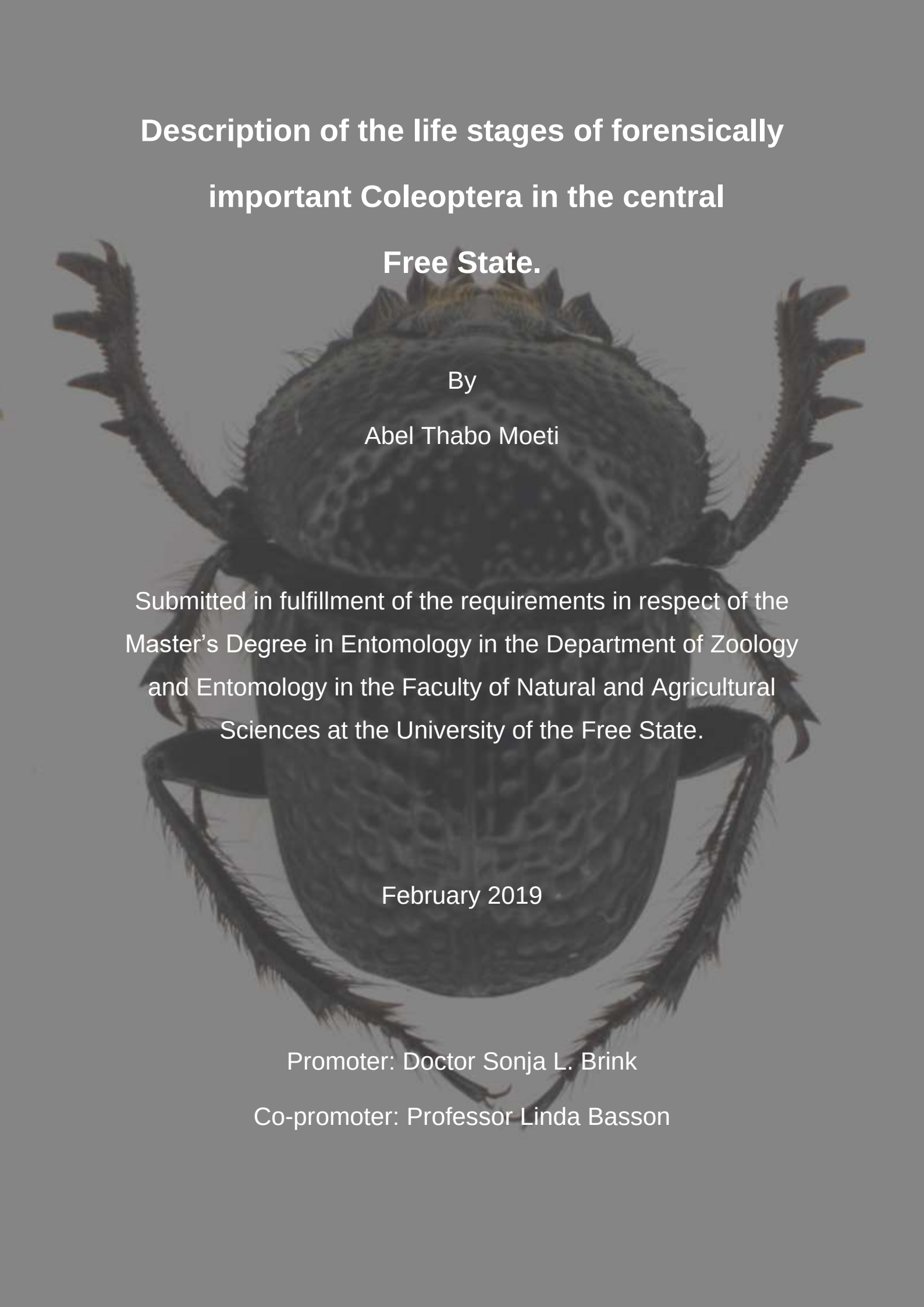

Description of the life stages of forensically important Coleoptera in the central Free State.

By

Abel Thabo Moeti

Submitted in fulfillment of the requirements in respect of the Master's Degree in Entomology in the Department of Zoology and Entomology in the Faculty of Natural and Agricultural Sciences at the University of the Free State.

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Promoter: Doctor Sonja L. Brink

Co-promoter: Professor Linda Basson

Declaration

I, Abel Thabo Moeti, declare that the Master's Degree research 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.

A.T Moeti

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Abstract

The identification and the development of beetles of forensic importance remain understudied when compared to the number of studies conducted on development and identification of the life stages of flies of forensic importance in central Free State. This hinders our understanding of what beetle species are associated with decomposing carcasses and how we can use their immature stages and their development to determine Post Mortem Interval. It is important to make correct species identification when calculating PMI because development data of one species cannot be used for the forensic significance of another species, even in closely related species. In recent successional studies that have been conducted in central Free State, beetles of forensic importance have been identified to family or genus level.

Carcasses used in this experiment were domestic pig (*Sus scrofa domesticus*) with a total of three pigs between the weights range of 32.5-49kg, Cape baboon (*Papio ursinus*) with a total of two baboons weighing 18 and 19kg and one sheep (*Ovis aries*) weighing 44kg. The carcasses were placed on the Western side of the campus of the University of the Free State. The carcasses were allowed to decompose and insects were collected twice a day during the decomposition period.

The aim of this project was to describe morphological characteristics, used to develop keys with which to differentiate between beetle species (adults and immatures) associated with decaying carcasses in central Free State.

A total of eighteen beetle species representing eight families of forensic importance (Silphidae, Staphylinidae, Histeridae, Dermestidae, Cleridae, Trogidae, Scarabaeidae, and Nitidulidae) were collected from the carcasses. Some beetle species were reared under laboratory conditions with the intention of obtaining immatures life stages that were not found in the field. The rearing temperature was set to 28 ± 2ºC and a photoperiod of 12L:12D was maintained in the insectarium. A 3 to 4cm soil layer was laid down in some breeding containers and moist cotton wool was

used to maintain the soil moisture levels. In some breeding containers, only sawdust and styrofoam were used as pupation refugia.

Of eighteen species collected, only two species completed their development under laboratory conditions. Some of the beetles that were collected are already described in literature, and these beetles were redescribed using both external and internal (internal male genitalia) morphological characteristics. Some of the species were only identified to genus level and, in future, the morphological characteristics and micrographs provided in this study will help with identification for both successional and developmental studies.

Keywords: Morphology, breeding, beetle, carcass decomposition, keys, male genitalia

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Chapter 1: Introduction and Literature Review

While much is known about the adults and immature stages of flies of forensic importance, the beetles of forensic importance remain understudied. The aim of this study is towards the most basic requirement necessary for any forensic entomological application, i.e. a correct species identification of adult and immature specimens found at a crime scene. The adult and immature stages of beetle species found associated with carrion in a central Free State ecosystem will be described and a key will be constructed that will aid with the identification of these beetles.

1.1 Post Mortem Interval

The role of beetles in carrion ecosystem determines if they are of forensic importance or not. Their importance is determined by appearance, feeding, breeding, and development at the carcass. Some species play a more important role in estimating the time of death than others (Collett 2015). This means that some species can give more accurate PMI estimation than the others. Beetles with a very short developmental cycle and those that arrive early on the carcass are more useful in the early stages of decomposition than those that have a long developmental cycle and arrive at later stages (Midgley 2007).

Determining a minimum Post Mortem is the most prominent of forensic entomology applications (Catts & Haskell 1990; Anderson & Van Laerhoven 1996; Schoenly *et al.* 1996; Strümpher *et al.* 2014). Determination of a PMI is the role of a qualified medical practitioner who specialises in forensic medicine. In cases where the degree of decomposition in corpses complicate matters for a medical pathologist to determine a time of death a forensic entomologist can be called upon to help, based on the analysis of the insects associated with a corpse, to determine a PMImin.

A forensic entomologist can determine PMI by means of two models: Insect Succession and Developmental Data model. Carrion insect succession is the

predictable pattern in which different insect species colonise a decomposing carcass and is defined by the presence as well as the abundance of species on decomposing carcass (Chapman & Sankey 1955; Bornemissza 1957; Payne 1965; Easton & Smith 1970; Lane 1975; Braack 1981; Rodriguez & Bass 1983; Braack 1986; Mann *et al.* 1990; Wells & Lamotte 2009). The developmental data model uses the developmental rates of the first generation of primarily blowflies and flesh flies present on a body (Van Laerhoven 2008).

The PMImin is based on the PIA (Period of Insect Activity), this is the time when the insects colonise the body. Period of insect activity is not always the same as the exact time of death. The PIA can be either longer or shorter than the actual PMI. This is because insects can infest the body when is still alive (myiasis) or after death. The PIA can be affected by biotic and abiotic factors (Amendt *et al.* 2007).

Species identification is of utmost importance in forensic entomology, especially when determining a PMImin through an insect succession model or a developmental data model. Although some information on species in other parts of the world can be used as baseline data for related species in our region, specifically the development data of one species cannot be used instead of that of another species (Ridgeway *et al.* 2014). Ahmed & Joseph (2016) also mentioned that the successional data from one region cannot be used in another region. In South Africa there are several studies that have been done in the past three decades focusing on beetle identification, visitation patterns of beetles to a carcass (for insect succession), description of both the immatures and the adults stages of beetles (for both PMI models), developmental thresholds of some species (for developmental model) as well as the distribution of beetle species (Braack 1981; Prins 1984a, b; Braack 1986; Louw & van der Linde 1993; Boucher 1997; Kelly 2006; Midgley 2007; Midgley *et al.* 2010; Villet 2011; Ridgeway *et al.* 2014; Collet 2015; Botham 2016; Daniel *et al.* 2017). Despite this, succession studies performed in our region was lacking regarding species identification of adult and immature beetles. Furthermore, none of these studies collected soil samples for immature stages of the beetles associated with the carcasses.

The pre-appearance interval is important when calculating a succession model-based PMI of beetles. Matuszewski & Madra (2015) explained that when calculating the PMImin, it is important to look at it as two parts; developmental interval and pre-appearance interval (PAI). The developmental interval is the time insect take to reach a certain stage of its developmental cycle. This interval can be influenced by whether the insects are exposed to a favourable environment or not. In order for us to know the developmental interval of beetles, we must (i) understand their life cycle and (ii) be able to distinguish between larval instars. If we know how to differentiate between larval instars and also understand environmental conditions required to develop from one instar to another, we will be able to effectively use beetles in PMI estimations. The pre-appearance interval is the time it takes for insects to make their first appearance at the carcass. This interval can be delayed by insects being unable to reach the body due to the environment the body is exposed to. The pre-appearance interval is volatile organic compounds (VOCs) dependent because beetles respond to certain chemicals that are released either by the body itself or other insects that are associated with decomposing bodies.

In order to calculate a PMI based on the developmental model for beetles, more development data are needed to meet the requirements of best practices for insect age estimation (Wang *et al.* 2017). Midgley & Villet (2009) and Ridgeway *et al.* (2014) have progressed in this regard and have constructed developmental diagrams of forensically important beetle species with the aim of using this data to determine a PMI based on the developmental model.

The knowledge on how beetles of forensic importance can be used more effectively in PMI estimations is emerging worldwide (Aecher 2003; Oliva 2001, Matuszewski 2011, Aballay *et al.* 2013, Fontenot *et al.* 2015, Matuszewski & Madra-Bielewick 2016, Aballay *et al.* 2016, Matuszewski 2017).

1.2 Decomposition with the emphasis on beetles in a carrion ecosystem

Carcasses/cadavers (human or animal) goes through a series of changes (chemical, biological and physical) as they decompose. At each decomposition stage very specific insect assemblages are present. When a person's body shuts down and cells breaks down during the process of autolysis certain chemicals will be released at the specific stage of decomposition and these are known as volatile organic compounds (VOCs). The rate of the release of the VOCs is temperature dependent, i.e. the higher temperature the more rapidly VOC's is released. These compounds play a vital role in the appearance of insects which follows a predictable pattern in terms of the decomposition of a carcass. LeBlanc and Logan (2010) distinguished between the olfactory cues that affects the way insects are attracted to a decomposing body as those from the decaying body itself and those produced by insects associated with the carcass. The VOC's emitted by a decomposing body are called apneumones. LeBlanc and Logan (2010) furthermore defined two other semiochemicals; pheromones that cause interactions between individuals of the same species and allelochemicals that cause interaction between individuals from different species. These semiochemicals affects mating (pheromones) or in the case of an allelochemical attracting a predator or a parasitoid to the insects present on the decomposing body. In their search of the literature LeBlanc and Logan (2010) could not pinpoint whether some insect colonisation patterns are due to volatiles released by insects or due to volatiles released by the decomposing body and which of these semiochemicals acts as attractants to some insects while being a repellent for others.

