Sarcophaga* are not able to survive below the soil for prolonged periods of time.

Excavation on day 120 (grave 6) showed an increased dipteran presence and diversity compared to day 60 (grave 6) (Figure 4.3.3.1). *Megaselia scalaris* was the most dominant species in regards to abundance (50-100 individuals). A large number of *M. scalaris* adult specimens together with *M. scalaris* larvae were sighted below the body. Adult members of Phoridae were found probing around the bottom side of the body where most moisture occurred in each grave. Predation by *Aleochara* sp. adults from day 21 with the exception on day 30, which had no insect activity whatsoever, were still taking place at this stage. Adults of *Aleochara* were also observed to feed on larval stages of *M. scalaris* during the excavation on day 120. A few adult representatives of *Leptocera* were also recorded during excavation (6-20 individuals) (Figure 4.3.3.1), suggesting later colonisation by these flies. No eggs, larvae or pupae of the *Leptocera* species were found on the buried carcass. *Leptocera* adults were observed in close association with the fungal growth on the body, similar to the observations of the winter trial. This may be an indication that *Leptocera* did not actively feed on the carcass and did not play a role as forensic feeders. Third instar members of *Sarcophaga* larvae were also found under the body (20-50 individuals) (Figure 4.3.3.1), with some larvae burrowing into the body cavity. Several empty pupal cases (found within 10 cm of the body) of members of Sarcophagidae were seen upon excavation, suggesting colonisation by representatives of Sarcophagidae during the period between day 60 and day 120.

During excavations, adult members of Phoridae were found probing around the bottom side of the body where most moisture occurred in each grave. However, no larvae were noted on the body until day 120 (grave 6). This may be due to the

presence of predators. Staphylinid larvae and adults of the genus *Aleochara* were seen on the body from day 21 of the summer trial. According to literature by Gennard (2007), members of the staphylinid genus *Aleochara* have been found to be predators of other insects. In the present study, active predation by these beetles on representatives of Phoridae occurred during excavations on days 21, 60 and 120. Adult beetles were seen to grab adult *M. scalaris*, immobilise the flies by ripping off their wings and legs, and then feeding on the flies. Adult members of *Aleochara* were also observed to feed on larval stages of *M.scalaris* during the excavation on day 120. The number of *Aleochara* individuals were noted to increase from day 21 (indicating colonisation of these predators between day 14 and 21) with the exception of day 30. The increase in predatory beetle numbers was seen to be larger than the increase in dipteran numbers. The effect of predation was seen to halt reproduction of some dipteran species on the body throughout the 120 day summer trial.

An absence of adult *Sarcophaga* on the buried body was recorded. However, newly emerged adult members of *Sarcophaga* were observed leaving the grave area between day 60 and day 120 to possibly disperse. Despite the presence of predaceous *Aleochara* beetles on buried carrion during the summer trial, *Sarcophaga* individuals were still seen in high numbers. Unlike the extensive effects of predation by *Aleochara* on Phoridae larvae, the presence of *Aleochara* on summer buried carcasses was not seen to have an effect on sarcophagid larval colonisation of the carcass. However, direct observations of the interactions between *Sarcophaga* and *Aleochara* still need to be recorded in order to prove whether *Aleochara* does not interact with *Sarcophaga* individuals, or whether some form of competition is displayed between the two species. *Sarcophaga* species individuals were seen to colonise a below ground carcass in active decay. In the parallel study by Botham (2016 unpubl.), it was observed that the grave carcass had only reached late active stages of decay by day 60 (grave 6) due to the clay soil nature of the grave, which resulted in waterlogging of the soil. This was seen to cause excess moisture around the body and may have, in turn, decreased the rate of decomposition by halting aerobic microbial action on the body.

According to previous studies, summer months have always yielded high abundance in species diversity and number of individuals (Oliva 2002; Kelly 2006 unpubl.; Carter

et al. 2007; Castro *et al.* 2011). This, however, is not true for all types of environments as differing activity and conditions can be observed with changes in environment (Dent *et* al. 2004; Carter *et al.* 2007; Carter *et al*. 2010). Warmer temperatures experienced during the summer trial generally resulted in the increase of overall insect activity. Although insect activity had increased during summer months of the present study, relatively few insect species observed in the area above ground proved to have the potential of reaching a body under wet and warm soil conditions. Dipteran adults may not be able to detect and reach a body through thoroughly wet clay soil as clay soil becomes clogged and water does not drain at a fast rate. High moisture levels in the soil decreases the few air pockets available for oxygen diffusion through the soil matrix, thus making the environment unfavourable to some species (Page 1952; Byrd & Castner 2010). This proved true for the present study, especially under wet conditions such as that observed on day 60 (grave 6). The number of species and abundance of individuals can be altered by soil composition, environmental factors and, in certain cases, climatic weather change (Turchetto & Vanin 2004; Voss *et al*. 2009; Pope 2010 unpubl.; George *et al.* 2013). This strongly suggests that further summer studies under different investigative focuses must be executed in order to gauge the full effects of factors that can be linked to summer conditions.

4.4 Section 3: Comparative analysis of results obtained during winter and summer trials

4.4.1. Overview of environmental conditions of winter and summer months

Ambient temperature and rainfall between winter and summer

Ambient temperatures and rainfall patterns experienced during each trial was seen to be noticeably different between summer and winter. Figure 4.4.1.1 show results for both summer and winter ambient temperatures and recorded rainfall plotted for comparison. As can be deduced by the diagram, winter ambient temperatures (between 18°C and 28°C) were generally lower in comparison to summer ambient temperatures (between 22°C and 46°C). Rainfall during summer trial period was observed to be significantly higher than winter rainfall, with more frequent rainfall days seen during the summer trial. Rainfall throughout the winter study was minimal, with only 8.5mm recorded throughout the 60 day period. In contrast, summer rainfall was recorded at 140 mm in total over the 60 day seasonal trial. The effect of rainfall on ambient temperature can clearly be seen in Figure 4.4.1.1. Frequent and heavy rain was noted to cause large drops in summer ambient temperature, thus explaining the drops in temperature below 30°C during the summer period.

The lack of rainfall, coupled with fairly warm temperatures, during the winter trial was noted to result in rapid drying of above ground carrion as well as the dry soil above buried carcasses. Dry conditions, such as the weather experienced during winter months of the trial, can influence the nature of a corpse and dries a cadaver quickly over a short period of time (Gennard 2007). Low levels of rainfall and constant temperatures may accelerate drying of carcasses above ground and increase the rate of mummification.

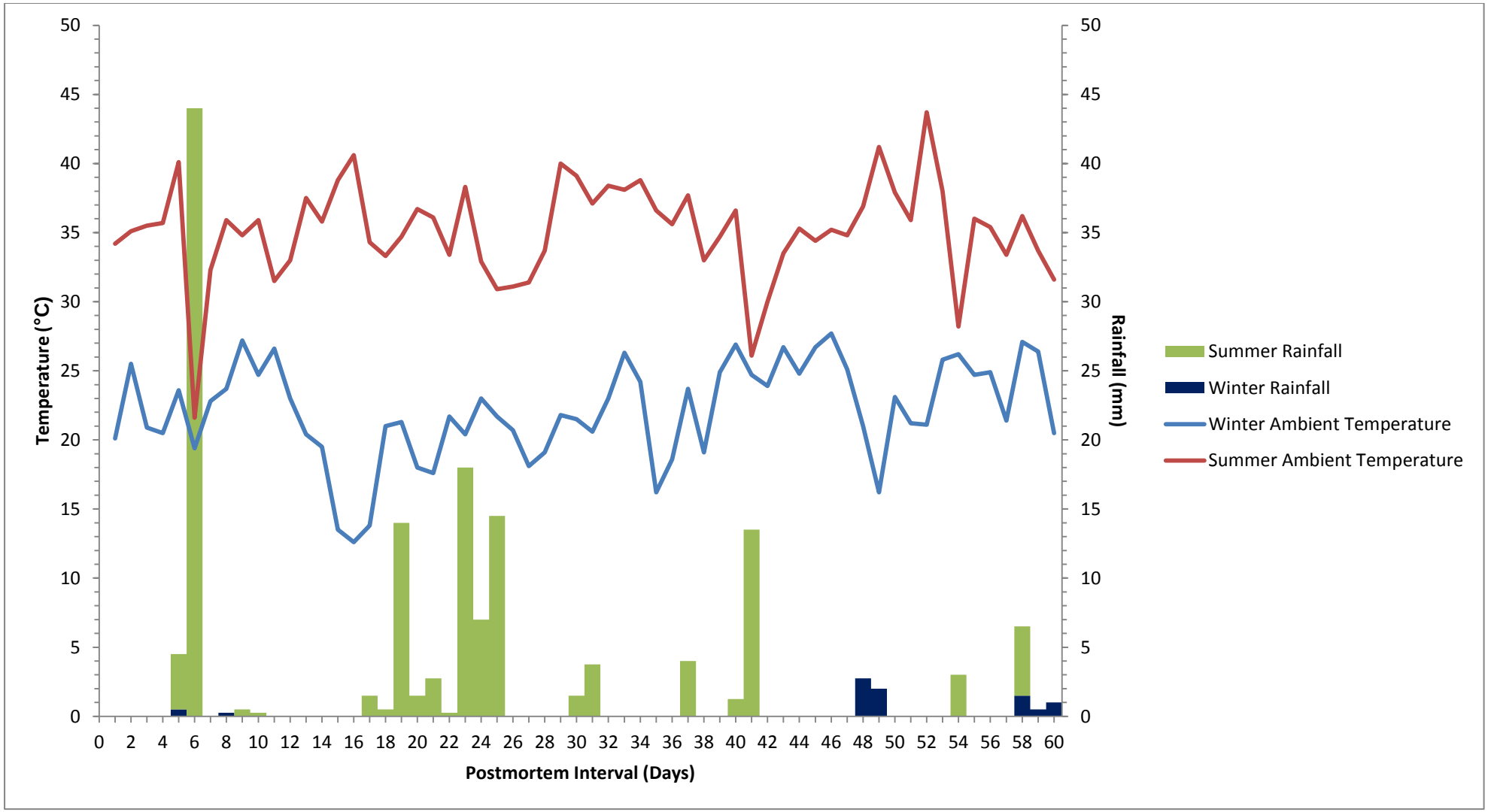


Figure 4.4.1.1: Recorded ambient temperatures and rainfall data taken directly from both seasonal study areas over a period of 60 days.

Changes in temperature and rainfall through the years and possible implications to forensic entomology

In order to get an idea of the changing climates over the years, it needs to be shown that some form of trend exists in past data.

In a past study conducted from 2003 to 2005, in the same area as the present study (Kelly 2006 unpubl.), field ambient temperatures were recorded as between 10°C and 15°C during winter months and between 25°C and 30°C during summer months. In the present study, which had taken place 9 years later, winter temperatures were seen to be in the range of between 18°C and 28°C, which was noted to be warmer than the temperatures recorded in the study by Kelly (2006 unpubl.). Summer temperatures in the present study were recorded at between 22°C and 46°C, which was also seen to be higher than that found by Kelly (2006 unpubl.). The comparison between temperatures taken in the past by Kelly (2006 unpubl.), and the present study temperature results, points to an increase in ambient temperatures over the years. This change in temperatures over the years may be representative of shifting climates over time, and emphasises the need to link climate studies with ecosystem analyses (SANBI 2014; Ziervogel *et al.* 2014).

The increase in temperature in the present study compared to past temperatures taken in past analyses may highlight a critical factor that needs to be taken into consideration when using past data. Entomological analysis for forensic cases is highly dependent on temperature data and the species that are present on and around a corpse (Catts & Goff 1992; Byrd & Castner 2001; Amendt *et al*. 2004; Archer 2004). Shifts in temperature may result in a shift in insect distribution as well as trigger new adaptation behaviour in an area (Harrington *et al*. 1999; Erasmus *et al*. 2002; Visser 2008). For example, species that were not present in a specific area in past records made a few years ago may be present in the same area in future records due to changes in temperature in an area, and *vice versa*. Living organisms are continually thriving towards adapting to shifting climates, which sometimes implies that species will often expand or shift their normal distribution areas to more favourable conditions (Harrington *et al*. 1999; Parmesan 2006). This is also applicable to forensically important species. Furthermore, temperature changes may change the interactions of species on a corpse. The implication of this is that the

findings of past studies may not be applicable to present day situations and future studies, and further studies should be conducted to fully gauge the effect of gradual temperature changes over the years.

Although yearly temperature data is valuable in tracing the changes in temperature over the years, it is only through comparison of species over the years that allow the deduction as to whether these changes have resulted in the shift in species occurrence. This will be discussed in section 4.2.2.

Weather conditions during different seasons play a large role in the behaviour of insects. This, in turn, may affect the insects that feed on dry remains and can alter the pattern of succession as well as the role-players that will be involved in the process (Byrd & Castner 2010). Warm temperatures and increased moisture, such as that experienced during summer months, may prove more favourable for dipteran species. It is important to keep track of data collected from specific areas on a continual basis, and not just data collected by researchers in past studies from other countries or regions. Changes in temperature and precipitation may strongly influence species activity, the time it takes for eggs to hatch, as well as length of life cycles and diapause (Turchetto & Vanin 2004). Temperature and moisture are thus considered as the major factors that drives the rate of oviposition as well as rates of dipteran activity on a cadaver (Smith 1986).