Feeding guilds and arthropods associated with the guild

Insects that are attracted by these VOCs to the carcass can be classified into different guilds based on their role in decomposition process (Fig. 1.1). Villet (2011) describe these guilds in terms of the significance (usefulness) of each organism as a source of evidence. The guilds are: necrophages, predators and parasites; omnivores, adventive species and incidentals (Villet 2011).

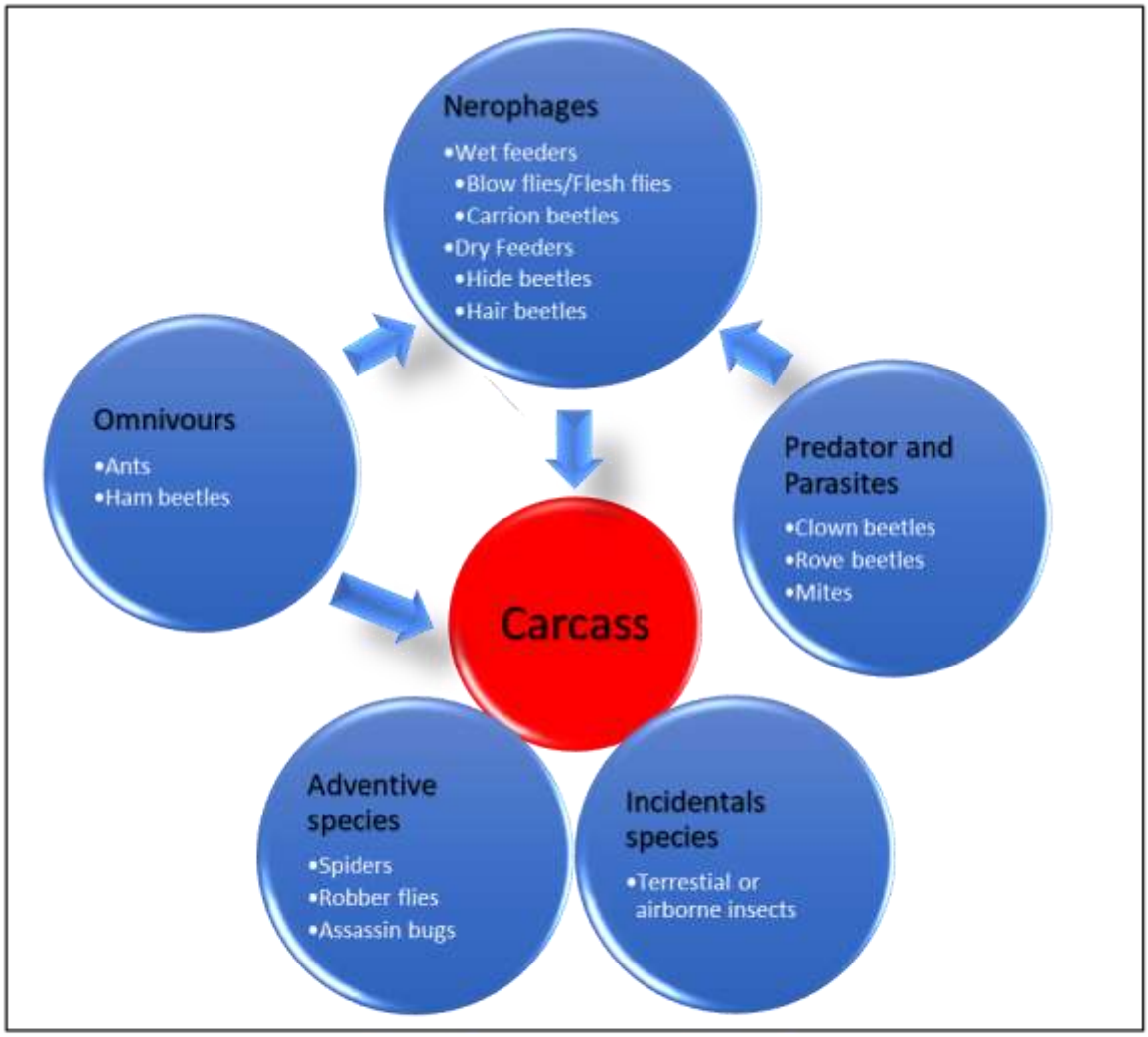

Figure 1.1: Some of the major arthropod carrion community components of a decomposing carcass and the interaction between members of the community and the carrion itself based on guild classification by Villet (2011). Arrows indicate primary feeding.

Necrophages are insects that feed directly on the carcass and these insects can be further divided into two groups: wet and dry feeders. Wet feeders are the first arrivals at the carcasses and they can be used to determine the PMI when the body has been dead for a short time. Wet feeders PMI estimation is the one that is closer to time of death. They feed on the carcass when there are still soft tissues. Examples of these are; maggots of blow flies (Calliphoridae), flesh flies (Sarcophagidae), and grub/larvae

of carrion beetles (Silphidae). Wet stages of decomposition begin after bloating, however, this does not imply that they only colonise the body during bloat stage.

The adults of wet feeding carrion insects can colonise the body as early as during the fresh stage (hours after death) and this also depend on factors such as; accessibility to carrion, area or location of the carcass and favourable conditions. Their role in that time is to lay their egg in orifices in the case of flies and in the soil underneath the carcass in the case of beetles. Carrion beetles (Silphidae) can act as a link between dry and wet stages because they have longer development cycle than flies and they can also be used to estimate long PMIs (Villet 2011).

Dry feeders are necrophage specialist which feed on dry tissue, hair, tendons, and ligaments of the carcass. The adults of dry feeders can co-occur with the wet feeders at the carcass but in smaller numbers. This includes species from families; Dermestidae (Hide beetles) and Trogidae (Hair beetles). Hair beetles are specialists known to digest keratin in hair and Robinson (2005) also mention that larvae of genus *Dermestes* (Dermestidae) can also digest keratin in the skin. This adaptation allows both families to utilise resources that cannot be utilised by other carrion insect families.

Predator and parasites feed mostly on necrophages and these includes; Cleridae (Ham beetles), Histeridae (Clown beetles), Staphylinidae (Rove beetles) and adults of Silphidae (Carrion beetles). They prey mostly on fly maggots and some can also prey on other beetle larvae/grubs. High numbers of these predators can affect PMI estimations if there are less numbers of prey. Predaceous beetles are attracted to the carrion by increase in number of maggots. For as long as these immature stages are present on the body, they will be a source of nutrition to parasitoids. Except for hymenopteran parasitoids (Fig. 1.2), some beetle species are also parasitoids of fly larvae and pupae. Grassberger & Frank (2003) recorded the development of this hymenopteran species.

Figure 1.2: Stereomicrograph of hymenopteran parasite (Pteromalidae: *Nasonia vitripennis*) pupa developing inside blowfly pupa (26/10/2017) (scale bar 2mm).

The members of genus *Aleochara* (Staphylinidae) are known to be parasitic to fly maggots and pupa. They complete their development in either the larvae or the pupa. Members of this genus can utilise one or more larvae or pupa to complete the development (Prins 1984a).

Phoretic mites (Fig. 1.3) also play a role in carrion ecosystem. They can be used as a valuable tool to determine PMI (Goff 1991). Diptera and Coleoptera are the carriers of these mites and their appearance at the carcasses can be correlated with the arrival of flies and beetles that are associated with the carrion. More research on mites and their association with the carrion in central Free State still need to be conducted in order for us to be able to use these arthropods in PMI estimations.

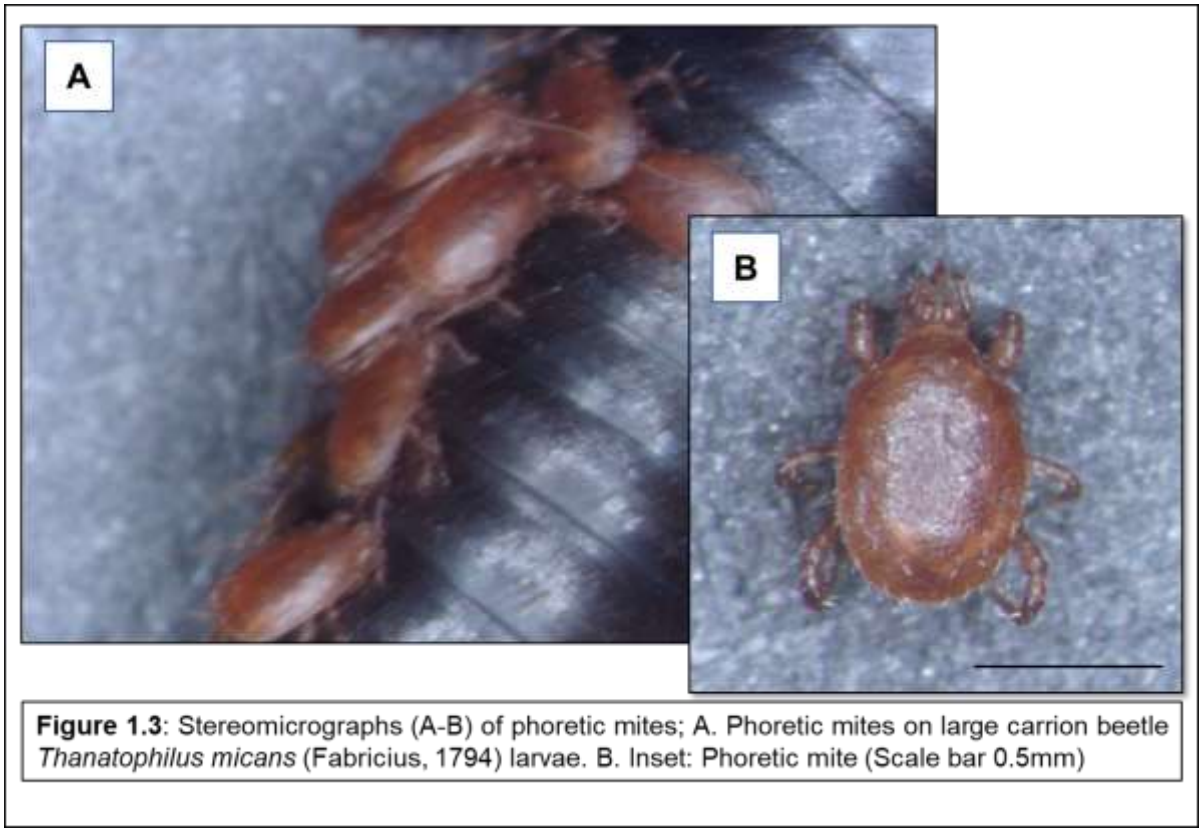

Figure 1.3: Stereomicrographs (A-B) of phoretic mites; A. Phoretic mites on large carrion beetle *Thanatophilus micans* (Fabricius, 1794) larvae. B. Inset: Phoretic mite (Scale bar 0.5mm)

Omnivores are insects that feed on both the carcass and other arthropods that are associated with the carcass. This guild mostly includes ants (Formicidae) as shown in (Fig. 1.4). They do not have strong forensic application for PMI estimations and their presence in high number on a carrion ecosystem affect PMI estimations negatively by preventing colonisation and by feeding on fly eggs.

Adventive species utilises a body for shade or shelter. This guild includes spiders, millipedes, assassin bugs and other insects. They are not associated with PMI directly but with a place or suspect. This includes mosquitoes which have been used to link possible suspects and the body (Spitaleri *et al*. 2006).

Figure 1.4: Photograph of pugnacious ant *Anoplolespis custodiens* (Formicidae) dragging a blow fly larva to the nest (27/09/2017).

Incidental species are species which occur accidentally they can be utilised in forensics by associating the carcass with the specific location or with the suspect during pre- or ante-mortem. An example is, when an aquatic insect is found on the carcass that is in a open field (carcass in grassland area) (Villet 2011).

Stages of decomposition

There are five stages of decomposition: fresh, bloat, active decay, advanced decay and dry/remains (Payne 1965, Gennard 2006, Villet 2011). These stages are based on the conditions of the carcass. Gennard (2006) stated that fresh stage starts from the moment the person's body shuts down and the cells being digested by enzymes such as lipases, proteases, and carbohydrases lasts to the first signs of bloating. The first insects to arrive are mostly the blow flies Calliphoridae: *Lucilia sericata* (Meigen), *L. cuprina* (Wiedemann, 1830), *Chrysomya albiceps* (Wiedemann, 1819), *C. chloropyga* (Wiedemann, 1818), *C. marginalis* (Wiedemann, 1830) (in summer months) and *Calliphora vicina* Robineau-Desvoidy, 1830 (in winter months). These flies (Fig. 1.4) have been recorded in insect ecological succession studies done in

central Free State (Louw & Van der Linde 1993; Boucher 1997; Kolver 2003; Kelly *et al*. 2008, 2009; Kolver 2009; Kelly 2011; Hoffman 2014; Botham 2016). Species of *Chrysomya putoria* (Wiedemann, 1830), *C. megacephala* (Fabricius, 1794)*, C. inclinata* Walker, 1861*,* and *Calliphora croceipalpis* Jaennicke, 1867 showed to have a limited distribution in South Africa they have only been recorded in other parts of the country (Williams 2003, Richards *et al.* 2009, Richards & Villet 2009, Villet 2015). Villet (2011) mentioned that the occurrence of these species in carrion ecosystems is dependent on thermo-physiological and geographical distribution of species.

The flies complete their development on a decomposing carcass. During the fresh stage of decomposition, primary adult flies will lay eggs in natural orifices because of moisture. Sometimes the eggs are laid underneath the carcass. The fresh stage is followed by microbial activity which causes the body to bloat by means of gases released. The torso swells and the whole body will start to stretch like an air-balloon. When the gases start to break down, the body will release the gases that attract more flies and they will lay more eggs. Predators of the families Staphylinidae, Histeridae, and Silphidae will be attracted to the body because of the eggs, maggots and pupa of the flies they will feed on (Gennard 2006).

According to Gennard (2006), following bloating the body will deflate due to high maggot activity and the skin of the corpse breaks. This will attract species of Cleridae, Dermestidae, Histeridae, and Silphidae which will then lay their eggs. During this stage, putrefaction and fermentation will generate butyric acid and caseic acid.

The body will transition to reach the advanced decay stage which can be indicated by increase in beetle species richness and relative abundance. At this stage there is a reduction of maggot activity because the soft tissues are digested. Furthermore, the bones are exposed and there is also high numbers of larvae of Dermestidae.

Figure 1.4: Photograph showing a batch of fly eggs and adults flies (Calliphoridae: *Chrysomya albicpes, C. marginalis and Lucilia sp*), (Muscidae: *Musca sp.*) and two adult beetle species (Histeridae: *Saprinus splendense* circled in blue and *S. flavipennis* circled in red) that found in central Free State (07/02/2017).

The last stage of decomposition is the dry/remains, and at this stage, only hair and bones left. Beetles which are associated with this stage are of the family Trogidae which feed on keratin. The beetles from the family Nitidulidae will then feed on decaying vegetation that has been caused by the ammonium released during decomposition (Gennard 2006).

Project Aims and Objectives

Aim

• To identify the beetles which forms part of a carrion ecosystem in the central Free State.

Objectives

• To provide morphological descriptions of the adult stages of beetles collected from carrion during the current study period; To construct a key that can be used to identify the adult beetles from a central Free State carrion ecosystem.

• To provide morphological descriptions of the larvae and pupae collected from around carrion during the current study period. To construct a key that can be used to identify larvae and pupae from a central Free State carrion ecosystem.

Chapter 2: Materials and Methods

2.1 Experimental area

The field trial was conducted in an open field shown by a red square located at the west campus of the University of the Free State (UFS), Bloemfontein (29°05'55.2"S 26°10'26.0"E, ± 1500 m above sea level) (Fig. 2.1). The area is approximately 24 hectares and can be classified as grassland with very little tree cover. The grass species are dominated by *Themeda triandra* Forsk., *Aristida congesta* Roem. et Schult., *Eragrostis lehmanniana* Nees, *Eragrostis capensis* (Thumb.) and *Chloris virgata* Sw. Also scattered trees found in the area are *Vachellia karroo* (Hayne) and *Rhus rehmanniana* Engl. It has cold winters and warm summers with an average rainfall of 450 – 500 mm per year (Kelly *et al*. 2009; South African Weather Services). A few goats, sheep and horses roamed in the area during the trial period. Low-level mongoose activity in the area were also noted.

Figure 2.1: Google map image showing the arial view of the study site (indicated by a red square) at University of the Free State West Campus (Google maps: accessed 13 Sep.17).

2.2 Experimental design

Carcasses used in this experiment were domestic pigs (*Sus scrofa domesticus* Erxleben, 1777) with a total of three pigs between the weights range of 32.5-49 Kilograms[1], Cape baboon (*Papio ursinus* (Kerr, 1792)) with a total of two baboons weighing 18 and 19kg respectively and one sheep (*Ovis aries* Linnaeus, 1758) weighing 44kg. Although many forensic entomology studies use pigs as human models, other animals also have been used to study different insects that colonise the decomposing carcasses (Chapman & Sankey 1955; Payne 1965; Braack 1984; Aecher 2003; Midgley *et al*. 2012; Keough *et al.* 2017). In this study the cape baboon and the sheep were used to see if there are different insect species of forensic importance which can be collected from other animals other than pig carcasses.

Carcasses used for this study were acquired from the Animal Research Unit at the University of Free State. The carcasses were obtained already euthanised and were stored in a frozen state in an industrial freezer at the Animal Research Unit. There were no observable trauma wounds on the carcasses. The carcasses used in this study were previously used in medical experiments and they were re-purposed in this study to further reduce resources used for scientific research. There was no ethical approval needed for the carcasses used for this study. Protocol regarding ethical approval for other aspects of the study (entomological sampling) was followed (Appendix 3).

Pig carcasses are internationally recognised and used as human models in forensic entomology investigations (Catts & Goff 1992, Aecher 2003). Moreau *et al.* (2015) stated that there are four reasons why we use pigs in forensic entomology studies: (1) the chest cavity of above 23kg pig approximates a fully grown human torso, (2) their skin is not as hairy as that of primates which are genetically closely related to humans and we have the same gut microbes, (3) they are easy to purchase, and (4) during the experiments pig carcasses do not draw much attention of both public and the media.

[1] The size of the pig that will model a full-grown human (Catts & Goff 1992)

However, there are also many other forensic entomology studies that have been done on other animal species to provide an insight on decomposition and arthropod associated with decomposing carcasses.

2.3 Study methodology

2.3.1 Study

At the beginning of each trial season, two carcasses (Table 2.1) were placed at the experimental site between 09:00 am and 12:00 am. Metal-framed cages (1.6 x 0.9 x 0.9m), covered with poultry wire mesh (15mm mesh size) were used to contain each carcass. This was done to prevent disturbance to the carcasses by large scavengers. The cages were placed approximately 100m apart. This was done in order to ensure minimal migration of insects from one carcass to another. Anderson & Van Laerhoven (1996) advised that the minimum distance between two carcasses should be at least 50m apart. However, Moreau *et al.* (2015) countered that the minimum distance between carcasses as suggested by Anderson & Van Laerhoven (1996) will only prevent the exclusion of migrating larvae between sites, but that it will not exclude flying insects from migrating from one carcass to another. At each successive trial, care was taken to maintain a 100m buffer zone from previous experimental sites.

Table 2. 1: Carcass used and placement dates during the study.

Season trial	**Carcass**	**Dates**
Autumn	1 pig	11 March – 24 April (2016)
	1 baboon	
Summer	2 pigs	06 February – 16 March (2017)
Spring	1 baboon	18 September – 26 October (2017)
	1 sheep	

At each carcass, two pitfall traps with glycerol were buried. The first trap was buried close to the anterior side and the second pitfall trap was buried close to the ventral

side of the carcasses (Fig. 2.2). Glycerol was used because it is colourless and odourless. In additional to pitfall traps, soil sampling and manual sampling were used to collect arthropods.

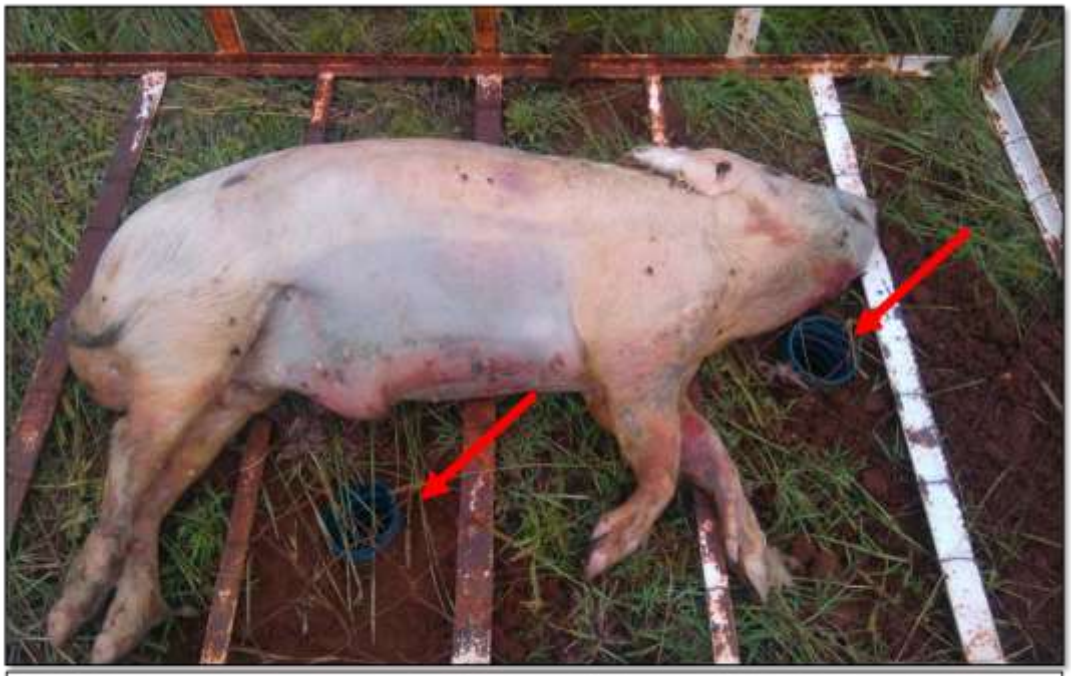

Figure 2.2: Photograph of a Male domestic pig (*Sus scrofa domesticus* L.) showing two pitfall traps used to collect arthropods during summer studies (06/02/2017).

The pitfall traps were emptied on a daily basis (starting on day 2 of the trials) in the mornings (between 09:00 am and 12:00 am) and afternoons (between 16:00 pm and 18:00 pm) after observations were done. The observations made were to record the species that are present on the carcass and to assess the conditions of the decomposition carcass. Although this was a Coleoptera taxonomic study, all insects associated with the carcasses were recorded and not only the beetles of forensic importance. Soil was sampled from beneath the head and beneath the torso from the bloat stage (i.e. when the carcass started to release body fluids into the soil) and thereafter with 5-day interval until dry stage of decomposition. Beetles were also manually sampled from underneath the carcass and also a few metres from around the carcass. All samples (pitfall, soil, and manual) from the field were then transported to the laboratory.

In the laboratory adult beetles of forensic importance and the immatures collected were sorted according to morphospecies and placed into different containers with 75% ethanol for preservation. These beetles were then identified and their numbers were recorded. Stereomicrographs were taken of specimens that could not be identified and were send out to the experts for identification. A reference sample was kept and other samples were used for morphological studies. Some of the species that were collected at very low numbers are not present in the reference samples because the samples were used for description purposes.

In this study, all the species of beetles of forensic importance were recorded regardless of the number of the individuals present on the carcasses. This was done because the aim of this study is to record all beetle species that were associated with the decomposing carcasses at the study site during the study period.

2.3.2 Weather data

The temperature recordings were provided by the South African Weather Services (SAWS) and Department of Soil, Crop and Climate Science, UFS. The UFS weather station the data is approximately 1.9 km from the study site. Weather data are not required for this type of study, but for completeness and the weather data during the trial periods is contained in Appendix 2.