Below ground soil temperatures

Grave temperatures of the winter and summer trials showed differences between seasons. Figures 4.2.1.3 (refer to 4.2 Section 1) and Figure 4.3.1.3 (refer to 4.3 Section 2) show the grave temperatures taken during the winter and the summer trials. Winter temperatures taken from deeper layers of soil were noted to be generally lower in comparison to internal soil temperatures of the summer graves. This may be an indication that excavated soil is affected by seasonal changes. The effect of seasonal change on loose soil temperature may be dependent on the soil properties of the graves, including soil composition, moisture, and depth of the soil (Carter *et al*. 2007). The clay soil of each grave during both studies was noted to be generally clumpy, forming air pockets in the soil. This may have allowed heat from the above ground environment to diffuse into the graves, resulting in differences in grave temperatures between summer and winter months.

Although carcass temperatures and below ground temperatures coincided with each other within seasons, as was discussed in Sections 1 and 2, no similar patterns were noted between winter and summer temperatures of the grave. Body temperatures of below ground carcasses also showed no similar patterns between the summer and the winter trial. Temperatures observed during the winter and the summer trials, and the lack of similarity patterns between seasonal temperatures, suggests that ground temperature and carcass temperatures may vary between seasons and may be dependent on soil properties.

Soil temperatures may have an impact on dipteran species present on below ground carrion as well as the decomposition of carcasses. Burial has been found to restrict below ground carcasses to a reduced set of temperatures as the soil layer above buried carcasses forms a barrier to solar radiation (Lowe 2010 unpubl.). Colder temperatures have been found to hinder growth and development of dipteran species and sometimes can halt growth completely (Wall *et al.* 1992; Ames & Turner 2003; Zuha *et al*. 2012).Warmer temperatures are more preferable to many Diptera of forensic importance and allows for optimal growth and development of immature specimens (Ames & Turner 2003; Donovan *et al*. 2006).

In a study by Lowe (2010 unpubl.) carcasses buried during summer months displayed an increased rate of decomposition due to higher temperatures and increased insect activity, compared to carcasses buried during winter months. It was assumed that winter months would yield less insect species due to decreased temperature. The present study, however, showed a deviation to the observed results of Lowe (2010 unpubl.). The present winter study yielded more dipteran species despite the decreased temperatures recorded below ground and summer was noted to have less dipteran species despite increased temperatures. Decomposition rates of the present study coincided with the observations by Lowe (2010 unpubl.), where summer grave carcasses decomposed faster, possibly due to heightened temperatures, in comparison to winter grave carcasses subjected to lower grave temperatures. Since the stage of decomposition affects the attraction and the dispersal of dipteran species on a carcass (Byrd & Castner 2010), it is important to investigate the factors that affect the decomposition rate of a carcass, including temperature changes within the environment.

4.4.2. Comparison between above ground carcasses of present and past studies

Comparison of above ground carcass succession between seasons

Figure 4.2.2.1 (refer to 4.2 Section 1) and Figure 4.3.2.1 (refer to 4.3 Section 2) shows the recorded quantitative data over each seasonal trial period of dipteran succession on the above ground carcasses. The winter trial (Figure 4.2.2.1) showed isolated occurrences of members of Diptera, with very few individuals seen each day. This was unlike the summer trial (Figure 4.3.2.1) that was seen to have the largest colonisation periods during the first 21 days of the trial, before showing a general absence in dipteran species on the above ground carcass. In winter, only *Sarcophaga* maggots were present on the carcass. This was very different to the maggot masses recorded on the summer above ground carcass, which was found to consist of several calliphorid species, including *Lucilia* spp., *C. marginalis*, *C. albiceps* and *C. chloropyga*. *Musca domestica* adults were noted to be present

during both summer and winter trials, mainly between the bloat stages and active stages of decomposition, but no oviposition occurred.

An interesting observation made was the presence of a few *M. scalaris* adults (<5 individuals) on the above ground carcass during the winter trial (Figure 4.2.2.1). Although only a few individuals were seen during isolated occasions on the winter above ground carcass, this was seen to differ from the summer results as no *M. scalaris* individuals were found around the summer above ground carcass.

During winter, dipteran abundance was low over the 60 day period, whereas summer showed an increase in the number of dipteran adults. Colder and drier weather experienced during winter, coupled with winds and cold fronts in the area, resulted in lower rates of dipteran activity on the above ground carcass. Delays in dipteran colonisation were observed during the winter trial, with the first sightings of Diptera noted from day 9 of the study. In contrast, warmer temperatures and increased rainfall during the summer trial was more favourable, resulting in increased fly activity and adults were seen around the carcass from as early as day 1 of the summer trial. No dipteran species were found on the above ground carcasses of both summer and winter trials on day 120. This could be attributed to the fact that both summer and winter carcasses had reached the dry remains stage, a condition unfavourable for dipteran utilisation. This could be considered as a typical successional pattern as this specific pattern of succession has been observed during past studies (Arnaldos *et al.* 2001; De Carvalho & Linhares 2001; Oliva 2002; Tabor *et al.* 2004; Kelly 2006 unpubl.; Carter *et al.* 2007; Castro *et al*. 2011; Corréa *et al.* 2014).

Seasonal changes have a major impact on the conditions within an ecosystem, in turn affecting the faunal colonisation of a carcass (Byrd & Castner 2010). Slight changes in temperature can change the presence or absence of species.

Above ground succession of the present study compared to past studies within the same area

In past studies, distinct seasonal differences in blowfly presence and abundance have been observed in the Bloemfontein area (Kelly 2006 unpubl.). Species such as

Lucilia sericata (Meigen 1826) and *C. albiceps* have been found to be tolerable to temperatures up to 50°C and are more active in warmer temperatures, whereas species such as *C. chloropyga* are intolerant of colder temperatures and are likely to favour warmer conditions (Richards *et al.* 2009). Despite this, members of Calliphoridae present during the full study trial were seen during both seasons.

Competition is also a major factor that may affect the rate of succession on a carcass. During the present study, winter yielded very few dipteran specimens on the above ground carcass, possibly due to competition with *D. maculatus* beetles throughout the winter trial and cold temperatures. Temperatures between 20°C and 30°C experienced during the winter trial proved favourable to the beetle species, resulting in the species thriving on the dry carcass (Richardson & Goff 2001). In contrast to winter, flies during the present study were observed to colonise the body over a short period of time during summer months and competed for the food source more strongly. This may be attributed to warmer temperatures experienced during this time, serving more favourable to dipteran species (Richards *et al.* 2009).

Dermestes maculatus beetles have displayed competition against other insect species for a food source, but aggression is highly dependent on temperature, moisture levels and the initial density ratio of species individuals within an area (Odeyemi 1997). In a past study by Hoffman (2014 unpubl.), large numbers of *D. maculatus* beetles were observed on above ground winter carcasses, possibly affecting the colonisation of dipteran species of forensic importance. Dipteran species during this past study were only noted to colonise the body during isolated periods of time and in very small numbers in comparison to summer species. In the summer trial of this past study, very few *D. maculatus* beetles were noted during warmer temperatures on the carcasses, whereas dipteran species such as *C. albiceps* and *C. chloropyga* thrived on the above ground carcasses. These results were similar to the results recorded during the present study for both summer and winter. Summer succession results by Hoffman (2014 unpubl.) showed a similar colonisation trend to that of the present summer study results, with the main colonisation occurring during the first few days of each trial.

In the present study, very few dipteran individuals were recorded during the winter trial. A similar result was observed in a study by Kelly (2006 unpubl.), where dipteran

species were observed in low numbers over the winter trial period. However, larvae were noted throughout that study, unlike the present winter study where only Sarcophagidae larvae colonised the carcass after 30 days of the trial. A study by Kolver (2009 unpubl.) showed very few dipteran individuals over two winter seasons, coinciding with the results obtained during the present winter study.

During the present winter study, it was noted that the calliphorid species *C. vicina*, commonly associated with winter months, was only present once during the full 120 days and in very small numbers (<5 individuals) (Figure 4.2.2.1). This was similar to findings by Kelly (2006 unpubl.) in a 2003 winter trial and by Kolver (2009 unpubl.) for a 2004 winter trial. The second winter trial conducted in 2005 by Kolver (2009 unpubl.) found no *C. vicina* adults on the carcass. A similar pattern was also seen by Hoffman (2014 unpubl.), where only a few *C. vicina* individuals were seen over the course of 14 days. This may suggest that *C. vicina* may not be specifically a winter species in the Bloemfontein area, or may have other preferences in regards to food sources in the area.

Calliphoridae species, *C. marginalis*, was noted to only occur on the summer above ground carcass, and were present in large numbers during the first 7 days of the trial. This was similar to the findings by Kelly (2006 unpubl.), where *C. marginalis* adults were seen to dominate the carcass during the first 5 days of the summer trial. A similar finding was also found by Kolver (2009 unpubl.), where *C. marginalis* individuals were seen to dominate summer above ground carcasses during 2005 and 2006. In the present study, new emergence of *C. marginalis* was noted during day 9 and day 19, suggesting that larvae of *C. marginalis* were able to complete their life cycles on the carcass. It can be assumed that *C. marginalis* is a primary coloniser capable of competing against more competitive species such as *C. albiceps*.

The sighting of *M. scalaris* on the winter above ground carcass of the present study can be considered as an unusual finding in comparison to past studies. In studies conducted by Kelly (2006 unpubl.), Kolver (2009 unpubl.) and Hoffman (2014 unpubl.) within the same Bloemfontein area in which the present study was conducted, no members of Phoridae were noted on above ground carcasses during both winter and summer months.

Generally, present study results from the above ground control carcasses during the summer and winter studies were similar to past studies in the same area conducted by Kelly (2006 unpubl.), Kolver (2009 unpubl.) and Hoffman (2014 unpubl.). Dipteran species found were not seen to differ considerably from past studies, suggesting that studies for the area within the last 10 years are still valid for use despite the shifts in climate within the region. This may also suggest that temperature changes observed over the years have not had a noticeable effect on the occurrence of forensically important species. However, no two cases or studies are alike (Kolver 2009 unpubl.), meaning that results throughout the years and even between seasons within the same year may vary and species abundance and diversity may change depending on the conditions of a specific case (Byrd & Castner 2010).

4.4.3. Comparison of below ground dipteran succession

Comparison of below ground succession

Figure 4.2.3.1 (refer to 4.2 Section 1) and Figure 4.3.3.1 (refer to 4.3 Section 2) shows quantitative data for both winter and summer trials over a course of 120 days of dipteran species in buried carcasses. Winter (Figure 4.2.3.1) yielded the most results in regards to dipteran abundance over a course of 120 days. A constant increase in species abundance was observed between day 21 and day 120 of the winter study. This is unlike the results obtained during the summer trial (Figure 4.3.3.1), where a steady increase was only observed from day 60. Although colonisation was seen to take place on day 21 of each study, only a small number of Diptera was observed on the summer carcass. This could be due to increased predation by staphylinid *Aleochara* species adults on the summer carcasses.

Members of Phoridae were noted to show a similar arrival pattern during both the winter and the summer trial, indicating no noticeable difference in the detection and colonisation of below ground carrion at 60 cm. Winter months proved to have the highest abundance in Phoridae individuals and displayed signs of species completing their life cycles on the grave carcass.

Shared species were observed between summer and winter burial trials. Phoridae species *M. scalaris* was observed to colonise buried carcasses from day 21 of both the winter and summer trials. Although the species were shared between the winter

and summer burial trials, different colonisation patterns were observed in regards to the present of larval instars and pupae. Only adult *M. scalaris* individuals were noted on day 21 of the summer trial. Larvae of *M. scalaris* were only noted on day 120 of the summer trial, which was unlike the winter period that saw larval occurrence on below ground carcasses from day 21 of the winter trial.

No pupae were observed on summer burial carcasses throughout the 120 day trial. This could be due to increased predation by *Aleochara* species adults on *M. scalaris* larvae on the summer carcasses, preventing larval representatives of *M. scalaris* from reaching pupal stages. Both larval and adult members of *M. scalaris* were noted on the winter buried carcass excavated on day 21 (Figure 4.2.3.1). Pupae of *M. scalaris* were noted on day 60 of the winter trial. It was noted that members of *M. scalaris* thrived on winter carcasses in the absence of predators. In contrast to the winter results, members of Phoridae during the summer trial were seen above and below the body. Adults were seen to mainly aggregate below the body, similarly to the behaviour of phorid species during the winter trial. However, adult *M. scalaris* were noted to move over the top side of the grave carcass as well as the lower side of the body. Summer soil above the body was moist compared to that of the winter study. Another shared species noted on buried carcasses was a member of *Leptocera*, found during both the winter and summer trials. The *Leptocera* species individuals were noted during the later stages of the burial trials. However, no immature stages of the species were found throughout the winter and summer burial trials. The shared species between summer and winter trials could indicate possible standardised species of use in estimating PMI.