2.3.3 Rearing of field collected immature states and Breeding experiments

Both the rearing of field collected immature stages and the breeding colonies beetles were kept under a constant controlled temperature (28ºC±2), relative humidity (50%) and light-to-darkness regiment of 12:12 hours. Breeding conditions were not chosen based on the species threshold temperature but were kept at 28ºC which is the average summer temperatures in Bloemfontein (SAWS). All containers with beetles were covered with a nylon screen mesh to allow air to access the container and to prevent beetles from escaping the containers.

The immature stages that were collected from soil samples and for which a definitive identification could not be made were reared to adulthood in the insectarium. These immature stages were kept in individual containers to prevent possible incidents of predation or cannibalism.

Colonies of adult beetles were kept in containers with a layer of soil from the study site. The main colony of collected beetle species were kept in a single container to simulate the condition as they co-occur in their natural environment. Breeding colonies housing the beetles of specific species were kept in separate containers. Laboratory breeding was attempted to obtain life stages that were not found in sufficient numbers at the decomposing carcasses. The observations on developmental data gathering for the laboratory breeding experiment were done twice a day. Pig muscle tissue and chicken livers were provided every day as food source. First instar larvae of flies were sometimes provided as food source to the facultative predator colonies. When the larvae reached the pupal stage, the pupa were removed and placed in a separate container for further development. This was done to protect the pupae from possible interference from the rest of the colony.

2.3.4 Morphological descriptions

Images were taken using a Nikon AZ100 multizoom stereomicroscope fitted with a digital camera. Drawings were produced using Corel Draw 10; the drawing were traced on Scholars Tracing Pad using Uni Pin Fine Line pens (0.1, 0.3, 0.5 & 0.8 pin). The measurements were made using stereo, scanning electron microscopy and Fragram digital calliper rounded off to 1 decimal place in millimetres.

Larger and heavily sclerotised specimen were dissected using a number 11 surgical blade. Soft bodied parts and the mouthparts were dissected using different sizes of insect pins. The male aedeagus were dissected and treated in a 25% solution of KOH. This was done according to the procedure by Özdemir & Sert (2008) to remove excessive membranes attached to the genitals.

For the preparation of specimens for scanning electron microscope (SEM), the specimens were immersed directly in 97% ethanol overnight to dehydrate. This was done as a measure to retain the size of the specimens. They were dried using a Tousimis Critical Point Dryer (Rockville, Maryland, U.S.A.) with ethanol dehydration and carbon dioxide drying gas. They were stored in an airtight container with moist absorbing crystals until they were ready for mounting. Specimens were mounted on clean studs using double sided-sole tape for adhesion. Larger specimens were mounted using commercial Pratley glue (quickset clear). Small specimens of the same species were mounted on one studs to directly compare the differences in morphological characteristics. Specimens were sputter coated at 50-60nm (Coater: BIO-RAD (Microscience Division) Coating System (London, UK) Au/Ar). A Shimadzu SSX-550 (Kyoto, Japan) scanning electron microscope was used to make measurements and to produce photographs.

Morphological characteristics that are used in the descrptions were based on those of Prins (1984a, b); Lackner (2010) and Lawrence *et al*. (2011). The letter designations used in the text were based on those used by Lackner (2010) and are as follows:

APW = width between anterior angles of pronotum;
EL = length of elytron along sutural line/midline;
EW = maximal width between outer margins of elytra;
PEL = length between anterior angles of pronotum and apices of elytra;
PPW = width between posterior angles of pronotum.
n = Number of the individuals examined.

Chapter 3: Results and Discussion

There are eight forensically important beetle families recorded from decomposing carcasses in central Free State (Louw & Van der Linde 1993, Boucher 1997, Kolver 2003, Kelly *et al.* 2008, 2009, 2011, Kolver 2009, Hoffman 2014, Botham 2016). Beetles from all of these families were also recorded during the course of the current study.

A total number of 15383 adult beetles belonging to 8 families and 18 species were collected during the trial period (Fig. 3.1, Table 3.1). Except for one pig carcass placed during the summer of 2017 and sheep carcass during spring 2017 the carcasses presented with the same beetle species richness. The species diversity was higher for the pig carcass placed during the summer and sheep carcass placed during the spring of 2017 where three additional species were recorded. It should be noted that only one specimen each of *Platydracus hottentotus, Carpophilus obsoletus* and *Scaptobius* sp. were recorded from carcasses.

The most abundant species collected were: *Dermestes maculatus* (14%), *Necrobia rufipes* (13%), *Thanatophilus micans* (14%), *Saprinus splendens* (14%), *S. cupreus* (13%), *Onthophagus sp.* (7%), *Scarabaeus sp.* (6%), *Philonthus caffer* (10%) *and P. longicornis* (9%) (Table 3.1). These species were the dominant component from all carcasses that was placed as carrion during the current trial period as well as being the dominant species recorded for all succession studies conducted at the same site in the past (Table 3.2).

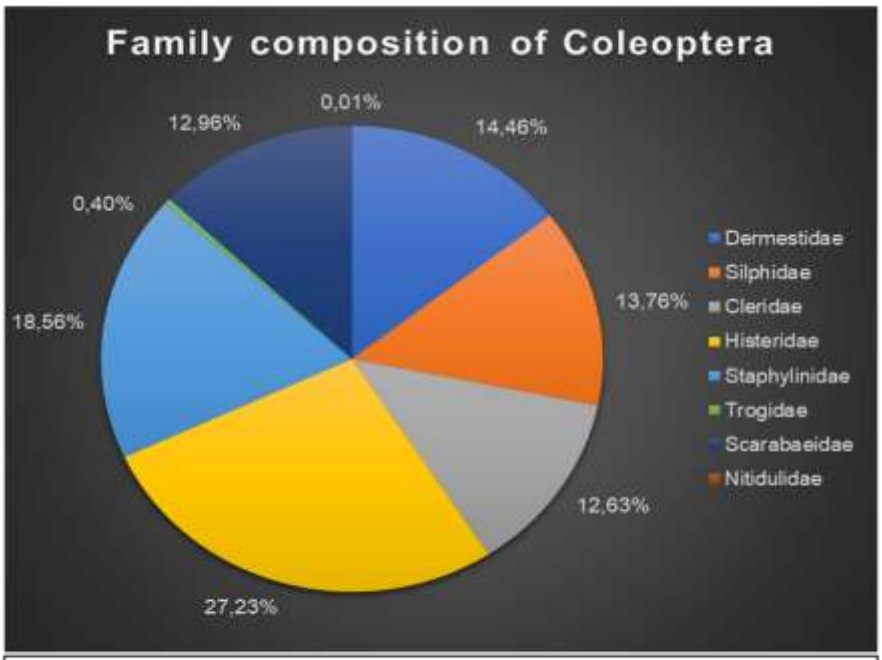

Figure 3.1: Coleoptera families of importance found during the study represented as percentages: Silphidae (14%); Staphylinidae (19%) Histeridae (27%); Dermestidae (14%); Cleridae (13%); Troidae (<1%); Nitidulidae (<1%) and Scarabaeidae (13%).

Table 3.1: Adult beetle species collected from the study site during the trial period 2016 – 2017.

Family	Genus species	Number of individuals (%)
Cleridae	*Necrobia rufipes*	1943 (13%)
Dermestidae	*Dermestes maculatus*	2224 (14%)
Histeridae	*Hister nomas*	65 (<1%)
	Pachylister heros	44 (<1%)
	Saprinus cruciatus flavipennis	13 (<1%)
	Saprinus cupreus	1932 (13%)
	Saprinus splendens	2135 (14%)
Nitidulidae	*Carpophilus obsoletus*	1 (<1%)
Scarabaeidae	*Onthophagus* sp.	1101 (7%)
	Scaptobius sp.	1 (<1%)
	Scarabaeus sp.	891 (6%)
Silphidae	*Thantophilus micans*	2117 (14%)
Staphylinidae	*Aleochara* sp.	15 (<1%)
	Philonthus caffer	1530 (10%)
	Philonthus longicornis	1309 (9%)
	Platydracus hottentotus.	1 (<1%)
Trogidae	*Afromorgus squalidus*	21 (<1%)
	Phoberus strigosus	40 (<1%)

Table 3.2: Families, genera, and species of forensic importance found at the study site during the successional studies.

Family	Species	Reference study	Trial period	Scope of the study (Carcass)	Season
Cleridae	*Necrobia rufipes*	Boucher (1997)	1994 – 1995	Under sunny/ under shaded	Autumn/Winter/ Spring/Summer
		Kolver (2003)	1999; 2001	Hanging in sun/Hanging in shade/ Laying in sun	Summer/Winter/Spring
		Kelly (2006)	2003 – 2004	Not clothed/ clothed/wrapped without clothes/wrapped with clothes	Autumn/Winter/Spring/Summer
		Kelly (2006)	2004 – 2005	Not clothed/clothed/stab wounds not clothed/stab wounds clothed/severe trauma not clothed/severe trauma clothed	Autumn/Winter/Spring/Summer
		Kolver (2009)	2004 – 2006	Not burnt/slightly burnt/moderate burnt/heavily burnt	Summer/Winter/Spring/Autumn
		Hoffman (2014)	2007 – 2008	Multiple trauma	Autumn/Winter/Spring/Summer
		Botham (2016)	2014 – 2015	Unburied	Winter/Summer
Dermestidae	*Dermestes maculatus*	Boucher (1997)	1994 – 1995	Under sunny/ under shaded	Autumn/Winter/ Spring/Summer
		Kolver (2003)	1999; 2001	Hanging in sun/Hanging in shade/ Laying in sun	Summer/Winter/Spring
		Kelly (2006)	2003 – 2004	Not clothed/ clothed/wrapped without clothes/wrapped with clothes	Autumn/Winter/Spring/Summer
		Kelly (2006)	2004 – 2005	Not clothed/clothed/stab wounds not clothed/stab wounds clothed/severe trauma not clothed/severe trauma clothed	Autumn/Winter/Spring/Summer

Table 3.2: Families, genera, and species of forensic importance found at the study site during the successional studies (Cont).

		Kolver (2009)	2004 – 2006	Not burnt/slightly burnt/moderate burnt/heavily burnt	Summer/Winter/Spring/Autumn
		Hoffman (2014)	2007 – 2008	Multiple trauma	Autumn/Winter/Spring/Summer
		Botham (2016)	2014 – 2015	Unburied	Winter/Summer
Histeridae	*Antholus* sp.	Boucher (1997)	1994 – 1995	Under sunny/ under shaded	Autumn/Winter/ Spring/Summer
	Adelopygus sp.	Boucher (1997)	1994 - 1995	Under shaded	Winter/Spring
	Euspilotus sp.	Botham (2016)	2014 – 2015	Unburied	Winter
	Hister caulidus	Boucher (1997)	1994	Under sunny/ under shaded	Autumn
	Hister nomas	Boucher (1997)	1994 - 1995	Under sunny	Summer
	Macrolister sp.	Botham (2016)	2014 – 2015	Unburied	Winter/Summer
	Pachylister nigrita	Boucher (1997)	1994 – 1995	Under sunny/ under shaded	Autumn/Winter/ Spring/Summer
	Saprinus cupreus	Boucher (1997)	1994 – 1995	Under sunny/ under shaded	Autumn/Winter/ Spring/Summer
	Saprinus splendens	Boucher (1997)	1994 – 1995	Under sunny/ under shaded	Autumn/Winter/ Spring/Summer
	Saprinus sp.	Kelly (2006)	2004 – 2005	Not clothed/clothed/stab wounds not clothed/stab wounds clothed/severe trauma not clothed/severe trauma clothed	Autumn/Winter/Spring/Summer
	Saprinus sp. a	Boucher (1997)	1994 – 1995	Under sunny/ under shaded	Autumn/Winter/ Spring/Summer
	Saprinus sp. b	Boucher (1997)	1994 – 1995	Under sunny/ under shaded	Autumn/Winter/ Spring/Summer

Table 3.2: Families, genera, and species of forensic importance found at the study site during the successional studies (Cont).

	Saprinus sp. c	Boucher (1997)	1994	Under sunny	Autumn
	Histeridae spp.	Kolver (2003)	1999; 2001	Hanging in sun/Hanging in shade/ Laying in sun	Summer/Winter/Spring/Autumn
		Kelly (2006)	2004 – 2005	Not clothed/clothed/stab wounds not clothed/stab wounds clothed/severe trauma not clothed/severe trauma clothed	Autumn/Winter/Spring/Summer
		Kolver (2009)	2004 – 2006	Not burnt/slightly burnt/moderate burnt/heavily burnt	Summer/Winter/Spring/Autumn
		Hoffman (2014)	2007 – 2008	Multiple trauma	Autumn/Winter/Spring/Summer
Nitidulidae	*Lasiodactylus* sp.	Boucher (1997)	1994 – 1995	Under shaded	spring
Scarabaeidae	*Ontherus* sp.	Botham (2016)	2014 – 2015	Unburied	Summer
	Onthophagus sp.	Boucher (1997)	1994 – 1995	Under sunny/ under shaded	Autumn/Winter/ Spring/Summer
	Scarabaeidae spp.	Kolver (2003)	1999; 2001	Hanging in sun/Hanging in shade/ Laying in sun	Summer/Winter/Spring/Autunm
		Kelly (2006)	2004 – 2005	Not clothed/clothed/stab wounds not clothed/stab wounds clothed/severe trauma not clothed/severe trauma clothed	Autumn/Winter/Spring/Summer
		Kolver (2009)	2004 – 2006	Not burnt/slightly burnt/moderate burnt/heavily burnt	Summer/Winter/Spring/Autumn
	Cetoniinae sp.	Boucher (1997)	1994 – 1995	Under sunny/ under shaded	Spring/Summer
		Kolver (2003)	2001	Hanging in sun/Hanging in shade	Summer

Table 3.2: Families, genera, and species of forensic importance found at the study site during the successional studies (Cont).

Silphidae	*Thanatophilus micans*	Boucher (1997)	1994 – 1995	Under sunny/ under shaded	Autumn/Winter/ Spring/Summer
		Kelly (2006)	2004 – 2005	Not clothed/clothed/stab wounds not clothed/stab wounds clothed/severe trauma not clothed/severe trauma clothed	Autumn/Winter/Spring/Summer
		Kelly (2006)	2004 – 2005	Not clothed/clothed/stab wounds not clothed/stab wounds clothed/severe trauma not clothed/severe trauma clothed	Autumn/Winter/Spring/Summer
		Kolver (2009)	2004 – 2006	Not burnt/slightly burnt/moderate burnt/heavily burnt	Summer/Winter/Spring/Autumn
		Hoffman (2014)	2007 – 2008	Multiple trauma	Autumn/Winter/Spring/Summer
	Thanatophilus mutilatus	Botham (2016)	2014 – 2015	Unburied	Winter/Summer
	Silphidae spp.	Kolver (2003)	1999; 2001	Hanging in sun/Hanging in shade/ Laying in sun	Summer/Spring/Autumn
Staphylinidae	*Aleochara bipustulata*	Boucher (1997)	1994 – 1995	Under sunny/ under shaded	Autumn/Winter/ Spring/Summer
	Aleochara sp.	Botham (2016)	2014 – 2015	Buried	Summer
	Anotylus sp.	Boucher (1997)	1994 – 1995	Under sunny/ under shaded	Autumn/Winter/ Spring
	Atheta sp.	Boucher (1997)	1994	Under sunny	Autumn/Winter
	Belonchus sp.	Botham (2016)	2014	Unburied	Winter
	Eulissas sp.	Botham (2016)	2014 – 2015	Unburied	Summer

Table 3.2: Families, genera, and species of forensic importance found at the study site during the successional studies (Cont).

	Phacophallus sp.	Boucher (1997)	1994 – 1995	Under sunny/ under shaded	Autumn/Spring
	Philonthus caffer	Boucher (1997)	1994 – 1995	Under sunny/ under shaded	Autumn/Winter/ Spring/Summer
	Philonthus labdanus	Boucher (1997)	1994 – 1995	Under sunny/ under shaded	Autumn/Winter/ Spring
	Philonthus sp.	Botham (2016)	2014	Unburied	Winter
	Oxytelus planus	Boucher (1997)	1994 – 1995	Under sunny/ under shaded	Winter/Spring Summer
Trogidae	*Trox sulcatus*	Botham (2016)	2014	Unburied	Winter
	Histeridae spp.	Boucher (1997)	1994 – 1995	Under sunny/ under shaded	Autumn/Winter/ Spring/Summer

3.1 Silphidae

The sub-family Silphinae is of forensic significance in relation to the colonization of larger carrion, unlike individuals from the sub-family Necrophorinae that colonise small carrion, such as rodents, (Prins 1984a; Gennard 2006; Byrd & Castner 2009; Navarrete-Heredia & Contreras 2011). Large carrion beetles arrive at almost the same time as the blow and flesh flies at the decomposing body. They have longer developmental interval than flies, this make members of this family to be the link between wet stages of decomposition and the dry stages of decomposition (Ridgeway *et al*. 2013). Developmental models for *Thanatophilus micans* and *T. mutilatus* were recorded by Midgley & Villet (2009) and Ridgeway *et al.* (2013). The morphological characteristics of these two species were supplied by Schawaller 1981 (adults); Prins 1984a (adults and larvae) and Daniel *et al*. 2017 (larvae and key to the larval instars).

Thanatophilus micans adults are necrophages, they primarily feed on the carcass (Fig. 3.2) and they also feed on fly maggots (Villet 2011). Larvae of *T. micans* are necrophagous but facultative cannibalism was observed (personal observation). They can colonise dead bodies as soon as 24 hours after death depending on accessibility to the body and environmental conditions (Ridgeway *et al*. 2013). Adults colonise from fresh to the advanced stages of decomposition and the larvae will be present until the dry stage of decomposition.

Three Afrotropical species from the subfamily Silphinae are found in South Africa; *T. micans, T. mutilatus* and *Silpha punctulata* (Prins 1984a; Schawaller 1987; Daniel *et al*. 2017). During the course of the trial period, only specimens of *T. micans* were collected. *Thanatophilus micans* made up a major component (14%) of the beetle specimens collected from the carcasses during the current trial period and was also recorded in all the trials conducted previously at the study site (Table 3.1). *Thanatophilus mutilatus,* an endemic South African species (Schawaller 1981; Prins 1984a; Villet 2011, Ridgeway *et*

al. 2013) was only recorded at this study site by Botham (2016) from his above ground carcass. *Silpha punctulata* was not recorded during the current trial period or during succession trials that ran previously (Table 3.2) at the study site.

Figure 3.2: Photograph of a domestic pig (*Sus scrofa domesticus* L.) showing the adults of *Thanatophilus micans* (Fabricius, 1794) circled in red on Day 1 of decomposition (11/03/2016).

Thanatophilus micans larvae were collected from soil samples recovered from beneath the carcasses. Furthermore, these larvae were noted roaming around the carcass and were collected through active sampling. No pupae were collected from the field. Unfortunately, only three larval instars were produced in the insectarium; third instar larvae did not pupate. For these reasons, a description of the pupae could not be supplied currently.

Thanatophilus micans (Fabricius, 1794)

Larval description

Diagnosis: Campodeiform; larvae easily recognised by heavily sclerotised body; have brown to blackish colouration except after moulting; head orientated prognathous (Fig. 3.3); head length 0.9-2mm and head width 1.1-2.4mm, n=30.

Head: Antenna with three segments; second antennomere with apical setae; labrum emarginated with four setae on each side (Fig. 3.4C); mandible robust, with two ventral setae and one basal seta; mandible molar region without teeth; incisor lobe with two teeth (Fig. 3.4B); lacina with series of spines apically and base of mesal area with denticles; maxilla with four maxillary palpomeres (Fig. 3.4A).

Thorax: Dorsal shield apices pointing towards posterior side of individual; legs well developed for running with tarsal segments fused together to form tarsungulus; tarsungulgus with two spines, one posteriorly and other one anteriorly; both femur and tibia armed with longitudinal spines (Fig. 3.4F); mesospiracle with two spiracular setae (Fig. 3.4E).

Abdomen: Urogomphus with three segments present at last second abdominal sternite (Fig. 3.4G).

Remarks: Descriptions of the larvae of *T. micans* are provided by Prins (1984a) and Daniel *et al.* (2017). In both studies they showed that the larvae of *T. micans* have two spiracular setae on the mesospiracle. This was also supported by descriptions provided by this study. When we look at the setae on the mandibles, both studies found that there are two setae on each mandible and the current study showed that there are actually three setae on each mandible when the mandible is viewed from the ventral side (Fig. 3.4D). On the ventral side of the mandible in Fig. 3.4D, one of the two apical seta is broken and the basal setae but their insertions can be seen. Two setae are visible when the mandible is viewed dorsally (Fig. 3.4B).

Figure 3.3: Stereomicrograph of the dorsal view of the larvae of *Thanatophilus micans* (Fabricius, 1794) (Scale bar = 3mm).

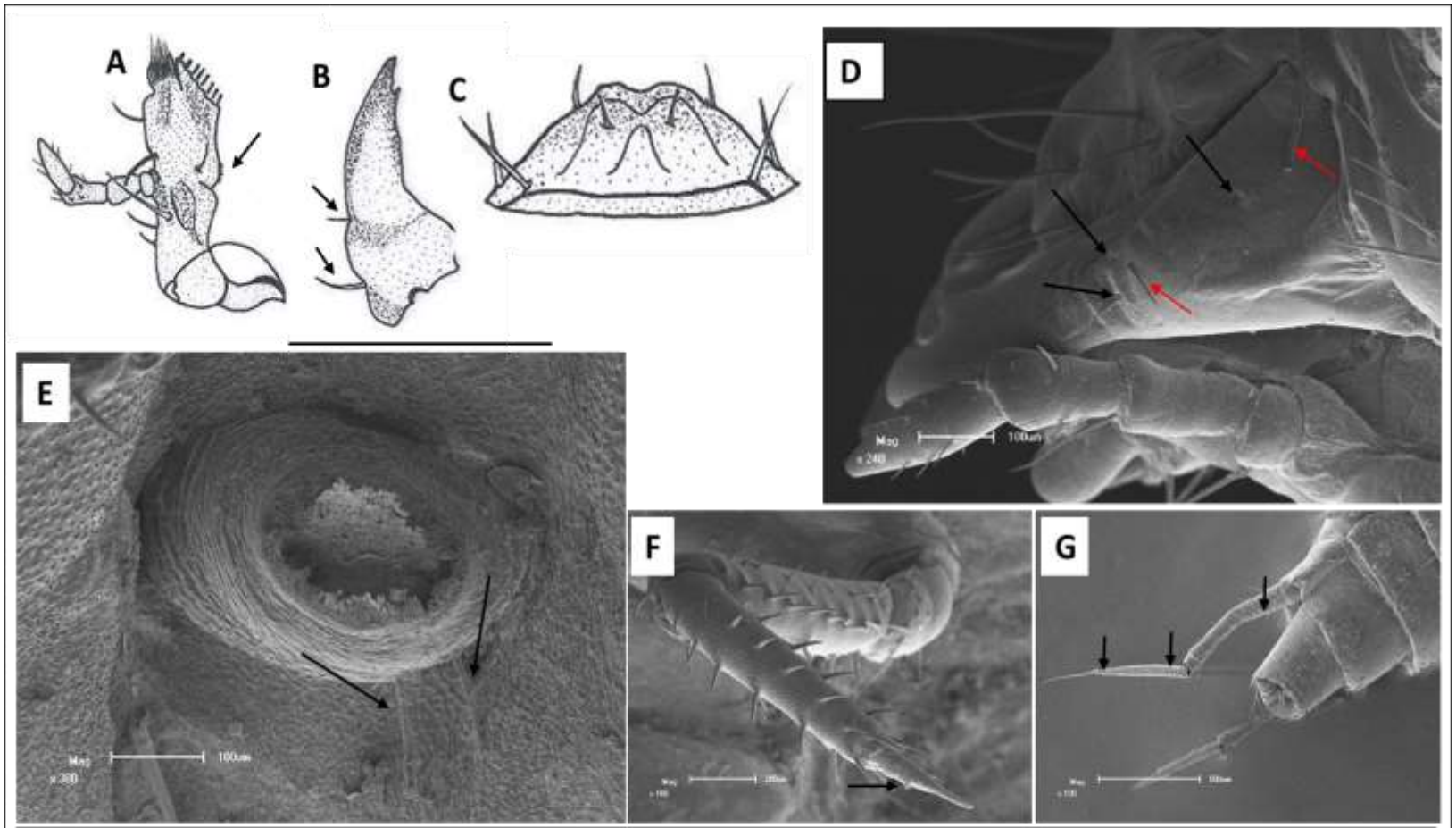

Figure 3.4: Diagrammatic drawings (A-C) and Scanning electron micrographs (D-G) of the larvae of *Thanatophilus micans* (Fabricius, 1794). A. Left maxilla, arrows indicating denticles on the masal base (dorsal view) . B. Left mandible, arrows indicating visible setae (dorsal view). C. Labrum (dorsal view) (Scale bar = 0,5mm). D. Mandible, black arrows indicating insertion of setae (two apical setae and one basal seta) and red arrows indicating broken setae at x240 magnification (lateral view). E. Mesospiracle, arrows indicating two mesospiracular setae at x300 magnification (lateral view). F. Foreleg, arrow indicating anterior tarsangulus at x160 magnification. G. Urogomphi, arrows indication three segments at x100 magnification.