It is important to identify species that could be seasonal species for use in the absence of standardised species. Unique species between the winter and summer burial trials were noted. Phoridae species *C. tibialis* were noted only during the winter burial trial. Carcasses during the winter burial trial was noted to be drier compared to summer buried carcasses, proving more favourable for the colonisation of *C. tibialis*. The tendency of representatives of *C. tibialis* to stay on a carcass for several generations makes the species useful in PMI estimation. However, estimations may be less reliable as time progresses due to the difficulty in determining the number of generations that may have been present since burial of a body. Another species found only on winter buried carcasses was the muscid

species *M. stabulans*, noted during the later stages of the study. The presence of larvae and, later, pupae indicates that the below ground carcasses were colonised and fed upon successfully by *M. stabulans* individuals. *Muscina stabulans* individuals were absent during the wetter summer months. This may have been due to the increased moisture levels of top soil of the graves. Clay soil of the graves during summer was often seen as waterlogged and may have resulted in the drowning of dipteran eggs and hatched larvae laid on the surface of the graves. Although findings by Rodriguez & Bass (1985) suggests that blowflies are more likely to oviposit into the soil directly above buried remains when the soil was still moist following heavy rainfall, it was noted during the study that waterlogged top soil proved fatal to muscid larvae. It is important to note the moisture levels of a grave and its effect on some dipteran species. The presence of muscid larvae could be an indication that the species can be used in PMI estimation in winter burial cases. However, the tendency of adults to leave the grave after emergence could mean that *M. stabulans* may not be a reliable way to estimate PMI over a long term period. During the summer burial trial, one unique species to summer burial Diptera was noted. Members of *Sarcophaga* were found during later stages of the summer trial. The finding of empty pupal cases indicated colonisation of the species between day 30 and day 60 of the summer trial. Wet soil due to increased rainfall during the summer months proved to be more favourable to larvae deposited into the soil by female *Sarcophaga* individuals. Larvae require certain moisture levels in order to survive (Byrd & Castner 2010). In the winter study, soil above the buried carcasses was dry within 40 cm of the graves, which may have prevented effective larviposition and subsequent colonisation by members of Sarcophagidae. Similar to the behaviour of newly emerged *M. stabulans* individuals, newly emerged adults of *Sarcophaga* sp. were seen to leave the grave and did not stay around the buried carcass. The presence of larvae could be an indication that the species can be used in PMI estimation in burial cases. However, the tendency of adults to leave the grave after emergence could indicate that *Sarcophaga* species found in burial cases may not be a reliable way to estimate PMI over longer periods of time. Despite the presence of predaceous *Aleochara* beetles on buried carrion during the summer trial, *Sarcophaga* individuals were still seen in high numbers. Unlike the extensive effects of predation by *Aleochara* on Phoridae larvae, the presence of *Aleochara* on summer buried

carcasses was not seen to have an effect on sarcophagid larval colonisation of the carcass.

Distinct differences in abundance as well as species diversity can be seen in the results. However, the number of factors that may have attributed to these differences is still unclear. It is difficult to conduct burial studies in a natural environment with constant variables and therefore slight changes in experimental factors should be taken into consideration when analysing obtained results.

During heterotrophic succession, entomophagous predators tend to increase as succession progresses (Payne 1965). In past studies, the most common predators noted in a soil environment are beetles of the families Staphylinidae and Carabidae (Gennard 2007; Byrd & Castner 2010). Summer trial observations of the present study are representative of the effect of predation on a corpse, and displayed a delay in dipteran colonisation that could be the result of high levels of predation. Large numbers of predators on the corpse before dipteran colonisation may have prevented flies from ovipositing on the carcass and establishing a colony during early days of the summer trial. The findings by Turner *et al.* (2013) showed that members of *Aleochara* species have been noted to thrive in a clay soil environment during warmer months, such as the summer graves of the present study.

Differences in soil type were noted in the study field even amongst graves within the same season, which may have resulted in subtle changes in the succession process. In a study by Turner *et al.* (2013), sandy soil with a lower moisture level yielded the most insect results in regards to species diversity. Graves during winter months of the present study were generally sandy and dry, proving more favourable to Diptera of the grave. Colonisation of summer carcasses was observed to take place during drier periods of the study. However, excess shale deposits in certain summer graves were seen to cause impassable barriers that delayed detection and colonisation of the body.

Results gained from a study by Pastula (2012 unpubl.) in East Lansing, Michigan, coincided with some dipteran results gained in the present study. Results from Pastula (2012 unpubl.) showed that species such as *Hydrotaea* sp. (Diptera: Muscidae) and *M. scalaris* (Diptera: Phoridae) were able to colonise carrion at 60 cm within a grassland area in Michigan. *Megaselia scalaris* was the most prominent

dipteran species throughout the winter and summer trials of the present study. The similarity between certain species of the study by Pastula (2012 unpubl.) in the northern hemisphere and the present study conducted in the southern hemisphere suggests that burial species of forensic importance can be the same in different regions.

Below ground carrion studies have mainly focussed on identifying the species of forensic importance capable of reaching a cadaver (Huchet & Greenberg 2010; Gunn & Bird 2011; Martín-Vega *et al*. 2011; Pastula & Merritt 2013; Mariani *et al*. 2014). Although factors such as pH, temperature, soil moisture and nutrient content has been taken into consideration in past studies, it is still unclear as to what the full effect of even slightly differing factors have on decomposition and the ecology of burial Diptera.

Members of *M. scalaris* were seen to be present on the above ground carcass as well as on the below ground carcasses during the summer months. However, larvae of the species were only seen on the buried carcass on day 120 during this period. The absence of immature stages of Phoridae species on above ground carcasses limits the possibility of using representatives of Phoridae for forensic analysis in above ground cases.

Comparison of below ground and above ground dipteran succession

Winter above ground vs winter below ground

Table 4.4.3.1 shows the presence and absence data of species that occurred during the winter trial on the above ground control carcass and the below ground buried carcasses. Results show very little similarity between the above ground dipteran species and the below ground dipteran species, with the highest species diversity observed on the above ground carcass. Members of Calliphoridae noted during the winter trial were seen to be excluded from grave carcasses, suggesting that members of Calliphoridae are incapable of successfully detecting and colonising carrion at a depth of 60 cm during winter months within the Bloemfontein area. Most members of the Muscidae were found on the above ground carcass, with only one shared species, *M. stabulans*, occurring on both the above ground and below ground

carcasses. However, the absence of immature stages of *M. stabulans* on above ground carcasses limits the possibility of using this species for forensic analysis in above ground cases. Members of *Sarcophaga* were only found on the above ground carcass during the winter trial. Phoridae species were found to be more dominant on below ground carcasses, with two species occurring on buried carcasses. Only one species of Phoridae was noted to occur on the above ground carcass. However, the absence of immature stages of Phoridae species on above ground carcasses limits the possibility of using representatives of Phoridae for forensic analysis in above ground cases. Unique species were seen to occur on the above ground control carcass. A representative of Piophilidae was seen only on the above ground carcass. In addition to the presence of *P. casei*, all listed members of Calliphoridae, and representatives of *Hydrotaea* and *M. domestica* (Diptera: Muscidae) were seen to be exclusive to the above ground carcass. Species exclusive to the below ground carcasses were also noted. Species members of *C. tibialis* (Diptera: Phoridae) and *Leptocera* (Diptera: Sphaeroceridae) were seen to only occur on below ground carcasses.

Summer above ground vs summer below ground

Table 4.4.3.1 shows the presence and absence data of species that occurred during the summer trial on the above ground control carcass and the below ground buried carcasses. Results show very little similarity between the above ground dipteran species and the below ground dipteran species, with the highest species diversity observed on the above ground carcass. Members of Calliphoridae were seen to be excluded from grave carcasses, suggesting that members of Calliphoridae are incapable of successfully detecting and colonising carrion at a depth of 60 cm during summer months within the Bloemfontein area. All observed members of the Muscidae were found on the above ground carcass only. Members of *Sarcophaga* were found on both the above ground carcass and the below ground carcasses during the summer trial. Only one species of Phoridae, *M. scalaris*, was noted to occur on the below ground carcass. Unique species were seen to occur on the above ground control carcass. A representative of Piophilidae was seen only on the above ground carcass. In addition to *P. casei*, all listed members of Calliphoridae and Muscidae were seen to be exclusive to the above ground carcass. Species exclusive to the below ground carcasses were also noted. Species members of *M.*

scalaris (Diptera: Phoridae) and *Leptocera* (Diptera: Sphaeroceridae) were seen to only occur on below ground carcasses.

Shared dipteran species noted during the winter and the summer study can be considered as resilient species capable of living in enclosed spaces, such as a grave, as well as open environments, such as above ground areas. However, results show that dipteran faunal abundance and diversity on above ground carrion differs considerably in comparison to below ground fauna. This may emphasise the need for investigation on species relevant to burial cases in order to assist in the estimation of PMI.

Table 4.4.3.1: Presence and absence data comparing species occurrence on the winter above ground carcass and species found on the winter burial carcasses, as well as species occurrence on the summer above ground carcass and species found on the summer burial carcasses.

Species ID	Winter Trial		Summer Trial	
	Above ground carcass	Below ground carcasses	Above ground carcass	Below ground carcasses
Calliphoridae				
Lucilia spp.	X		X	
Chrysomya albiceps	X		X	
Chrysomya chloropyga	X		X	
Chrysomya marginalis	X		X	
Calliphora vicina	X			
Muscidae				
Hydrotaea sp.	X		X	
Muscina stabulans	X	X	X	
Musca domestica	X		X	
Sarcophagidae				
Sarcophaga sp.	X		X	X
Phoridae				
Megaselia scalaris	X	X		X
Conicera tibialis		X		
Piophilidae				
Piophila casei	X		X	
Sphaeroceridae				
Leptocera sp.		X		X

4.4.4. Statistical analysis

To fully quantify the differences between summer and winter data, a Sørenson's test was conducted to test the level of similarity. Table 4.4.4.1 shows the coefficient of similarity for different sets of data over both 120 day trial periods. Results show the least similarity in species between summer above ground and summer graves, with only a 16.67% similarity observed between the above ground carcass fauna and the below ground carcass fauna of the summer trial. This may be due to the lack of dipteran species in the summer graves. Only one species, *Sarcophaga* sp., was found to be shared between the summer above ground fauna and the summer grave fauna. Similarly, winter data also displayed a low level of similarity between winter above ground and winter graves, with a 26.67% similarity between winter graves and the winter above ground carcass. The low levels of similarity found between both summer graves and the summer above ground, as well as winter graves and the winter above ground, suggests that there is a significant difference between species that occurs above ground and species that occur below ground during both seasons.

Above ground carcass data between seasons displayed the highest similarity, possibly due to favourable conditions experienced during both seasons, with a 90.00% similarity noted between the above ground carcass fauna of the winter and summer trials.

Graves between seasons were seen to have a moderate level of similarity at 57.14%. Differences in temperature and rainfall between seasons were noted to affect species occurrence and activity on carcasses. The increased rainfall and high temperatures experienced during summer months resulted in less dipteran species occurring on the summer grave carcasses, whereas drier conditions and mild temperatures during winter months were seen to result in more dipteran species occurring on buried carcasses during the winter trial. Members of *M. scalaris* were noted to be a shared species between summer and winter on below ground carcasses, thus suggesting the use of *M. scalaris* as a prime species for burial analysis during summer and winter seasons.

Table 4.4.4.1: Sørenson's coefficient of similarity values generated using the results of the present study, showing percentage of similarity within seasons and between seasons.

Similarity between graves and above ground carcasses within same season	
Winter above ground *vs* Winter graves	26.67%
Summer above ground *vs* Summer graves	16.67%
Similarity between different seasons	
Summer above ground *vs* Winter above ground	90.00%
Summer graves *vs* Winter graves	57.14%

Although members of *Leptocera* were noted during both seasons on below ground carcasses, adult flies were seen at different time intervals. This suggests that this species may play a role in burial cases, either as a potential forensically important species or as an indicator species.

The difference observed between summer graves and winter graves may emphasise the presence of species unique to summer graves and other species unique to winter graves. However, similarity percentage values only represent the similarity in species that were present and do not represent the number of species that were present during the summer and winter burial studies. The presence *versus* absence data is also not representative of the life stages that occurred on each group during each period. It is important to consider the species abundance as well as the species diversity when monitoring faunal composition in forensic cases, as both abundance and diversity may affect the succession on a carcass as well as the interactions on and around a cadaver.

4.5 Section 4: Interactions and biology of Diptera of the grave

Members of Diptera often adopt different strategies for survival and change these strategies according to certain external conditions. These strategies include competition between species (interspecific competition) and even among individuals of the same species (intraspecific competition), adaptation to their surroundings based on biotic and abiotic factors, as well as strategies for reproduction. In order to understand the importance of species on a cadaver, it is important to document the interactions of species over time. Individuals within a species may change their behaviour according to their surroundings in order to survive under different circumstances. Variations in the nature of interactions are often noted as behavioural patterns shift to adjust to their surroundings (Wajnberg *et al*. 2008).

4.5.1 Resource utilisation and competition

The two phorid species, *M. scalaris* and *C. tibialis*, only utilised the below-ground carcass as a food source during winter at the same time. Although adults of *M. scalaris* and *C. tibialis* were observed below the body, they were noted to reside on different sections of the body and were rarely seen in the same area. Adult *C. tibialis* showed preference to the spinal area and posterior parts of the body, whereas adult *M. scalaris* were generally noted on the abdominal area as well as around the legs. It is possible that competition between Phoridae species may have resulted in resource partitioning on the carcass. Resource partitioning is often the result of niche overlap and competition between species, which forces each competing species to move to certain areas of a niche and subsequently utilise different isolated areas of the niche to avoid further competition (Schoener 1974; Griffin & Silliman 2011). The extent of resource partitioning is dependent on the level of competition between species and the area in which species can coexist (Ives 1988). Individuals of the species *M. scalaris* and *C. tibialis* were seen to prefer the lower side of the body, suggesting that the habitable area, or niche, was restricted to the underside of the body. Species were partitioned into separate areas below the body.

Larvae of *M. scalaris* and *C. tibialis* were noted to scatter across preference areas and were often only found in small groups of 3-5 larvae per cluster. No prominent

maggot masses were observed on the body. This is contrasting to the behaviour of above ground forensically important blowfly larvae that form large clusters on above ground cadavers. Overlapping areas of feeding between each species were noted on edges of preference areas. However, only single isolated larvae were found in these areas. This also points to the possibility of resource partitioning of the carcass and avoidance behaviour resulting in coexistence on a single food source.

However, preference areas may depend on many factors, including soil conditions, time of species colonisation as well as the number of individuals of each species present, and therefore the areas where these species were found below the body during this study cannot be considered as permanent preference sites.

In the breeding study mentioned in chapter 3, members of *M. scalaris* were observed displaying high levels of intraspecific competition once the colony had reached the F_1 generation. Competition was seen to drive certain behavioural cues, including the attempt to expand territory and finding a new food sources when the primary living space became overpopulated. Subsequent interspecific competition was then seen between *M. scalaris* and the other test species when *M. scalaris* individuals started occupying other breeding cages. *Megaselia scalaris* individuals were observed to be rapid colonisers of breeding mediums once specimens entered the cages of other species during the breeding trial. The competitive behaviour of *M. scalaris* was especially seen to have a large impact on *C. tibialis*. No F_2 generation of *C. tibialis* was seen during the breeding trial, and may have been the result of interspecific competition with *M. scalaris*. *Conicera tibialis* was noted to have a more solitary behaviour and did not show any attempts to expand their territory. The species was soon outcompeted by invading *M. scalaris* individuals. This may suggest that *C. tibialis* prefers more isolated food sources and thrive better under non-competitive conditions.

In a laboratory study by Prawirodisastro & Benjamin (1979), smaller food sources were noted to cause overcrowding and competition between larval stages of *M. scalaris*, resulting in longer larval and pupal periods and higher mortality rate of outcompeted individuals. Crowding of blowfly larvae on carrion and competition for food sources is commonly seen in above ground cases (Lane 1975; Ireland & Turner 2006), often resulting in the absence of smaller forensically important species such

as *M. scalaris*, which can be easily outcompeted by larger species, on above ground carrion. The larger food source within the graves, together with the soil preventing larger species from accessing the carcass was noted to provide an optimal environment for growth and development of *M. scalaris*. This may point to the possibility of concealed carrion being a more preferable food source to species such as *C. tibialis* and *M. scalaris*. In literature by Byrd & Castner (2010), a similar suggestion was made that *C. tibialis* may prefer underground or enclosed environments over above ground environments that are open to a larger number of species.

Adult *Leptocera* were seen to overlap with Phoridae species. However, no adult *Leptocera* individuals were seen to compete with Phoridae adults within the overlapping space. This may indicate that *Leptocera* individuals did not need to compete for a food source. Despite the overlap with Phoridae species on grave carcasses, the lack of interactions between *Leptocera* adults and Phoridae adults was noted throughout both the summer and winter trials due to possible difference in what each species feeds on.

Muscina stabulans larvae were found burrowing into the fold of skin of the back left leg. However, larvae showed no aggregation behaviour and only showed preference to the areas between the legs and among folds of the legs. In literature by Gunn & Bird (2011), it is suggested that the tendency of *M. stabulans* larvae to feed below the body could be a way to reduce competition with other larger species.

Due to the fact that larvae were not seen directly on buried carcasses of undisturbed graves, it is difficult to map the resource utilisation of members of *Sarcophaga* without further investigation.

4.5.2 Predation

Predation below ground was only noted during summer months.

Although literature commonly discusses predation of beetles on larvae (Slansky & Rodriguez 1987; Kočarek 2003; Gennard 2007), an interesting observation was made of the active feeding of *Aleochara* adults on *M. scalaris* adults. Adult *M. scalaris* were noted to have the ability to burrow through soil near the carcass. During excavations, adult *M. scalaris* individuals were seen actively burrowing deeper into the soil upon uncovering of the carcass. This could be used as a way to escape predators on the carcass. However, adult beetles in the soil were seen to locate *M. scalaris* adult individuals, grab the adult flies and immobilize them by ripping off their wings and legs. This would prevent flies from escaping and allowed *Aleochara* adults and larvae to feed on the flies. It is uncertain whether the active feeding was an adapted method of predation on burial species of Diptera.

Staphylinid larvae and adults of the genus *Aleochara* were seen on the body from day 21 of the summer trial. According to literature by Gennard (2007), members of the staphylinid genus *Aleochara* have been found to be predators of other insects. In the present study, active predation by these beetles on representatives of Phoridae occurred. Adult beetles were seen to grab adult *M. scalaris*, immobilise the flies by ripping off their wings and legs, and then feeding on the flies. Larvae of *Aleochara* were also observed to feed on larval stages of *M.scalaris* during the excavation on day 120.

Despite the presence of predaceous *Aleochara* beetles on buried carrion during the summer trial, *Sarcophaga* individuals were still seen in high numbers. Unlike the extensive effects of predation by *Aleochara* on Phoridae larvae, the presence of *Aleochara* on summer buried carcasses was not seen to have an effect on larval colonisation of the carcass.

No active feeding was noted by *Aleochara* sp. on *Leptocera* sp. individuals upon excavation due to *Leptocera* sp. adults escaping upon the uncovering of the body. However, it may be possible that *Aleochara* individuals did, in fact, feed on *Leptocera* sp. adults below ground.

4.5.3 Influence of soil conditions on species behaviour

Wet soil caused by seepage of the body proved to be more preferable to all life stages of Phoridae species.

In the present study, members of the Muscidae and members of *C. tibialis* were only found during the winter trial, which were noted to be drier than summer months. *Conicera tibialis* and *M. stabulans* individuals were absent during the wetter summer months. This may have been due to the increased moisture levels of top soil of the graves. Clay soil of the graves during summer was often seen as waterlogged and may have resulted in the drowning of muscid eggs on the surface of the soil, as well as *C. tibialis* adults in the grave soil.

Members of Sarcophagidae were only seen to colonise below ground carrion during wetter summer months. Larvae require certain moisture levels in order to survive (Byrd & Castner 2010). In the winter study, soil above the buried carcasses was dry within 40 cm of the graves, which may have prevented effective larviposition and subsequent colonisation. Moistened soil seen during summer months proved to be more favourable to *Sarcophaga* larvae that were larviposited directly into the soil. The adaptation of a sunken spiracular plate in larvae may also be a factor that allowed larval members of *Sarcophaga* to occupy buried carcasses even in wetter soil. Larvae of *Sarcophaga* are able to burrow into confined areas and close off the posterior spiracular area in order to access food sources in more moist areas. The wet soil condition seen during summer months was therefore not a hindrance to species individuals and did not prevent colonisation by *Sarcophaga* individuals.

Members of *Leptocera* sp. were not seen to be affected by drier or wetter soil. It is uncertain as to the effects of other soil factors on the burial species of *Leptocera*.

4.5.4 Reproduction strategies of Diptera of the grave

Phoridae species, such as *M. scalaris* and *C.tibialis*, are seen to remain on the body throughout all life stages. No migration of new adults out of the graves was seen during each trial period. The sighting of new generations of larvae on below ground carcasses, coupled with the presence of adult individuals on a carcass, may indicate

the possibility of mating and reproduction taking place within the confines of the grave. However, the reproduction and mating of Phoridae species on buried remains still needs to be investigated in order to prove that adult individuals mate below the soil and do not leave the grave to mate.

In the present study, no adult members of *M. stabulans* were noted on the body during excavation. However, adult individuals were observed burrowing out of the graves. This may point to the possibility of female *M. stabulans* mating above ground and burrowing into the soil to oviposit. Oviposited eggs would hatch and larvae would burrow down to the carrion to feed and develop. This has been found to be true in an experiment by Gunn & Bird (2011), where adult *M. stabulans* were seen to refrain from burrowing deep into the soil and larvae were noted to colonise carrion from eggs laid on the soil surface. In the present study, empty pupal cases were found on day 120 of the winter trial, indicating that adults had emerged between day 60 and 120. No adults were present in the grave or on the carcass. This may be an indication that adults burrowed out of the grave once they had emerged and left the area.

In a parallel study by Botham (2016 unpubl.), *Sarcophaga* larvae were noted to colonise a disturbed grave from day 38 of a summer study, coinciding with colonisation between day 30 and day 60 (Figure 4.5.4.1). This explains the large number of empty *Sarcophaga* pupae found in grave 6 on day 60. Empty *Sarcophaga* pupae were found within 10 cm of the body, indicating migratory behaviour of postfeeding larvae for pupation. No adults or larvae were found on the body on day 60. *Sarcophaga* adults were seen to probe the grave surface and burrow into the shallow parts of the soil. This was commonly to larviposit into the soil, allowing larvae to burrow down to the carrion to feed. It is uncertain when larviposition had taken place as larviposition has been observed at night in a past study as was found by Singh & Bharti (2008). It is also uncertain as to how long it takes *Sarcophaga* larvae to reach buried carrion at 60 cm. Newly emerged adults were observed burrowing out of the grave and leaving the grave area between day 30 and day 60. It was assumed that the adult individuals were newly emerged *Sarcophaga* adults as large numbers of adult individuals were seen leaving the grave.

Figure 4.5.4.1: Photograph of *Sarcophaga* Meigen 1826 adult probing the body after the excavation on day 60 of the present study (circled in black) (top left); third instar *Sarcophaga* Meigen 1826 larva discovered on an excavated carcass after 38 days of burial (bottom right; circled in red) (Botham 2016 unpubl.).

Members of *Leptocera* sp. did not reproduce and effectively colonise the body. Reproductive strategies for the species could therefore not be determined.

Chapter 5: Concluding remarks

“Lots of things are mysteries. But that doesn’t mean there isn’t an answer to them. It’s just that scientists haven’t found the answer yet.”

- Mark Haddon, The Curious Incident of the Dog in the Night-Time

Concluding remarks

Chapter 3: Morphology

Suitable preservation and breeding methods for Diptera of the grave

Overview

From the results in Chapters 3, it can be deduced that regularly used preservation methods, such as ethanol solution and formaldehyde solution, are not suitable for immature members of Diptera of the grave. Adult specimens were successfully preserved in 70% ethanol and specimens were kept in acceptable condition for identification. These species require further experimentation in order to establish suitable preservation methods for larvae for field use.

Breeding of carrion feeding Diptera of the grave was successful using a basic animal tissue based medium under constant laboratory conditions. This proved sufficient for identification purposes and can be used by field investigators. However, the study does not prove that the conditions were optimal for each species and further breeding studies are needed to document developmental data and optimal conditions for each species.

Preservation and breeding

Results showed a majority failure rate in preservation with the preservation mediums used. Gradual dehydration proved slightly more successful, however, still resulted in a majority failure rate. The failure of preservation of immature stages of Diptera of the grave in the study using commonly used field preservation mediums may make it difficult to follow conventional protocols for burial cases. Preservation of adult members of the Diptera in a 70% ethanol solution proved successful and can be used for burial species. Breeding trials using bait breeding mediums in the present study proved effective in obtaining a positive identification of burial species of the Diptera, suggesting that identification of burial species may be more reliable if immature samples are bred to adulthood and subsequently identified from the resulting adult individuals.

It could be suggested that larval samples obtained from a burial crime scene should be kept alive and sent to a temperature regulated breeding environment. Samples should be bred to adulthood using carrion bait breeding mediums at a temperature of 25°C and 50% humidity, and the resulting adults should be sampled and stored in a 70% ethanol solution for analysis.

Identification of Diptera on the grave based on morphological characteristics

Overview

Species were identified using external morphological characteristics and identification keys were compiled for dipteran species of the grave found in the Free State area.

Identification of Diptera of the grave

Identification of three species, *M. scalaris*, *C. tibialis* and *M. stabulans,* down to species level was achieved using external morphological characteristics. However, members of Sarcophagidae and Sphaeroceridae were only identified to family and genus level respectively as external morphology alone was not reliable enough to characterise these representatives from other species. According to other sources, this can be achieved by the dissection of male genitalia and characterising of unique structures. However, this is not a plausible method for use by untrained forensic investigators and would not prove “user-friendly”.

Identification key

A pictorial identification key using the morphological characteristics of adult Diptera of the grave found during the study was compiled, providing a guided checklist for the purpose of identifying adult Diptera of the grave, for use by non-specialist forensic investigators. In addition to this, a written key has been compiled as can be seen in Table 5.1.