Adult description

Diagnosis: Adults blue to green body colouration with head visible when viewed dorsally (Fig. 3.5A); body length: APW 3.5mm – 3.7mm; EL 7.0mm – 9.4mm; EW 6.1 – 7.9mm; PEL 10.6mm – 12.4mm; PPW 5.1mm – 5.9mm, n=30.

Head: Head visible from above and not concealed by pronotum; compound eyes large and convex; compound eyes extending laterally on each side when viewed from dorsal side; compound eyes undivided by clypeus into lower and upper parts; antenna clavate with 12 antennomeres and visible from above; three apical antennomeres enlarged to form club (Fig. 3.5A); frons covered with hairs; labrum visible from above and not concealed by clypeus; labrum well sclerotised and labral apex emerginate; base of labrum plain and separated from clypeus by complete line; mandibles visible from above; molar region with teeth; mandible with spines on ventral side (Fig. 3.6B); maxillary lobe consist of lacinia and galea; lacinia with hook; maxillary palp with three palpomeres; apical maxillary palpomere almost same size as preapical and basal palpomere; galea with fine hairs at apex (Fig. 3.6A); labium with three labial palpomeres; articulation of labial palps visible when head viewed ventrally.

Thorax: Pronotum with fine hairs; pronotum with greenish to black colouration; anterior angles of pronotum obtuse and anterior angles of pronotum do not form solid rounded curve with pronotal carinae; lateral carinae separate pronotal disc and hypomeron; pronotal carinae smooth; posterior angles of pronotum obtuse; pronotal hypomeron asetose; scutellum visible between elytral bases and large; scutellum anteriorly plain and posteriorly acute; elytra exposing more than three complete tergites; elytral disc with three longitudinal striae (Fig. 3.5A); elytra with pointy apex and elytra meet at midline when wings at rest; forelegs cursorial and adapted for running; protibial with series of spines surrounding it and two apical spurs; mesotibia and metatibia not widened, both meso and metatibia have series of spines surrounding tibia and two apical spurs; tarsal segment 5-5-5 and all legs pretarsal claws toothed at claw base.

Abdomen: Usually more than three abdominal sternites can be seen when viewed ventrally; pygidium exposed (Fig. 3.5A).

Male genitals: Trilobate type; medial lobe broad with slightly pointy apex; parameres heavily sclerotised and curved towards medial lobe apex; parameres and basal lobe medial lobe almost same size; basal lobe heavily sclerotised; inner sac present and round (Fig. 3.6C; Appendix 1, Fig. K).

Remarks: Adults can be differentiated by their last abdominal sternites (Fig. 3.5B) males have convex sternites and females have concaved sternites. Descriptions provided in present study supports descriptions of adults of *T. micans* provided by Schawaller (1981) and Prins (1984a). Prins (1984a) found smaller specimens with length between 12.6mm – 13.8mm compared to the current study were larger specimen were found with length between 17mm – 21mm.

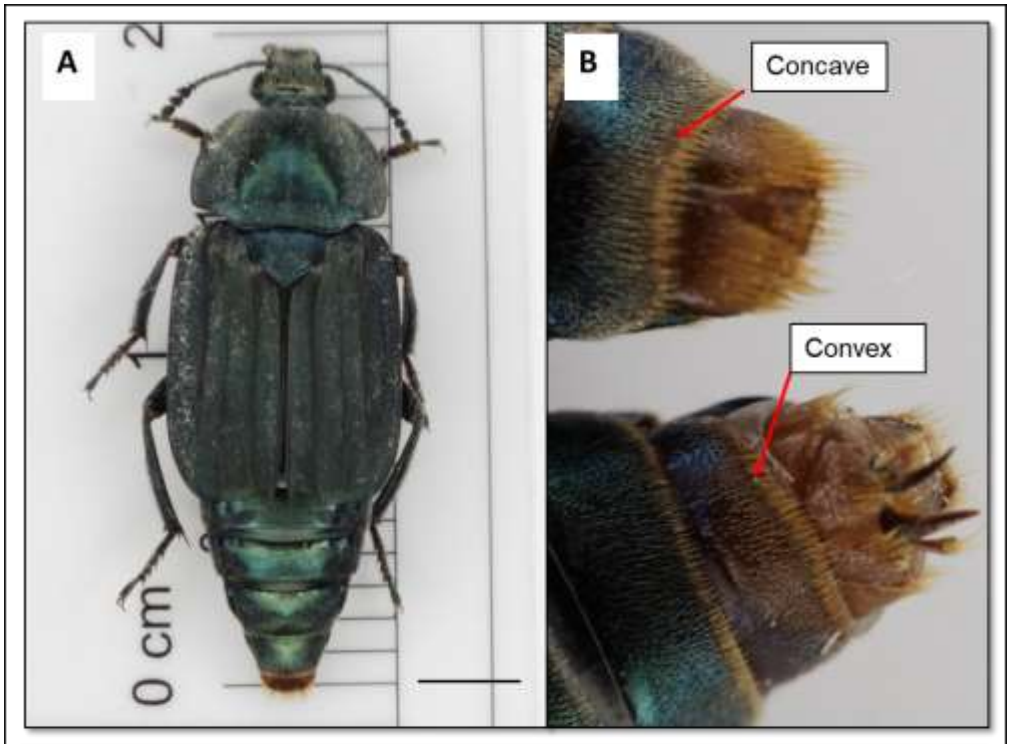

Figure 3.5: Stereomicrographs of the adult of *Thanatophilus micans* (Fabricius, 1794) (A-B) A. Adult dorsal view (Scale bar = 3mm). B. Female, arrow indicate concaved seventh sternite (top) and male, arrow indicating convexed seventh sternite (bottom).

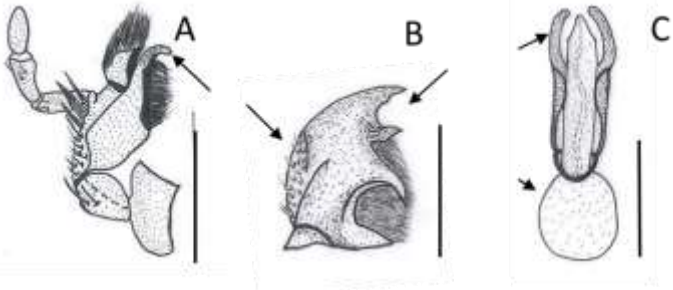

Figure 3.6: Diaromatic drawings of the adult of *Thanatophilus micans* (Fabricius, 1794) A. Left maxilla, arrow indicating lacina tooth (dorsal view) (Scale bar = 0.5mm) B. Left mandible, arrow indicating tooth on the molar region (dorsal view) (Scale bar = 0.5mm). C. Adult male aedeagus, arrows indicating curved parameres and the presence of rounded inner sac (dorsal view) (Scale bar = 2.5mm).

3.2 Staphylinidae

There are between 45 000 and 54 000 species of staphynilids described (Dekeirsschieter *et al.* 2013). Most staphylinid species are predaceous on fly eggs, maggots and immature stages of other beetles; members of the genus *Aleochara* are parasites of maggots and fly pupae (Prins 1984a; Braack 1986; Villet 2011; Dekeirsschieter *et al*. 2013). Taxonomic studies on the Afro-tropical species from the carrion associated genus *Philonthus* are described by Hromadka (2009; 2012).

Specimens collected during the course of this study were represented three Staphylinidae genera (*Aleochara*, *Philonthus* and *Platydracus*). Only a few adult specimens of an *Aleochara* species were collected from carcasses during the course of this study (Table 3.1). Adult individuals of the two *Philonthus* species (*P. caffer* (10%) and *P. longicornis* (9%)) were abundant at all the carcasses. Only one adult specimen of *Platydracus hottentotus* was collected from a carcass. Specimens representing two of the three genera were also collected during the course of previous succession studies conducted

at the same site (Table 3.2). Boucher (1997) and Botham (2016) recorded specimens from the staphylinid genera *Anotylus, Atheta, Phacophallus, Belonuchus* and *Oxytelus* which was not collected during the trial period of the current study.

Members of the genus *Aleochara* are known parasites of maggots (Prins 1984a; Braack 1986; Villet 2011; Dekeirsschieter *et al*. 2013) therefore, fly pupae collected from the carcasses were dissected to look for signs of parasitism. No immature stages of this genus were recovered from fly pupae or from soil samples. Specimens of *Philonthus sp.* larvae were collected from soil samples. The live portion of the larval sample could not be reared to the adult stage to make a species identification from the emerging adults. These larvae died during the pupal stage and were degraded to the extent that a pupal description could not be generated. Furthermore, the breeding of the adults of these particular species failed to produce immature stages. No immature stages of *P. hottentotus* were collected from soil samples, furthermore since only one adult specimen of this species was collected from a carcass breeding for immature stages was not possible.

Philonthus sp. Stephens, 1829

Larval description

Diagnosis: Campodeiform; larvae easily recognised by soft bodies; tergites with grey colouration; head orientated prognathous (Fig. 3.7A); head length 0.3 – 0.7mm and head width 0.3 – 0.9mm, n=18.

Head: Antenna with three segments; mandibles slender with no tooth; retinaculum present.

Thorax: Thorax soft; legs well developed for running with tarsal segments fused together to form single claw.

Abdomen: Urogomphus present on last abdominal segment and urogomphus segmented (Fig. 3.7B).

Remarks: Larvae of *Philonthus* sp. has a soft body and head which is orientated prognathous like those of *Saprinus splendens* and *Necrobia rufipes,* but the larvae differ from *Saprinus splendens* and *Necrobia rufipes* by a head that is somehow rounded when mandibles are at rest. *Philonthus* sp. larvae can be differentiated from the larvae of *S. splendens* by a pointy urogomphus at the apex rather than rounded urogomphus. *Philonthus* sp. larvae can be differentiated from the larvae of *N. rufipes* by absences of sclerotised plate on the last abdominal segment.

Figure 3.7: Stereomicrographs of dorsal view of the larvae of *Philonthus* sp Stephens, 1829 (A-B) A. head and the pronotum (Scale bar = 1mm). B. Abdominal segments showing segmented urogomphus (Scale bar = 1mm).

Philonthus caffer Boheman, 1848

Adult description

Diagnosis: Adults elongated; brown and black body colouration (Fig. 3.8); body length: APW 0.6mm – 0.8mm; EL 0.8mm – 1.1mm; EW 1.5 – 1.9mm; PEL 2.6mm – 3.0mm; PPW 0.9mm – 1.0mm, n=30.

Head: Mouthparts orientated prognathous; head not concealed in pronotum; occiput long and separates head from pronotum; head shiny metallic black; head with scattered punctation on lateral side; few punctures on frontal disc; compound eyes flat and not extending laterally on both sides; compound eyes visible from above; compound eye not divided into lower and upper parts; antennae moniliform with 11 antennomeres (Fig. 3.8); frontoclypeal suture complete; labrum and the clypeus separated by membrane; labrum plain at base and emarginate at apex with setae on labrum margin; mandibles visible from above; mandibles slightly robust and pointy at apex; mandibles both dentate with series of setae from mandible base to mid-mandible on lateral side; maxilla with three maxillary palpomeres and second and fourth palpomeres longer than first and third palpomeres; labium with three labial palpomeres; apical labial palpomere longer than pre apical palpomere and basal palpomere; labial articulation of labial palpomeres visible from below.