Table 5.1: Morphological key to Diptera associated with buried remains in the Free State area

1.	Wing venation simplified; no crossveins present; branches of the radial vein (*R*) strongly thickened and crowded into anterior base of wing; thorax with a humpbacked appearance	4
1'.	Wing venation not simplified; crossveins present	2
2(1').	Incomplete wing venation; longitudinal veins not reaching distal portion of wing; lower-calypter absent; subcostal (*Sc*) vein of wing incomplete, not reaching the costal vein (*C*); basal segment of hind tarsi short, swollen and shorter than second segment; distinct apical bristle on first tarsomere of mid-leg; distinct preapical seta on mid-tibia	*Leptocera* sp. (Sphaeroceridae)
2'.	Complete wing venation; longitudinal veins not as preceding description; lower-calypter present and enlarged	3
3(2').	First radial (R_1) vein independent of subcostal (*Sc*) vein; fifth radial (r_5) cell narrowed distally; medial 1 and 2 (M_{1+2}) vein angled sharply towards radial veins 4 and 5 (R_{4+5}); three black stripes present on dorsal side of thorax; 3-4 notopleural bristles (2 primary, 2 subprimary); pteropleuron bristles present; multiple elongate setae on anterodorsal side of hind-tibia	Sarcophaginae (Sarcophagidae)
3'.	First radial (R_1) vein branching from subcostal (*Sc*) vein; fifth radial (R_5) cell not narrowed distally; medial 1 and 2 (M_{1+2}) vein not angled sharply but curves slightly but distinctly towards radial 4 and 5 (R_{4+5}) vein; cubitus vein and second anal vein *(Cu2 + A_2)* and third anal *(3A)* vein short and fading out before wing margin; third anal *(A_3)* vein not curved forward on a trajectory that could intersect cubitus vein and second anal vein *(Cu2 + A_2)* if extended; pteropleuron bare; single elongated seta on anterodorsal side of hind-tibia	*Muscina stabulans* (Muscidae)
4(1).	Radial sector vein (*Rs*) forked to form veins *Rs* and first radial (R_1); anterodorsal side of hind-tibia absent of bristle-like hairs	5 (Genus: *Megaselia*) (Phoridae)
4'.	Radial sector vein (*Rs*) unforked; hind-tibia with two true-dorsal bristles and one anterodorsal bristle near upper one	6 (Genus: *Conicera)* (Phoridae)
5(4).	Anal tube of hypopygium pointed	♂ *Megaselia scalaris*
5'.	Dorsal tergite 6 elongated horizontally and flattened	♀ *Megaselia scalaris*
6(4').	Anal tube of hypopygium with irregular lobe, rounded and not tapered; pit of sense-organ on mid-femur large and apical process long	♂ *Conicera tibialis*
6'.	Dorsal tergite 6 pointed distally; upper dorsal bristle of hind-tibia below upper anterodorsal bristle and usually longer than bristle just below middle quarter	♀ *Conicera tibialis*

Chapter 4:

Documented differences in dipteran succession: above ground and below ground

Overview

Significant differences in species abundance and diversity was noted between above ground carcasses and below ground carcasses. Below ground data showed a prominence in smaller dipteran individuals on carcasses buried at 60 cm, including members of Phoridae, Sphaeroceridae and Muscidae. One larger species was found during summer months. However, only pupal cases, and later larvae, were found during the summer trial. These results were contrasting to above ground data, where a prominence of Calliphorid species was seen in higher species abundance and diversity.

Species that were most abundant in diversity and number of individuals on below ground carcasses belonged to the family Phoridae, with *M. scalaris* being most prevalent during both the summer and winter trials from the first day of colonisation. Members of Phoridae were deduced as the major feeders on the carcass. Although *Sarcophaga* and *Leptocera* were prominent during the summer and winter trial respectively, these species were only prevalent during the later stages of the study.

Capability of Diptera reaching carrion at 60 cm depth

Observations of active burrowing by adult flies to a depth of 60 cm, such as *M. scalaris* and *C. tibialis*, highlight the ability of these flies to burrow down to buried carrion. This is a significant finding as it was originally thought that Diptera of the grave oviposit into the shallow layers of the soil to allow hatched larvae to burrow down into the grave and colonise carrion. The findings of *C. tibialis* in past studies as adults and immature stages may strengthen the theory of Phoridae species being able to burrow down to a cadaver. Members of the Phoridae that played a role in below ground succession were seen to remain on the below ground carcasses, completing their life stages on the carrion and dying within the soil closest to the carcasses. Over time, empty pupal casings as well as dead Phoridae adults amassed within the graves. Phoridae adult specimens of the grave showed a common trend of staying within 5 cm of carrion in below ground cases. Below

ground evidence is not well documented and it is possible that certain members of the family Muscidae, for example, may possess the ability to detect and colonise a body below the soil. The presence of *M. stabulans* at a depth of 60 cm on day 60 of the study may suggest that these flies are able to colonise a body below ground after a delayed period of time and survive in the fairly anaerobic and compact environment such as a grave.

Below ground Diptera different to above ground Diptera

Results in the winter study showed little similarity between faunal data of the above ground carcass and the below ground carcasses, with two species being found on both above ground and below ground. Members of Calliphoridae were noted to be prominent on the above ground carcass, whereas the below ground carcasses were dominated by Phoridae species individuals. The above ground carcass also yielded the highest diversity in dipteran species.

Results in the summer study similarly showed differences between faunal data obtained from the above ground carcass and the below ground carcasses, with only one species common to both above and below ground. Several representatives of Calliphoridae and Muscidae were abundant on the above ground carcass. In contrast, below ground data contained few dipteran individuals, with only three species found during the summer trial.

Overall, it can be concluded that above ground succession involves members of Calliphoridae as the dominant feeder of carrion, whereas below ground succession yields different drivers of succession, strongly inclusive of members of Phoridae.

Dominant species of below ground carrion

Members of *M. scalaris* were noted to play a role during summer and winter months, showing coinciding periods of initial colonisation. Generally, adult Phoridae were commonly seen to colonise carcasses that had reached active decay and advanced decay, indicating their roles as primary colonisers of

buried carrion. However, members of *C. tibialis* were seen to be absent during the wetter summer months. This could have been due the inability of adult members of *C. tibialis* to survive in very moist soil caused by the high level of rainfall during the summer months in the Bloemfontein area.

Two Phoridae species were found during the winter study, being present in constantly high numbers (>100 individuals per excavation) from the initial colonisation date. Although this was not seen in summer results, it is evident that the presence of predators may have altered the dominance of members of Phoridae. It could be argued that members of Phoridae are therefore a dominant group in below ground cases, especially during the winter months in the Free State area.

Documented differences in dipteran succession: winter versus summer

Overview

Winter above ground carcass data showed less species individuals than that found on the summer above ground carcass. This was not the case with the below ground carcasses during winter and summer. Winter data showed more dipteran results than summer data on below ground carcasses. Species individuals were less during summer months due to excessive predation by *Aleochara* larvae and adults found on the below ground carcasses. Species found during winter months thrived on the below ground carcasses due to isolation, sufficient moisture retained around the body, and lack of predators around the food source.

Abundance of burial Diptera

A marked difference was noted in the results obtained from below ground carcasses during winter and summer. In comparison, the winter study showed more dipteran results than the summer study during burial analysis, in regards to both species abundance and number of individuals. Colonisation of larger numbers of members of Phoridae (between 50-100 individuals in total) was seen on the below ground carcasses by day 21 of the winter study, which contrasts the small number of *M. scalaris* individuals (<5 individuals) seen on day 21 of the summer study. An increase in species individuals was

subsequently noted in the winter study as time progressed, with the addition of two other species by day 60 of the winter trial. Data from the summer trial did not show heightened patterns of dipteran activity, instead only showing established colonisation in moderate numbers (between 20-50 individuals in total) from day 60 of the summer trial.

Limitations and shortcomings

Preservation methods

Only two preservation methods were used in the preservation study. This could be considered as insufficient to make accurate assumptions about preservation, but still serves as a solid preliminary introduction into the difficulties in preserving Diptera of the grave. As discussed in Chapter 3, there are other mediums successfully used as fixatives and preservatives of biological specimens. However, many of these chemical compounds require controlled environments and stringent safety procedures to ensure the effective use of these compounds, as well as to ensure the safety of the user. In addition, obtaining compounds such as Osmium tetraoxide is costly. Cost constraints and operating limitations can prevent the use of alternative chemical compounds for preservation, and may not be viable.

Consistency of data in field studies

It is a challenge ensuring consistency in variables within a natural environment. For example, soil type and composition may vary even in a small area of land. It was seen that grave soil composition differed slightly despite the placement of the graves being in close vicinity of each other. This shows that soil type can differ even in the same area, making it difficult to do controlled variable studies in a natural environment. Other factors that cannot be controlled in a natural environment, such as temperature, pH, and moisture, may have significant effects in burial cases but cannot be fully investigated in a non-controlled environment. It could be suggested that laboratory tests should be done to accurately gauge the effects of certain variables at different levels, and could strengthen the findings in natural field studies. However, laboratory studies should be in conjunction with field analyses as insects

will behave generally different in a controlled environment in comparison to natural environments and result in varying behaviour.

Soil barrier and observations

Although burial has been investigated in northern parts of the world, much is still unknown in regards to the interactions that soil macrofauna with their surroundings in a soil environment. It is difficult to make accurate deductions about the extent of below ground interactions as it is near impossible to observe insects on buried carrion without disturbing the soil environment. Alternative studies could be conducted using opaque cases to try and combat the problem. However, this may influence the system and act as an unwanted barrier.

Avoiding disturbance and natural succession

The current study utilised several different pigs buried within a small area and was excavated on separate days in order to minimalize the effects of disturbance on the succession process as well as eliminate the chances of false results due colonisation of above ground species colonising the carcasses during excavation and sampling. However, this may have arguably caused other problems. As mentioned before, soil type was noted to be different even within a small vicinity and resulted in slightly differing results, especially seen during the wetter summer months. The soil properties of each grave may have altered accessibility to each carcass especially during rainy periods, in turn altering the succession process. It is possible to import similar soils into a test area and replace excavated soil with brought-in soil in order to ensure that there is consistency in soil types. However, in this case, the natural environment will be altered and cannot be considered as a true representation of the area.

Carcass number and excavation

Excavations were only performed on predetermined dates, leaving periods of no activity and times where no below ground observations were made. This was due to the limited number of carcasses available for each season of the study. The limited carcass availability resulted in some cases of uncertainty in regards to the exact interactions below ground. In order to counter this in future studies, more carcasses could be buried each season in order to increase the frequency of excavations and

allow for more frequent observations. However, cost constraints, ethical permissions and land availability could be limiting factors of this design.

Summary of contributions

Breeding, preservation and identification of adult Diptera of the grave

- Conventional methods of preservation using mediums such as ethanol and formaldehyde solution on larvae of Diptera of the grave are not effective enough to preserve immature stages of Diptera of the grave.
- Gradual dehydration of larvae using an ethanol concentration increase dehydration technique resulted in deformation of larvae.
- Breeding of below ground carrion flies is successful with the use of a carrion bait breeding medium.
- Not all Diptera of the grave may be carrion feeding flies.
- Adult dipteran samples taken from buried carcasses could be identified using adult external morphological characteristics.
- A simplified pictorial key was successfully compiled using pictorial guidelines for use by crime scene investigators that may not have been exposed to forensic entomology in the past (seen in Chapter 3, Section 2).
- In addition to a simplified key, a basic morphological key was successfully compiled for use by trained forensic entomologists (Figure 5.1).

Soil properties and the effect of soil environments on dipteran succession

- Clay soil alters the rate of carrion colonisation during peak periods of rainfall.
- Large structures in soil, such as shale deposits, can alter dipteran succession, and can even halt the insect succession process.

Interactions and biology of forensically important Diptera

- Phoridae, such as *M. scalaris* and *C. tibialis*, are able to burrow down to buried carrion and oviposit directly on the carcass.
- Sampling of members of Phoridae are typically found within 5 cm of carrion in below ground cases
- Competition between burial dipteran species of the grave causes shifts in species distribution on a carcass, resulting in resource partitioning on a carcass.
- Predators, such as *Aleochara* (Coleoptera: Staphylinidae) can greatly impact dipteran occurrence on buried carrion and even cause the complete absence of dipteran species on buried carrion.
- Members of Phoridae thrive in the presence of sufficient moisture and the absence of predators.

Diptera of the grave: Role players and arrival times in the Free State area

- Members of Phoridae can be described as primary colonisers of buried carrion
- Members of *Sarcophaga* can be considered as secondary colonisers of buried carrion during summer months.
- Members of *M. stabulans* can be considered as secondary colonisers of buried carrion during winter months.
- *Leptocera* species are possibly not carrion feeders, but can be used as forensic indicators.

Main Study outcomes and proved hypothesis

- Hypothesis I proven false: Preservation in ethanol solution was not successful.
- Hypothesis II proven initially true: Breeding was successful in the F_0 generation. However, further breeding to the F_2 generation was not successful.

- Hypothesis III proven true for three of the five species investigated: *M. scalaris*, *C. tibialis* and *M. stabulans* was successfully identified using only external morphological characteristics.
- A user friendly key was successfully compiled using morphological characters for use by forensic investigators.
- Hypothesis iv proven true: Members of the Diptera are capable of reaching carrion at a depth of 60 cm during summer and winter months.
- Hypothesis v proven true: Burial faunal composition was seen to be different to that of above ground fauna.
- Hypothesis vi proven true: Members of Phoridae were the most dominant members of the Diptera on buried carrion.
- Hypothesis iii proven false: Burial members of the Diptera were more abundant during winter months than during summer months.

"We are organisms; we're conceived, we're born, we live, we die, and we decay. But as we decay we feed the world of the living: plants and bugs and bacteria."

- William M. Bass, Death's Acre: Inside the legendary forensic lab the body farm where the dead do tell tales

References

ADAMS, Z.J.O. & HALL, M.J.R. 2003. Methods used for the killing and preservation of blowfly larvae, and their effect on post-mortem larval length. *Forensic Science International* **138**: 50-61.

ALDRICH, J.M. 1916. *Sarcophaga and allies in North America*. Thomas Say Foundation. La Fayette, Indiana.

AMENDT, J., KRETTEK, R. & ZEHNER, R. 2004. Forensic entomology. *Naturwissenschaften* **91**: 51-65.

AMENDT, J., RICHARDS, C.S., CAMPOBASSO, C.P., ZEHNER, R. & HALL, M.J.R. 2011. Forensic entomology: application and limitations. *Forensic Science, Medicine, and Pathology* **7**: 379-392.

AMES, C. & TURNER, B. 2003. Low temperature episodes in development of blowflies: implications for post-mortem interval estimation. *Medical and Veterinary Entomology* **17**: 178-186.

ANDERSON, G.S. 1995. The use of insects in death investigations: an analysis of forensic entomology in British Columbia over a five year period. *Canadian Society of Forensic Science Journal* **28**: 277-292.