Thorax: Pronotum completely metallic black in colouration; pronotum punctate and each punctation with hair; anterior angles of pronotum forming truncate shape with pronotal carinae; pronotal lateral carinae separates pronotal disc and hypomeron; pronotal carinae smooth; posterior angles of pronotum rounded; pronotum disc somehow oval with anterior being narrower than posterior; hypomeron asetose; elytra exposing more than three complete tergites (Fig. 3.8); forelegs cursorial and adapted for running; protibial with series of spines surrounding it and two apical spurs; mesotibia and metatibia not widened, both have series of spines surrounding tibia and two apical spurs; tarsal segment 5-5-5 and all legs pretarsal claws toothed at claw base.

Abdomen: Abdominal tergites black and brown and visible from above; pygidium not covered by elytra (Fig. 3.8)

Male genitals: Medial lobe broad; basal lobe heavily sclerotised; two apical setae and series of peg setae on each parameres (Appendix 1, Fig. B).

Remarks: Descriptions of this species supports the descriptions given by Hromádka (2009).

Figure 3.8: Stereomicrograph of dorsal view of the adult of *Philonthus caffer* Boheman, 1848 (Scale bar = 2mm).

Philonthus longicornis Stephens, 1832

Adult description

Diagnosis: Adults with black body colouration; head visible when viewed dorsally; body elongated (Fig. 3.9); body length: APW 0.6mm – 0.9mm; EL 0.7mm – 1.3mm; EW 1.7 – 2.1mm; PEL 2.6mm – 3.1mm; PPW 0.9mm – 1.1mm, n=30.

Head: Head visible from above and not concealed by pronotum; compound eyes flat; compound eyes not extending laterally on each side when viewed from dorsal side; compound eyes undivided by clypeus into lower and upper parts; antenna moniliform with 12 antennomeres; antennas visible from above and not concealed by frontal ridges (Fig. 3.9); frons metallic black and punctate; labrum visible from the above and not concealed by the clypeus; labrum well sclerotised and labral apex emerginate; base of labrum plain and separated from clypeus by complete line; mandibles visible from the above; molar region with teeth; maxillary lobe consist of lacinia and galea; lacinia with fine hairs; maxillary palp with three palpomeres; apical maxillary palpomere longer than preapical and basal palpomere; articulation of maxillary palpomeres visible when head viewed ventrally; galea with fine hairs at apex; labium with three labial palpomeres; articulation of labial palpomeres visible when viewing head ventrally.

Thorax: Pronotum completely black with scattered punctation; four punctures on the dorsal-central of pronotum disc; anterior angles of pronotum forming truncate shape with pronotal carinae; pronotal lateral carinae separates pronotal disc and hypomeron; pronotal carinae smooth; posterior angles of pronotum rounded; hypomeron asetose; scutellum visible between elytral bases; scutellum anteriorly plain/straight and posteriorly acute; elytra very short and truncated; elytra exposing more than three complete tergites; forelegs cursorial and adapted for running (Fig. 3.9); protibial with series of spines surrounding it and two apical spurs; mesotibia and metatibia not widened, both have series of spines surrounding tibia and two apical spurs; tarsal segment 5-5-5 and all legs pretarsal claws toothed at claw base.

Abdomen: More than three abdominal tergites black and visible from above; pygidium exposed (Fig. 3.9).

Male genitals: One paramere present; when viewed ventrally paramere skewed to right; paramere with peg setae at apex (Appendix 1, Fig. D)

Remarks: The adult descriptions provided in this study supports the description of adults of *Philonthus longicornis* group are given by Hromádka (2012).

Figure 3.9: Stereomicrograph of dorsal view of the adult of *Philonthus longicornis* Stephens, 1832 (Scale bar = 2mm).

Aleochara sp. Stephens, 1832

Adult description

Diagnosis: Adults black and orange body colouration with head visible when viewed dorsally (Fig. 10); body length: APW 0.3mm – 0.4mm; EL 0.4mm – 0.5mm; EW 1.0mm – 1.2mm; PEL 0.9mm – 1.1mm; PPW 0.4mm – 0.5mm, n=15.

Head: Head visible from above and not concealed by pronotum; compound eyes flat; compound eyes not extending laterally on each side when viewed from dorsal side; compound eyes undivided by clypeus into lower and upper parts; antenna clavate with 12 antennomeres and visible from above and not concealed by frontal ridges; seven of 12 antennomeres gradually increasing to antennal apex (Fig. 3.10); antennal sockets between compound eyes; frons black and covered with hairs; labrum visible from above

and not concealed by clypeus; labrum well sclerotised and labral apex emerginate; base of labrum plain and separated from clypeus by complete line; mandibles visible from above; maxillary palp with four palpomeres; apical maxillary palpomere smaller than preapical palpomere; preapical palpomere enlarged; articulation of maxillary palpomeres not visible when viewing head ventrally; galea with fine hairs at apex; labium with three labial palpomeres; articulation of labial palpomeres visible when viewing head ventrally.

Thorax: Pronotum with fine hairs and brownish to black colouration; anterior angles of pronotum obtuse and do not form solid rounded curve with pronotal carinae; lateral carinae separates pronotal disc and hypomeron; pronotal carinae smooth; posterior angles of pronotum obtuse; pronotal hypomeron asetose; scutellum visible between elytral bases and small; elytra expose more than three complete tergites; elytral disc orange and covered with hairs; elytra truncated and meet at midline when wings at rest; forelegs cursorial and adapted for running (Fig. 3.10); protibial with series of spines surrounding it and two apical spurs; mesotibia and metatibia not widened, both have series of spines surrounding tibia and two apical spurs; tarsal segment 5-5-5.

Abdomen: Abdomen black and brown; more than three abdominal sternites visible when viewed ventrally; pygidium exposed (Fig. 3.10).

Male genitals: Parameres crossing at apex; medial lobe curved and shorter than parameres (Appendix 1, Fig. C).

Remarks: This species differs from other species collected of Staphylinidae by having antenna sockets that are between the compound eyes and antennomeres gradually increasing apically. This species was identified to genus level based on the general morphological descriptions of the genus *Aleochara* given by Almeida & Mise (2009).

Figure 3.10: Stereomicrograph of dorsal view of the adult of *Aleochara* sp. Stephens, 1832 (Scale bar = 1mm).

Platydracus hottentotus (Nordman, 1837)

Adult description

Diagnosis: Adults brown and black with head orientated prognathous; body elongated; body length: APW 3.5mm; EL 4.0mm; EW 5.5; PEL 8.1mm; PPW 4.6mm, n=1.

Head: Head visible from above and not concealed by pronotum; compound eyes flat; compound eyes not extending laterally on each side when viewed from dorsal side; compound eyes undivided by clypeus into lower and upper parts; antenna moniliform with 12 antennomeres and visible from above and not concealed by frontal ridges; frons brown and covered with hairs; labrum visible from above and not concealed by clypeus; labrum

well sclerotised and labral apex emerginate; base of labrum plain and separated from clypeus by complete line; mandibles visible from above; maxillary palp with four palpomeres; basal maxillary palpomere smaller than three apical palpomeres; articulation of maxillary palpomeres visible when viewing head ventrally; galea with fine hairs at apex; labium with three labial palpomeres; apical labial palpomere longer than basal and preapical palpomeres; articulation of labial palpomeres visible when viewing head ventrally.

Thorax: Pronotum with fine hairs and brown; anterior angles of pronotum obtuse and do not form solid rounded curve with pronotal carinae; lateral carinae separates pronotal disc and hypomeron; pronotal carinae smooth; posterior angles of pronotum rounded; pronotal hypomeron asetose; scutellum visible between elytral bases and U-shaped; elytra expose more than three complete tergites; elytral disc brown and covered with hairs; elytra truncated and meet at midline when wings at rest; forelegs cursorial and adapted for running; protibial with series of spines surrounding it and two apical spurs; mesotibia and metatibia not widened, both have series of spines surrounding tibia and two apical spurs; femur black dorsally and brown ventrally; tarsal segment 5-5-5.

Abdomen: Abdomen black with hairs; six abdominal tergites exposed when viewed dorsally; pygidium exposed.

Male genitals: No male specimen found.

Remarks: This is the first time this species is recorded at the study area and only one female specimen was recorded. The preliminary descriptions given in the current study are based on single female specimen. The specimen is very large, reaching up to 20mm in length. Unfortunately, I could not locate a published article of book to which to compare the morphological descriptions given in the current study. Stereomicrographs of this specimen were sent to Harald Schillhammer for the idenfication. Collett (2015) did molecular identification of this species.

Figure 3.11: Stereomicrograph of dorsal view of the adult of *Platydracus hottentotus* (Nordman, 1837) (Scale bar = 3mm).

3.3 Histeridae

Histeridae beetles are referred to as clown beetles. Histerids can be observed as early as the fresh stage feeding on fly eggs. In a carrion ecosystem they are classified as predators. Summerlin & Fincher (1988) mentioned that histerids are also found in cow dungs and they are attracted to flies that lay their eggs on fresh cow dung because they feed on larvae as well as the eggs. Histerids can be seen in high numbers during the wet stages of decomposition when there is a high number of dipteran larvae at the carcass.

Braack (1984) during his study on decomposition of impala at the Kruger National Park mentions that the number of histerids on the carcass increased when the number of dipteran larvae increased and decreased when there were low numbers of dipteran species on the carcass left. Morphological identification of some South African histerids was provided by Prins (1984a) and molecular studies has been done by Collett (2015).

Individuals from the following histerid genera are attracted to carrion: *Acritus, Atholus, Chaetabraceus, Chlcionellus, Hister, Hyppocacculus, Pachylister, Paratropus* and *Saprinus* (Villet 2011). Histerids beetles were recorded in all the previous succession studies (Table 3.2) conducted at the study area. Boucher (1997) recorded 10 histerids species (*Antholus sp., Adelopygus sp. Hister calidus, H. nomas, Patchylister nigrita, Saprinus* sp. a*, Saprinus* sp.b*, Saprinus* sp.c, *Saprinus cupreus, S. splendens*). Boucher did not record the two unspecified histerids species (*Macrolister sp*. and *Euspilotus sp*.) Botham (2016) recorded during his study. Thus, of the nine genera specified by Villet (2011) to be associated with carrion only four (*Antholus, Hister, Pachylister,* and *Saprinus*) were recorded for the study area. Boucher (1997) recorded *Adelopygus sp.* and Botham (2016) recorded *Macrolister* sp*.* and *Euspilotus* sp*.* found to be associated with carrion that was not listed by Villlet (2011). However, this might not be because individuals of these species were not recorded by the researchers quoted by Villet (2011). Many researchers only record the presence of a specific species if the number of individuals has reached the minimum number they require for their analysis. The minimum number of species present is important for successional studies because the higher the number of specific species present, the higher the chance to give accurate PMI estimates. Of the seven genera that were recorded from the study site by previous researchers (Table 3.2) only three genera were also recorded during the course of this study. These three genera were *Patchylister (P. heros), Saprinus (S. splendens, S. cupreus and S. cruciatus flavipennis)* and *Hister (H. nomas)*. Individuals of *Patchylister* and *Hister* made up less than 1% of beetles recorded on carcasses during the current trial period. *Saprinus splendens* and *S. cupreus* were abundant on carcasses (i.e. 14% and 13% respectively). Some reasons why the species were not found during the current

study's trial period is: *Adelopygus sp* recorded by Boucher (1997) was only found associated with shaded carcasses whereas the current study's carrion was in direct sunlight; Botham (2016) recorded species from the genus *Euspilotus* during his winter studies whereas sampling was not done during winter for the current study; the rest of the species recorded by Boucher (1997) and Botham (2016) which was not collected during the current study were simply not caught in the pitfall traps or through active sampling because individuals from these species are present at a low abundance at each carrion.

It should be noted that both Boucher (1997) and Botham (2016) identified some Histeridae beetles only to the genus level, whereas Kolver 2003; Kelly 2006; Kolver 2009 and Hoffman 2014 only identified beetles to the family level. The fact that beetles in this family were not always recorded to the species level highlights the problem of carrion beetle species identification. If beetles are to be used more extensively in PMI estimation work, a correct species identification is vital.