ARCHER, M.S. 2004. Annual variation in arrival and departure times of carrion insects at carcasses: implications for succession studies in forensic entomology. *Australian Journal of Zoology* **51**: 569-576.

ARNALDOS, I., ROMERO, E., GARCIA, M.D. & LUNA, A. 2001. An initial study on the succession of sarcosaprophagous Diptera (Insecta) on carrion in the southeastern Iberian peninsula. *International Journal of Legal Medicine* **114**: 156-162.

ARNALDOS, M.I., GARCÍA, M.D., ROMERO, E., PRESA, J.J. & LUNA, A. 2005. Estimation of postmortem interval in real cases based on experimentally obtained entomological evidence. *Forensic Science International* **149**: 57-65.

BANFI, E. & GALASSO, G. 2008. New combinations of *Vachellia* Wight & Arn., formerly *Acacia* Mill. s.s. (Fabaceae). *Atti della Societa Italiana di Scienze Naturali e del Museo Civico di Storia Naturale di Milano* **149**: 149.

BARNES, J.K. 1990. Life history of *Dohrniphora cornuta* (Bigot) (Diptera: Phoridae), a filth-inhabiting humpbacked fly. *Journal of the New York Entomological Society* **98**: 474-483.

BARROS DE SOUZA, A.S., KIRST, F.D. & KRÜGER, R.F. 2008. Insects of forensic importance from Rio Grande do Sul state in southern Brazil. *Revista Brasileira de Entomologia* **52**: 641-646.

BATTÁN HORENSTEIN, M., XAVIER LINARES, A., ROSSO DE FERRADAS, B. & GARCÍA, D. 2010. Decomposition and dipteran succession in pig carrion in central Argentina: ecological aspects and their importance in forensic science. *Medical and Veterinary Entomology* **24**: 16-25.

BAUMGARTNER, D.L. 1988. Spring season survey of the urban blowflies (Diptera: Calliphoridae) of Chicago, Illinois. *The Great Lakes Entomologist* **21**: 119-121.

BENBOW, M.E., TOMBERLIN, J.K. & TARONE, A.M. 2015. *Carrion ecology, evolution and their applications.* CRC Press, Boca Raton.

BENECKE, M. 2001. A brief history of forensic entomology. *Forensic Science International* **120**: 2-14.

BOEHME, P., AMENDT, J., DISNEY, R.H.L. & ZEHNER, R. 2010. Molecular identification of carrion-breeding scuttle flies (Diptera: Phoridae) using COI barcodes. *International Journal of Legal Medicine* **124**: 557-581.

BORROR, D.J., TRIPLEHORN, C.A. & JOHNSON, N.F. 1989. *An introduction to the study of insects*. Sixth Edition. Saunders College Publishing, Philadelphia.

BOTHAM, J.L. 2016 unpublished. Decomposition and arthropod succession on buried remains during winter and summer in central South Africa: Forensic implications and predictive analyses. M.Sc Dissertation. University of the Free State, Bloemfontein, RSA.

BOUREL, B., TOURNEL, G., HÉDOUIN, V. & GOSSET, D. 2004. Entomofauna of buried bodies in northern France. *International Journal of Legal Medicine* **118**: 215-220.

BOZZOLA, J.J. & RUSSELL, L.D. 1992. *Electron microscopy: Principle and techniques for biologists.* Jones & Bartlett, Boston, USA.

BRAACK, L.E.O. 1987. Community dynamics of carrion-attendant arthropods in tropical african woodland. *Oecologia* **72**:402-409.

BRINK, S.L. 2009 unpublished. Key diagnostic characteristics of the developmental stages of forensically important Calliphoridae and Sarcophagidae in central South Africa. Ph.D Thesis. University of the Free State, Bloemfontein, RSA.

BUCK, M. 1997. Sphaeroceridae (Diptera) reared from various types of carrion and other decaying substrates in southern Germany, including new faunistic data on some rarely collected species. *European Journal of Entomology* **94**: 137-151.

BUGAJSKI, K.N., SEDDON, C.C. & WILLIAMS, R.E. 2011. A comparison of blow fly (Diptera: Calliphordae) and beetle (Coleoptera) activity on refrigerated only versus frozen-thawed pig carcases in Indiana. *Journal of Medical Entomology* **48**: 1231-1235.

BYRD, J.H. & CASTNER, J.L. (Eds.) 2001. *Forensic entomology: The utility of arthropods in legal investigations*. First Edition. CRC Press, Boca Raton.

BYRD, J.H. & CASTNER, J.L. (Eds) 2010. *Forensic Entomology: The utility of arthropods in legal investigation.* Second Edition. CRC Press, Boca Raton.

CAMPOBASSO, C.P., DI VELLA, G. & INTRONA, F. 2001. Factors affecting decomposition and Diptera colonization. *Forensic Science International* **120**: 18-27.

CARTER, D.O. 2011. An overview of forensic taphonomy. University of Nebraska-Lincoln, USA. Second International Seminar of Forensic Anthropology. (http://forensicosteology.org).

CARTER, D.O., YELLOWLEES, D. & TIBBETT, M. 2007. Cadaver decomposition in terrestrial ecosystems. *Naturwissenschaften* **94**: 12-24.

CARTER D.O., YELLOWLEES, D. & TIBBETT, M. 2010. Moisture can be the dominant environmental parameter governing cadaver decomposition in soil. *Forensic Science International* **200**: 60-66.

CASTRO, C.P.E., SOUSA, J.P., ARNALDOS, M.I., GASPAR, J. & GARCÍA, M.D. 2011. Blowflies (Diptera: Calliphoridae) activity in sun exposed and shaded carrion in Portugal. *Annales de la Société entomologique de France* **47**: 128-139.

CATTS, E.P. & GOFF, M.L. 1992. Forensic entomology in criminal investigations. *Annual Review of Entomology* **37**: 253-272.

CATTS, E.P. & HASKEL, N.E. 1990. *Entomology and death: A procedural guide*. Joyce's Print Shop, Clemson.

CENTENO, N., MALDONADO, M. & OLIVA, A. 2002. Seasonal patterns of arthropods occurring on sheltered and unsheltered pig carcasses in Buenos Aires province (Argentina). *Forensic Sciences International* **126**: 63-70.

CHEN, C.D., LEE, H.L., NAZNI, W.A., RAMLI, R., JEFFERY, J. & SOFIAN-AZIRUN, M. 2010. First report of the house fly larvae, *Musca domestica* (Linnaeus)(Diptera: Muscidae) associated with the monkey carcass in Malaysia. *Tropical Biomedicine* **27**: 355-359.

CHESWORTH, W. 2007. *Encyclopedia of soil science*. Springer Science and Business Media, Berlin.

CHU, H.F. & WANG, L.Y. 1975. Insect carcasses unearthed from the Chinese antique tombs. *Acta Entomologica Sinica* **18**: 333-337.

COATES PALGRAVE, K. & COATES PALGRAVE, M. 2002. *Trees of Southern Africa.* Struik Publishers, Cape Town.

COYLER C.N. & HAMMOND C.O. 1951. *Flies of the British Isles*. Frederick Warne, London.

CORRÊA, R.C., ALMEIDA, L.M. & MOURA, M.O. 2014. Coleoptera associated with buried carrion: potential forensic importance and seasonal composition. *Journal of Medical Entomology* **51**: 1057-1066.

COUPLAND, J.B. & BAKER, G. 1994. Host distribution, larviposition behaviour and generation time of *Sarcophaga penicillata* (Diptera: Sarcophagidae), a parasitoid of conical snails. *Bulletin of Entomological Research* **84**: 185-189.

COURI, M.S. 2007. A key to the afrotropical genera of Muscidae (Diptera). *Revista Brasileira de Zoologia* **24**: 175-184.

COURI, M.S., DE CARVALHO, C.J.B. & PONT A.C. 2012. Taxonomy of the Muscidae (Diptera) of Namibia: a key to genera, diagnoses, new records and description of a new species. *African Invertebrates* **53**: 47-67.

D'ASSIS FONSECA E.C.M. 1968. Diptera Cyclorrhapha Calyptrata Section (b) Muscidae. In Dolling, W.R. & Askew, R.R. (Eds.) *Handbooks for the identification of British insects*. Volume 10, Part 4b. Royal Entomological Society of London, London.

DAVENPORT, G.C., FRANCE, D.L., GRIFFIN, T.J., SWANBERG, J.G., LINDEMANN, J.W., TRANUNELL, V., ARMBRUST, C.T., KONDRATEIFF, B., NELSON, A., CASTELLANO, K. & HOPKINS, D. 1992. A multidisciplinary approach to the detection of clandestine graves. *Journal of Forensic Sciences* **37**: 1445-1458.

DAY, D.H. & WALLMAN, J.F. 2008. Effect of preservative solutions on preservation of *Calliphora augur* and *Lucilia cuprina* larvae (Diptera: Calliphoridae) with implications for post-mortem interval estimates. *Forensic Science International* **179**: 1-10.

DE CARVALHO, C.J.B. & DE MELLO-PATIU, C.A. 2008. Key to the adults of the most common forensic species of Diptera in South America. *Revista Brasileira de Entomologia* **52**: 390-406.

DE CARVALHO, L.M.L. & LINHARES, A.X. 2001. Seasonality of insect succession and pig carcass decomposition in a natural forest area in southeastern Brazil. *Journal of Forensic Science* **46**: 604-608.

DEKEIRSSCHIETER, J., VERHEGGEN, F.J., GOHY, M., HUBRECHT, F., BOURGUIGNON, L., LOGNAY, G. & HAUBRUGE, E. 2009. Cadaveric volatile organic compounds released by decaying pig carcasses (*Sus domesticus* L.) in different biotopes. *Forensic Science International* **189**: 46-53.

DENT, BB., FORBES, S.L. & STUART, B.H. 2004. Review of human decomposition processes in soil. *Environmental Geology* **45**: 576-585.

DISNEY, R.H.L. 1983. Scuttle flies, Diptera: Phoridae (except *Megaselia*). In Fitton, M.G. *Handbooks for the identification of British insects*. Volume 10, Part 6. Royal Entomological Society of London, London.

DISNEY, R.H.L. 1989. Scuttle flies, Diptera: Phoridae (Genus *Megaselia*). In Dolling, W.R. & Askew, R.R. (Eds.) *Handbooks for the identification of British insects*. Volume 10, Part 8. Royal Entomological Society of London, London.

DISNEY, R.H.L. 2005. Duration of development of two species of carrion-breeding scuttle flies and forensic implications. *Medical and Veterinary Entomology* **19**: 229-235.

DISNEY, R.H.L. 2008. Natural history of the scuttle fly, *Megaselia scalaris*. *Annual Review of Entomology* **53**: 39-60.

DISNEY, R.H.L., GARCIA-ROJO, A., LINDSTRÖM, A. & MANLOVE, J.D. 2014. Further occurrences of *Dohrniphora cornuta* (Bigot) (Diptera: Phoridae) in forensic cases indicate likely importance of this species in future cases. *Forensic Science International* **241**: e20-e22.

DISNEY, R.H.L., PRESCHER, S. & ASHMOLE, N.P. 2010. Scuttle flies (Diptera: Phoridae) of the Canary Islands. *Journal of Natural History* **44**: 107-218.

DONG, H. & YANG, D. 2015. The genus *Leptocera* from China, with description of a new species (Diptera: Sphaeroceridae). *Zoological Systematics* **40**: 303-314.

DONOVAN, S.E., HALL, M.J.R., TURNER, B.D. & MONCRIEFF, C.B. 2006. Larval growth rates of the blowfly, *Calliphora vicina*, over a range of temperatures. *Medical and Veterinary Entomology* **20**: 106-114.

EBERHARDT, T.L. & ELLIOT, D.A. 2008. A preliminary investigation of insect colonisation and succession on remains in New Zealand. *Forensic Science International* **176**: 217-223.

ELTOUM, I., FREDENBURGH, J., MYERS, R.B. & GRIZZLE, W.E. 2001. Introduction to the theory and practice of fixation of tissues. *Journal of Histotechnology* **24**: 173-190.

ERASMUS, B.F.N., VAN JAARSVELD, A.S., CHOWN, S.L., KSHATRIYA, M. & WESSELS, K.J. 2002. Vulnerability of South African animal taxa to climate change. *Global Climate Change* **8**: 679-693.

GARCÍA-ROJO, A.M., MARTÍNEZ-SÁNCHEZ, A., LÓPEZ, R., GARCÍA DE LA VEGA, J.M., RICA, M., GONZÁLEZ, M. & DISNEY, R.H. 2013. A mathematical model applied for assisting the estimation of PMI in a case of forensic importance. First record of *Conicera similis* (Diptera: Phoridae) in a corpse. *Forensic Science International* **10**: 11-18.

GENNARD, D.E. 2007. *Forensic Entomology: an introduction*. First Edition. John Wiley & Son Ltd., England.

GEORGE, K.A., ARCHER, M.S. & TOOP, T. 2013. Abiotic environmental factors influencing blowfly colonisation patterns in the field. *Forensic Science International* **229**: 100-107.

GONZALEZ-VAINER, P., CASTRO, M., REMEDIOS, M., MORELLI, E., MARTÍNEZ, M. & DISNEY, R.H.L. 2012. First record of *Megaselia rufipes* (Meigen, 1804) (Diptera: Phoridae) from Uruguay with notes on biology. *Check list* **8**: 1299-1300.

GOULD, C. (Ed.) 2009. *Criminal (in)justice in South Africa: A civil society perspective*. Institute for Security Studies, Pretoria.

GREENBERG, B. & KUNICH, J.C. 2002. *Entomology and the law: flies as forensic indicators*. First Edition. Cambridge University Press, United Kingdom.

GREENBERG, B. 1990. Nocturnal oviposition behaviour of blowflies (Diptera: Calliphoridae). *Journal of Medical Entomology* **27**: 807-810.

GREENBERG, B. 1991. Flies as forensic indicators. *Journal of Medical Entomology* **28**: 565-577.

GRIFFIN, J.N. & SILLIMAN, B.R. 2011. Resource partitioning and why it matters. *Nature Education Knowledge* **3**: 49.

GUNN, A. & BIRD, 2011. The ability of the blowflies *Calliphora vomitoria* (Linnaeus), *Calliphora vicina* (Rob-Desvoidy) and *Lucilia sericata* (Meigen) (Diptera: Calliphoridae) and the muscid flies *Muscina stabulans* (Fallén) and *Muscina prolapsa* (Harris) (Diptera: Muscidae) to colonise buried remains. *Forensic Science International* **207**: 198-204.

HALL, R.D. & DOISY, K.E. 1993. Length of time after death: Effect on attraction and oviposition or larviposition of midsummer blowflies (Diptera: Calliphoridae) and flesh flies (Diptera: Sarcophagidae) of medicolegal importance in Missouri. *Annals of the Entomological Society of America* **86**: 589-593.

HARRINGTON, R., & WOIWOD, I. & SPARKS, T. 1999. Climate change and trophic interactions. *Trends in Ecology and Evolution* **14**: 146-150.

HARVEY, M.L., MANSELL, M.W., VILLET, M.H. & DADOUR, I.R. 2003. Molecular identification of some forensically important blowflies of southern Africa and Australia. *Medical and Veterinary Entomology* **17**: 363-369.

HOFFMAN, S. 2014 unpublished. The influence of multiple trauma related injuries on carcass decomposition and insect activity: A seasonal study. M.Sc Dissertation. University of the Free State, Bloemfontein, RSA.

HUCHET, J.B. & GREENBERG, B. 2010. Flies, mochicas and burial practices: a case study from Huaca de la Luna, Peru. *Journal of Archaeological Science* **37**: 2846-2856.

IRELAND, S. & TURNER, B. 2006. The effects of larval crowding and food type on the size and development of the blowfly, *Calliphora vomitoria*. *Forensic Science International* **159**: 175-181.

ITIS 2015. Online report (on line) available: http://www.itis.gov, accessed 23 July 2015.

IVES, A.R. 1988. Aggregation and the coexistence of competitors. *Annales Zoologici Fennici* **25**: 75-88.

JAKOVLEV J. 1994. Palaearctic Diptera associated with fungi and *Myxomycetes*. Karelian Research Center, Russian Academy of Sciences, Forest Research Institute. Petrozavodsk.

JOSEPH, I., MATHEWS, D.G., SATHYAN, P. & VARGHEESE, G. 2011. The use of insects in forensic investigations: an overview on the scope of forensic entomology. *Journal of Forensic Science* **3**: 89-91.

KARAPAZARLIOGLU, E. & DISNEY, R.H.L. 2015. First record of forensic species *Conicera similis* (Haliday, 1833) (Diptera: Phoridae) on exhumed rabbit carcasses in Turkey. *European Scientific Journal* **11**: 13-16.

KELLY, J.A. 2006 unpublished. The influence of clothing, wrapping and physical trauma on carcass decomposition and arthropod succession in central South Africa. Ph.D Thesis. University of the Free State, Bloemfontein, RSA.

KLOTZBACH, H., KRETTEK, R., BRATZKE, H., PÜSCHEL, K., ZEHNER, R. & AMENDT, J. 2004. The history of forensic entomology in German-speaking countries. *Forensic Science International* **144**: 259-263.

KOČÁREK, P. 2003. Decomposition and Coleoptera succession on exposed carrion of small mammal in Opava, the Czech Republic. *European Journal of Soil Biology* **39**: 31-45.

KOLVER, J.H. 2009 unpublished. Forensic entomology: The influence of the burning of a body on insect succession and calculation of the post-mortem interval. Ph.D Thesis. University of the Free State, Bloemfontein, RSA.

KUMARA, T.K., DISNEY, R.H.L., HASSAN, A.A., FLORES, M., HWA, T.S., MOHAMMED, Z., CHESALMAH, M.R. & BHUPINDER, S. 2012. Occurrence of oriental flies associated with indoor and outdoor human remains in the tropical climate of north Malaysia. *Journal of Vector Ecology* **37**: 62-68.

KURAHASHI, H. & KIRK-SPRIGGS, A. 2006. The Calliphoridae of Namibia (Diptera: Oestroidae). *Zootaxa* **1322**: 1-131.

LAL, R. & SHUKLA, M.K. (Eds.) 2005. *Principles of soil physics*. Marcel Dekker, Inc., New York.

LANE, R.P. 1975. An investigation into blowfly (Diptera: Calliphoridae) succession on corpses. *Journal of Natural History* **9**: 581-588.

LOWE, A.C. 2010 unpublished. A geophysical and biochemical investigation of buried remains in contrasting soil textures in southern Ontario. M.Sc thesis. University of Ontario Institute of Technology, Oshawa, Canada.

LUI, D. & GREENBERG, B. 1989. Immature stages of some flies of forensic importance. *Annals of the Entomological Society of America* **82**: 80-93.

MACKELLER, N., NEW, M. & JACK, C. 2014. Observed and modelled trends in rainfall and temperatures for South Africa: 1960-2010. *South African Journal of Science* **110**: 1-13.

MALGORN, Y., COQUOZ, R. 1999. DNA typing for identification of some species of Calliphoridae: an interest in forensic entomology. *Forensic Science International* **102**: 111-119.

MANN, R.W., BASS, W.M. & MEADOW, L. 1990. Time since death and decomposition of the human body: variables and observations in case and experimental field studies. *Journal of Forensic Science* **35**: 103-111.

MARIANI, R., GARCÍA-MANCUSO, R., VARELA, G.L. & INDA, A.M. 2014. Entomofauna of a buried body: study of the exhumation of a human cadaver in Buenos Aires, Argentina. *Forensic Science International* **237**: 19-26.

MARSHALL, S.A. 2012. *Flies: The natural history & diversity of Diptera*. Firefly Books Ltd., New York.

MARTÍN-VEGA, D., GÓMEZ-GÓMEZ, A. & BAZ, A. 2011. The “coffin fly” *Conicera tibialis* (Diptera: Phoridae) breeding on buried human remains after a post-mortem interval of 18 years. *Journal of Forensic Sciences* **56**: 1654-1656.

MCALPINE, J.F., PETERSOM, B.V., SHEWELL, G.E., TESKEY, H.J., VOCKEROTH, J.R. & WOOD, D.M. (Eds) 1981. *Manual of Neartic Diptera Volume 1*. Research Branch, Agriculture Canada, Minnesota.

MCALPINE, J.F., PETERSOM, B.V., SHEWELL, G.E., TESKEY, H.J., VOCKEROTH, J.R. & WOOD, D.M. (Eds) 1987. *Manual of Neartic Diptera Volume 2*. Research Branch, Agriculture Canada, Ontario.

MÉGNIN, P. 1887. La faune des Tombeaux. *Compte Rendu Hebdomadaire des Séances de l'Academie des Sciences* **105**: 948-951.

MÉGNIN, P. 1894. *La faune des cadavres*. Encyclopédie Scientifique des Aide-Memoire. Gauthier-Villars et Fils, Paris.

MEIKLEJOHN, K.A., WALLMAN, J.F. & DOWTON, M. 2011. DNA-based identification of forensically important Australian Sarcophagidae (Diptera). *International Journal of Legal Medicine* **125**: 27-32.

MEREDITH, D. (Ed). 1955. *The grasses and pastures of South Africa.* Central News Agency, Cape Times Limited, Cape Town.

MILLER, R.A. 2002 unpublished. The affects of clothing on human decomposition: implications for estimating time since death. M.Sc thesis. University of Tennessee, Knoxville, USA.

MOTTER, M.G. 1898. A contribution of the fauna of the grave: a study of 150 disinterments, with some additional observations. *Journal of the New York Entomological Society* **6**: 201-231.

MULLEN, G.R. & DURDEN, L.A. 2009. *Medical and veterinary entomology*. Second Edition. Academic Press, Cambridge.

NARCHUK, E.P. 1989. Family Sphaeroceridae (Borboridae, Cypselidae). In: Bei-Bienko, G.Y. & Steyskal, G.C. (Eds.) *Keys to the insects of the European part of the USSR*. Volume 4. E.J. Brill, New York, pp. 559-590.

NAWROCKI, S.P. 1996. An outline of forensic taphonomy. University of Indianapolis Archeology and Forensic Laboratory (http://archlab.uindy.edu).

NELSON, L.A., WALLMAN, J.F. & DOWTON, M. 2008. Identification of forensically important *Chrysomya* (Diptera: Calliphoridae) species using the second ribosomal internal transcribed spacer (ITS2). *Forensic Science International* **177**: 238-247.

ODEYEMI, O.O. 1997. Interspecific competition between the beetles *Dermestes maculatus* Degeer and *Necrobia rufipes* Degeer in dried fish. *International Journal of Tropical Insect Science* **17**: 213-220.

OLIVA, A. 2002. Insects of forensic interest in Buenos Aires (Argentina), Addenda et corrigenda. I. Phoridae (Diptera: Brachycera). *Physis* **60**: 138-139.

PACZKOWSKI, S., MAIBAUM, F., PACZKOWSKI, M. & SCHIITZ, S. 2012. Decaying mouse volatiles perceived by *Calliphora vicina* Rob.-Desv. *Journal of Forensic Sciences* **57**: 1497-1506.

PAGE, J.B. 1952. Role of physical properties of clays in soil science. *Clay and Clay Minerals* **1**: 167-176.

PANAGIOTAKOPULU, E. & BUCKLAND, P.C. 2012. Forensic archaeoentomology – an insect fauna from a burial in York Minster. *Forensic Science International* **221**: 125-130.

PAPP, L. 2012. A review of the Afrotrpical species of *Leptocera* Olivier (Diptera: Sphaeroceridae). *Acta Zoologica Academiae Scientiarum Hungaricae* **58**: 225-258.

PARMESAN, C. 2006. Ecological and evolutionary responses to recent climate change. *Annual Review of Ecology, Evolution, and Systematics* **37**: 637-669.

PASTULA, E.C. 2012 unpublished. Insect timing and succession on buried carrion in East Lansing, Michigan. M.Sc thesis. Michigan State University, Michigan, USA.

PASTULA, E.C. & MERRITT, R.W. 2013. Insect arrival pattern and succession on buried carrion in Michigan. *Journal of Medical Entomology* **50**: 432-439.

PAYNE, J.A. 1965. A summer carrion study of the baby pig *Sus scrofa* Linnaeus. *Ecology* **46**: 592-602.

PAYNE J.A., KING E.W. & BEINHART G. 1968. Arthropod succession and decomposition of buried pigs. *Nature* **219**: 1180-1181.

PITKIN, B.R. 1988. Lesser dung flies (Sphaeroceridae). In Dolling, W.R. & Askew, R.R. (Eds.) *Handbooks for the identification of British insects*. Volume 10, Part 5e. Royal Entomological Society of London, London.

POKINES, J. & SYMES, S.A. (Eds) 2013. *Manual of forensic taphonomy*. CRC Press, Boca Raton.

POPE, M.A. 2010 unpublished. Differential decomposition patterns of human remains in variable environments of the Midwest. Ph.D. thesis. University of South Florida, Tampa, USA.

PRADO E CASTRO, C., SERRANO, A., MARTINS DA SILVA, P. & GARCIA, M.D. 2012. Carrion flies of forensic interest: a study of seasonal community composition and succession in Lisbon, Portugal. *Medical and Veterinary Entomology* **26**: 417-431.

PRAWIRODISASTRO, M. & BENJAMIN, D.M. 1979. Laboratory study on the biology and ecology of *Megaselia scalaris* (Diptera: Phoridae). *Journal of Medical Entomology* **16**: 317-320.

RICHARD, E.N. 2001 unpublished. Spatial and temporal variation in carrion blow fly communities: applications to forensic entomology. Ph.D. thesis. Texas Tech University, Lubbock, USA.

RICHARDS, C.S., PRICE, B.W. & VILLET, M.H. 2009. Thermal ecophysiology of seven carrion-feeding blowflies in Southern Africa. *Entomologia Experimentalis et Applicata* **131**: 11-19.

RICHARDSON, M.S. & GOFF, M.L. 2001. Effects of temperature and intraspecific interaction on the development of *Dermestes maculatus* (Coleoptera: Dermestidae). *Journal of Medical Entomology* **38**: 347-351.

RODRIGUEZ, W.C. & BASS, W.M. 1985. Decomposition of buried bodies and methods that may aid in their location. *Journal of Forensic Science* **30**: 836-852.

ROHÁČEK, J., MARSHALL, S.A., NORRBOM, A.L., BUCK, M., QUIROS, D.I. & SMITH, I. 2001. *World catalog of Sphaeroceridae (Diptera)*. Slezské Zemské Museum, Opava.

S.A.P.S. 2015. SAPS Online Statistics, (online) available: http://www.saps.gov.za. Accessed 20 November 2015.

SANBI 2014. National climate change response white paper. Department of Environmental Affairs Report. Published 2014 by Department of Environmental Affairs.

SANFORD, M.R., PECHAL, J.L. & TOMBERLIN, J.K. 2011. Rehydration of forensically important larval Diptera specimens. *Journal of Medical Entomology* **48**: 118-125.

SCHMITZ, H. 1928. Occurrence of phorid flies in human corpses buried in coffins. *Natuurhistorisch Maandblad* **17**: 150.

SCHOENER, T.W. 1974. Resource partitioning in ecological communities. *Science* **185**: 27-39.

SERVICE, M. 2012. *Medical entomology for students*. Fifth Edition. Cambridge University Press, Cambridge.

SHIVEKAR, S., SENTHIL, K., SRINIVASAN, R., SURESHBABU, L., CHAND, P., SHANMUGAM, J. & GOPAL, R. 2008. Intestinal myiasis caused by *Muscina stabulans*. *Indian Journal of Medical Microbiology* **26**: 83-85.

SINGH, D. & BHARTI, M. 2008. Some notes on the nocturnal larviposition by two species of *Sarcophaga* (Diptera: Sarcophagidae). *Forensic Science International* **177**: 19-20.

SLANSKY, F. & RODRIGUEZ, J.G. (Eds.) 1987. *Nutritional ecology of insects, mites, and spiders*. John Wiley and Sons, Inc., New Jersey, USA.

SMITH, K.G.V. 1986. *A manual of forensic entomology*. Trustees of the British Natural History Museum, London.

SNYDER, F.M. 1955. Notes and descritpions of *Muscina* and *Dendrophaonia* (Diptera: Muscidae). *Annals of the Entomological Society of America* **48**: 445-452.

STOKER, R.L., GRANT, W.E & VINSON, S.B. 1995. *Solenopsis invicta* (Hymenoptera: Formicidae) effect on invertebrate decomposers of carrion in central Texas. *Environmental Entomology* **24** :817-822.

SUKONTASON, K., SUKOTASON, K.L., PIANGJAI, S., BOONCHU, N., KURAHASHI, H., HOPE, M. & OLSEN, J.K. 2004. Identification of forensically important fly eggs using a potassium permanganate staining technique. *Micron* **35**: 391-395.

SUKONTASON, K.L., NARONGCHAI, P., KANCHAI, C., VICHAIRAT, K., PIANGJAI, S., BOONSRIWONG, W., BUNCHU, N., SRIPAKDEE, D., CHAIWONG, T., KUNTALUE, B., SIRIWATTANARUNGSEE, S. & SUKONTASON, K. 2006. Morphological comparison between *Chrysomya rufifacies* (Macquart) and *Chrysomya villeneuvi* Patton (Diptera: Calliphoridae). *Forensic Science International* **164**: 230-234.

TABOR, K.L., BREWSTER, C.C. & FELL, R.D. 2004. Analysis of the successional patterns of insects on carrion in southwest Virginia. *Journal of Medical Entomology* **41**: 785-795.

THEVAN, K., DISNEY, R.H.L. & AHMED, A.H. 2010. First record of two species of Oriental scuttle flies (Diptera: Phoridae) from forensic cases. *Forensic Science International* **195**: e5-e7.

TOMBERLIN, J.K. & BENBOW, M.E. 2015. *Forensic entomology: international dimensions and frontiers*. CRC Press, Boca Raton.

TRIPLEHORN, C.A. & JOHNSON, N.F. 2005. *Borror and Delong's introduction to the study of insects*. Seventh Edition. Brooks/Cole, Belmont.

TURCHETTO, M. & VANIN, S. 2004. Forensic entomology and climatic change. *Forenisc Science International* **146**: 207-209.

TURNER, A.R., KARACAOGLU, E., NAMLI, A., KETEN, A., FARASAT, S., AKCAN, R., SERT, O. & ODABAŞI, A.B. 2013. Effects of different types of soil on decomposition: an experimental study. *Legal Medicine* **15**: 149-156.

TURNER, B. & WILTSHIRE, P. 1999. Experimental validation of forensic evidence: a study of the decomposition of buried pigs in a heavy clay soil. *Forensic Science International* **101**: 113-122.

VAIRO, K.P., DE MELLO-PATIU, C.A. & DE CARVALHO, C.J.B. 2011. Pictorial identification key for species of Sarcophagidae (Diptera) of potential forensic importance in southern Brazil. *Revista Brasileira de Entomologia* **55**: 333-347.

VANLAERHOVEN, S.L. & ANDERSON, G.S. 1999. Insect succession on buried carrion in two biogeoclimatic zones of British Columbia. *Journal of Forensic Science* **44**: 32-43.

VASCONCELOS, S.D. & ARAUJO, M.C.S. 2012. Necrophagous species of Diptera and Coleoptera in northeastern Brazil: state of the art and challenges for the forensic entomologist. *Revista Brasileira de Entomologia* **56**: 7-14.

VILLET, M.H. 2011. African carrion ecosystems and their insect communities in relation to forensic entomology. *Pest Technology* **5**: 1-15.

VISSER, M.E. 2008. Keeping up with a warming world; assessing the rate of adaptation to climate change. *Proceedings of the Royal Society* **275**: 649-659.

VOSS, S.C., SPAFFORD, H. & DADOUR, I.R. 2009. Annual and seasonal patterns of insect succession on decomposing remains at two locations in Western Australia. *Forensic Science International* **193**: 26-36.

WAJNBERG, E., BERNSTEIN, C. & VAN ALPHEN, J. 2008. *Behavioural ecology of insect parasitoids: from theoretical approaches to field applications*. John Wiley & Sons, Hoboken.

WALL, R., FRENCH, N. & MORGAN, K.L. 1992. Effects of temperature on the development and abundance of the sheep blowfly *Lucilia sericata* (Diptera: Calliphoridae). *Bulletin of Entomological Research* **82**: 125-131.

ZEHNER, R., AMENDT, J., SCHÜTT, S., SAUER, J., KRETTEK, R. & POVOLNÝ, D. 2004. Genetic identification of forensically important flesh flies (Diptera: Sarcophagidae. *International Journal of Legal Medicine* **118**: 245-247.

ZIERVOGEL, G., NEW, M., ARCHER VAN GARDEREN, E., MIDGLEY, G., TAYLOR, A., HAMANN, R., STUART-HILL, S., MYERS, J. & WARBURTON, M. 2014. Climate change impacts and adaptation in South Africa. *WIREs Climate Change* **5**: 605-620.

ZUHA, R.M., RAZAK, T.A., AHMED, N.W. & OMAR, B. 2012. Interaction effects on temperature and food on the development of forensically important fly, *Megaselia scalaris* (Loew)(Diptera: Phoridae). *Parasitology Research* **111**: 2179-2187.

ZUMPT, F. 1961. *Exploration du Parc National Albert. Mission G.F. De Wittte (1933-1935). Calliphoridae (Diptera Cyclorrhapha) Part III: Miltogramminae.* Institut des Parcs Nationaux du Congo et du Ruanda-Urundi. Bruxelles.

ZUMPT, F. 1965. *Myiasis in man and animals in the old world: a textbook for physicians, veterinarians, and zoologists.* Butterworths, London.

ZUMPT, F. 1965a. *Exploration du Parc National Albert. Mission G.F. De Wittte (1933 -1935). Calliphoridae (Diptera Cyclorrhapha) Part 1: Calliphorini and Chrysomyiini.* Institut des Parcs Nationaux du Congo Belge. Brussel.

ZUMPT, F. 1965b. *Exploration du Parc National Albert. Mission G.F. De Wittte (1933 -1935). Calliphoridae (Diptera Cyclorrhapha) Part II: Rhiniini.* Institut des Parcs Nationaux du Congo Belge. Brussel.

ZUMPT, F. 1972. *Exploration du Parc National des Virunga. Mission G.F. De Wittte (1933 -1935). Calliphoridae (Diptera Cyclorrhapha) Part IV: Sarcophaginae.* Foundation Pour Favoriser les Recherches Scientifiques en Afrique. Bruxelles.

URL references:

Sarcophaga bercaea photograph: https://upload.wikimedia.org/wikipedia/commons/b/b5/Sarcophaga_Bercaea2.jpg, visited on 22 December 2015.

Male *Megaselia scalaris* photograph: http://www.opsu.edu/Academics/SciMathNurs/NaturalScience/PlantsInsectsOfGoodwell/diptera/phoridae/11-10-22-3m_9439.jpg, visited on 22 December 2015.

Appendix 1: Ethical approval

The following ethical approval form is for the present study:

"The identification of Diptera of the grave and their succession patterns during winter and summer in central South Africa, with reference to forensic applications"

accepted under the Animal experiment nr 24/2014 by the University of the Free State Ethical Committee. Ethical application was done to allow euthanasia of live pigs for the study.

Project titles in application form were changed from application title:

Sylvia van der Merwe: "An analysis of seasonality and depth on the occurrence of dipteran specimens in burial cases, with special focus on Phoridae"

to the present approved title in January 2016.

UNIVERSITY OF THE
FREE STATE
UNIVERSITEIT VAN DIE
VRYSTAAT
YUNIVESITHI YA
FREISTATA

UFS
UV

Internal Post Box / Interne Posbus G40
Tel: (051) 401-7794
Faks / Fax (051) 4444359

E-mail address: viljoenjj@ufs.ac.za

Me / Ms H Viljoen

2014-10-28

DR SL BRINK
DEPARTMENT OF ZOOLOGY & ENTOMOLOGY
D206 C
FACULTY OF NATURAL AND AGRICULTURAL SCIENCES
UFS

Dear Dr Brink

ANIMAL EXPERIMENT NR 24/2014
RESEARCHER: DR SONJA L BRINK - DEPARTMENT OF ZOOLOGY & ENTOMOLOGY
PROJECT TITLE: (1) SYLVIA VAN DER MERWE: "AN ANALYSIS OF SEASONALITY AND DEPTH ON THE OCCURRENCE OF DIPTERAN SPECIMENS IN BURIAL CASES, WITH SPECIAL FOCUS ON PHORIDAE"
(2) JASON BOTHAM: "A COMPARISON OF ABOVE GROUND AND BELOW GROUND ARTHROPOD SUCCESSION, WITH SPECIAL FOCUS ON COLEOPTERA AND DECOMPOSITION"

You are hereby kindly informed that the Interfaculty Animal Ethics Committee approved the above study and it will be condoned at the meeting scheduled for 30 October 2014.

ANIMAL	*NUMBER*	*EXPIRY DATE*
Pigs	7	April 2016

Kindly take note of the following:

1. ***Fully completed and signed applications have to be submitted electronically to viljoenjj@ufs.ac.za and a hard copy has to be submitted too.***
2. ***A signed progress report with regard to the above study has to be submitted electronically to viljoenjj@ufs.ac.za while a hard copy has to be submitted to Ms H Viljoen, Room D115, Francois Retief building, Faculty of Health Sciences. A report has to be submitted when animals are physically involved and after completion of the study. Guidelines with regard to progress reports are available from the secretary and on the Faculty Intranet.***
3. ***Researchers that plan to make use of the Animal Experimentation Unit must ensure to request and receive a quotation from the Head, Mr Seb Lamprecht. A copy of the quotation has to be submitted with the application before the application will be considered for approval.***
4. ***Fifty (50%) of the quoted amount is payable when you receive the letter of approval.***

Regards

.....
CHAIR:
INTERFACULTY ANIMAL ETHICS COMMITTEE

Appendix 2: Full species list of below ground arthropods on buried carcasses during the winter and summer study

The species list below is compiled using information gained during the winter burial trial of the present study as well as information from the concurrent study by Botham (2016 unpubl.).

Colour coding guidelines:

Green: Dipteran species listed during the present study

Red: Non-dipteran species listed by Botham (2016 unpubl.) during the concurrent study; trophic interactions noted by Botham (2016 unpubl.)

Full species list

Dipteran species		
Order: Diptera		
Family	**Species**	**Possible trophic interaction**
Phoridae	*Megaselia scalaris*	Carrion feeder
Phoridae	*Conicera tibialis*	Carrion feeder
Sphaeroceridae	*Leptocera* sp.	Possible fungivore
Muscidae	*Muscina stabulans*	Carrion feeder
Non-dipteran species		
Order: Scolopendromorpha		
Family	**Species**	**Possible trophic interaction**
Scolopendridae	*Cormocephalus* sp.	Predator

The species list below is compiled using information gained during the summer burial trial of the present study as well as information from the concurrent study by Botham (2016 unpubl.).

Colour coding guidelines:

Green: Dipteran species listed during the present study

Red: Non-dipteran species listed by Botham (2016 unpubl.) during the concurrent study; trophic interactions noted by Botham (2016 unpubl.)

Full species list

Dipteran species		
Order: Diptera		
Family	**Species**	**Possible trophic interaction**
Phoridae	*Megaselia scalaris*	Carrion feeder
Sarcophagidae	*Sarcophaga* sp.	Carrion feeder
Sphaeroceridae	*Leptocera* sp.	Possible fungivore
Non-dipteran species		
Order: Coleoptera		
Family	**Species**	**Possible trophic interaction**
Staphylinidae	*Aleochara* sp.	Predator
Order: Acari		
Family	**Species**	**Possible trophic interaction**
Acaridae	*Sancassania mycophagus*	Possible scavenger

CONFERENCES

Parts of this study were presented at the following conferences:

The 19th Entomological Congress of the Entomological Society of Southern Africa, held in Grahamstown, 12-15 July 2015.

Oral Presentation: Sylvia S. van der Merwe, Jason L. Botham, Sonja L. Brink. An Analysis on the Effects of Seasonality on Arthropod Succession and Decomposition of Buried Cadavers in a Central Free State Grassland Area.