Of the species collected as adults, only *S. splendens* immatures (i.e. larvae) were collected from soil samples. *Saprinus splendens* larvae were sampled from soil a few centimetres away from the carcass and also from underneath the carcass. The larval stage of *Pachylister heros* and *Hister nomas* were not found at the carcasses. This might be because the adults of both of these species were observed at very low number of less than 10 at each carcass (throughout the trail period a total number of 44 *P. heros* and 65 *H. nomas* specimens were collected from all carcasses), as compared to the high abundance of *S. splendens* adults at the carcasses. Although *S. cupreus* adults were present in very high numbers at the carcasses their larvae were not found at the carcasses. Larval and pupal stages were described from the specimens collected from the field. The other histerid species collected as adults during the course of this study could not be bred under the artificial conditions of a laboratory, therefore their larval and pupal stages could not be described.

Pachylister heros (Erichson, 1843)

Adult description

Diagnosis: Adults large with black colouration; head visible from above with very large mandibles (Figs. 3.12A&B); body length: APW 3.0mm – 4.1mm; EL 6.3mm – 6.7mm; EW 8.0 – 9.6mm; PEL 10mm – 12mm; PPW 8.0mm – 9.0mm, n=30.

Head: Head visible from above and not concealed by pronotum; compound eyes flat; compound eyes not extending laterally on each side when viewed from dorsal side; compound eyes undivided by clypeus into lower and upper parts; occiput punctate; antenna geniculate and visible from above; antennal socket concealed by frontal ridges; labrum visible from above and not concealed by clypeus; frontal disc and clypeus impunctate; frontal stria emerginate (Fig. 3.13B); labrum somehow pointy or mucronate at apex; labrum well sclerotised; mandibles visible from above; mandibles very large and mandibles with tooth on molar region.

Thorax: Pronotum with pronotal stria and pronotal stria reaches posterior angles of the pronotum, but does not complete the pronotum; depressions with fine punctate present on each side of the pronotum; anterior angles of the pronotum acute; pronotal disc is impunctate; pronotal hypomeron setose; scutellum very small and triangular-shaped; elytra does not cover abdomen completely; elytra with five dorsal striae; stria 1 – 4 almost completing elytra and stria 5 shorter than striae 1 – 4; inner subhumeral stria present; sutural striae present and shorter than stria 5; apical elytral stria absent (Figs. 12A & 13A) mesosternum emerginate at apex; longitudinal suture of metasternum present and reaches meso-metasternal suture (Fig. 3.13C); tarsal segment 5-5-5; foreleg tibia fossorial and serrated with three large teeth; foreleg tibia punctate when viewed dorsally and impunctate ventrally; foreleg femur punctate at apex when viewed dorsally and impunctate at apex when viewed ventrally (Fig. 3.13G&H).

Abdomen: Five abdominal sternites visible when viewed ventrally; pygidium exposed from above (Fig. 3.12A); pygidium with depressions (Fig. 13D) lateral stria of first abdominal sternite narrow and truncated at anterior and wider at posterior side (Fig. 3.13C).

Male aedeagus: Basal piece one pieced; basal lobe hollow; heavily sclerotised basal lobe and tegmen; median lobe does not reach apex and forms hollow along tegmen (Fig. 3.13F); tegmen slightly curved when viewed from dorsal side (Fig. 3.13F); tegmen broad with scattered punctation at apex (Fig. 3.13E&F) (Appendix 1, Fig. G)

Remarks: Stereomicrographs of this species were sent out to an expert for identification. No morphological comparisons were made from literature.

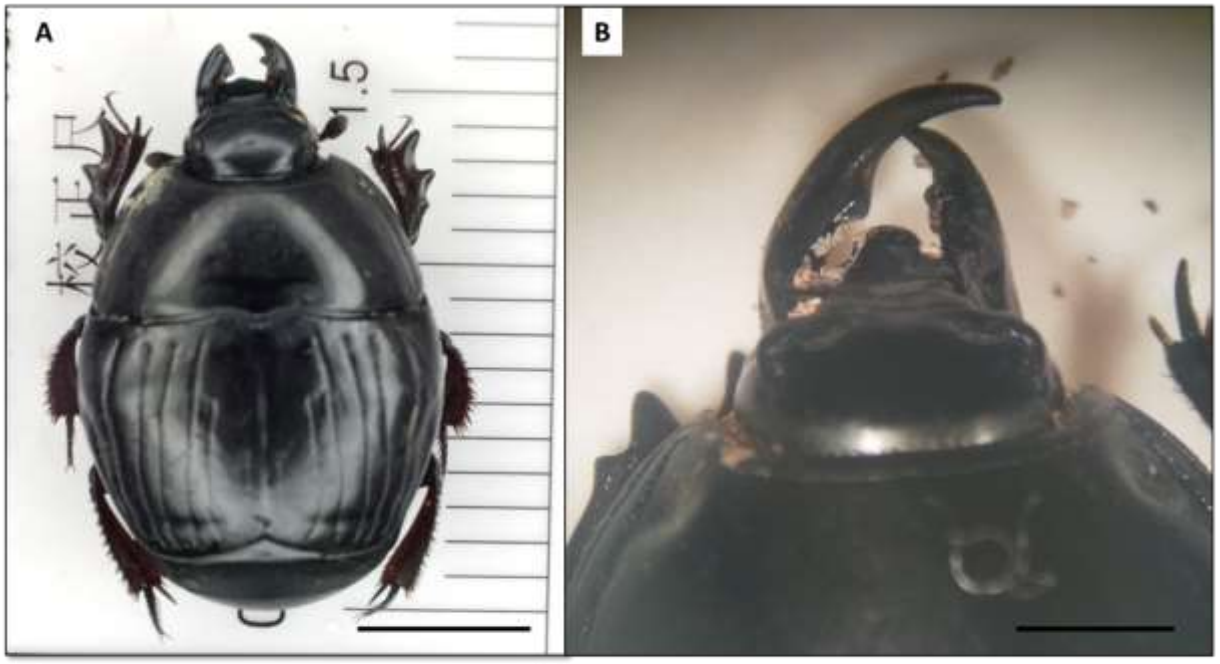

Figure 3.12: Stereomicrographs of dorsal view of the *Pachylister heros* (Erichson, 1843) (A) (Scale bar = 5mm) (B) (Scale bar = 2mm).

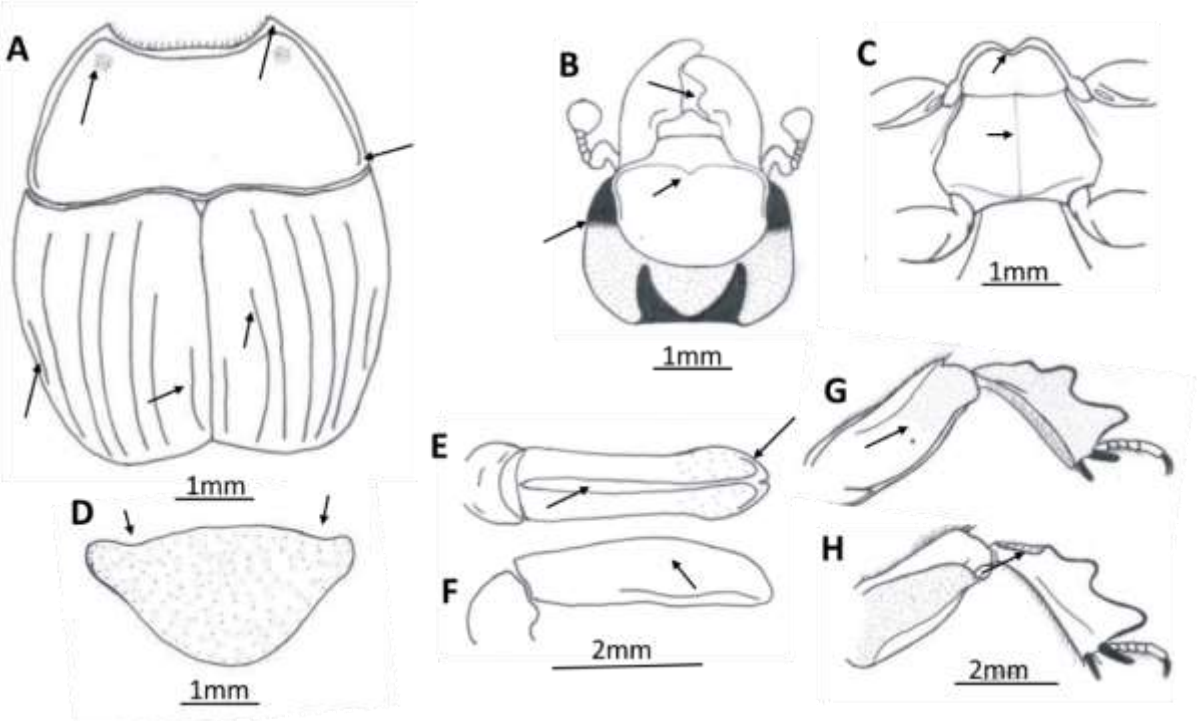

Figure 3.13: Diagrammatic drawings of *Pachylister heros* (Erichson, 1843) *A*dult A-H. A. Pronotum, arrows indicating acute pronotal angle, pronotal depression and pronotal stria & elytra, arrows indicating subhumeral stria, sutural stria and dorsal stria 5 (dorsal view). B. Female head, arrows indicating flat compound eye, emerginate frontal stria and toothed right mandible (dorsal view). C. Mesosternum, arrows indicating emerginate mesosternum apex and presence of longitudinal suture (ventral view). D. Pygidium, arrows indicating anterior depressions (dorsal view). E. Aedeagus, arrows indicating emerginate apex and hollow basal lobe (dorsal view). F. Aedeagus, arrow indicating a hump on mid tegmen (lateral view). G. Foreleg, arrow indicating punctation on femur (dorsal view). H. Foreleg, arrow indicating setae on protibia (ventral).

Hister nomas Erichson, 1834

Adult description

Diagnosis: Adults body oval and flattened; metallic black colouration on elytra and pronotum (Fig 3.14). Body length: APW 1.9 – 2.2mm; EL 2.7 – 3.1mm; EW 5.5 – 6.0mm; PEL 4.7 – 5.1mm; PPW 6.4 – 7.1mm, n=30.

Head: Head visible from above and not concealed by pronotum; compound eyes flat; compound eyes not extending laterally on each side when viewed from dorsal side; compound eyes undivided by clypeus into lower and upper parts; occiput densely

punctate; antenna geniculate and visible from above; antennal socket concealed by frontal ridges; clypeus truncated at apex; labrum deflexed and obtuse; mandibles robust, punctured and left mandible dentate; frontal disc and the clypeus impunctate; labrum visible from above and not concealed by clypeus; frontal disc and clypeus impunctate; frontal stria complete (Fig. 3.15B).

Thorax: Pronotum impunctate; pronotal stria present and reach base of pronotum; pronotal depressions present on each side of pronotum anteriorly; pronotal disc impunctate; pronotum anterior angles acute; scutellum small but visible and triangular-shaped; elytra with four dorsal, one humeral and one outer-humeral striae; sutural stria present and elytra impunctate; four dorsal striae almost completing elytra; dorsal stria five shorter than dorsal striae 1-4 and sutural stria; apical elytral stria absent (Figs. 3.14 & 3.15A); mesepimeron setose; discal marginal mesosternal stria slightly emarginate; longitudinal suture of metasternum present; mesosternum rounded at apex; mesosternum impunctate (Fig. 3.15C); tarsal segment 5-5-5; front tibia fossorial and serrated with up to four teeth; femur of foreleg with series of hairs on anterior side; both tibia and femur punctate dorsally and only femur punctate ventrally (Fig. 3.15G&H).

Abdomen: Pygidium exposed and carinate with black colouration; pygidium punctation same both anteriorly and posteriorly; pygidium posteriorly convex with depressions on both sides (Fig 3.15D); five abdominal sternites visible when viewed ventrally; each abdominal sternites impunctate anteriorly and punctate posteriorly.

Male genitals: Basal piece one pieced; tegmen apex emerginate; median lobe does not reach apex and median lobe heavily sclerotised (Fig. 3.15E); tegmen slightly curved through medio-distal (Fig. 3.15F) (Appendix 1, Fig. E).

Remarks: Stereomicrographs of this species were sent out to an expert for identification. No morphological comparisons were made from literature.

Figure 3.14: Stereomicrograph of dorsal view of the adult of *Hister nomas* Erichson, 1843 (Scale bar = 2mm).

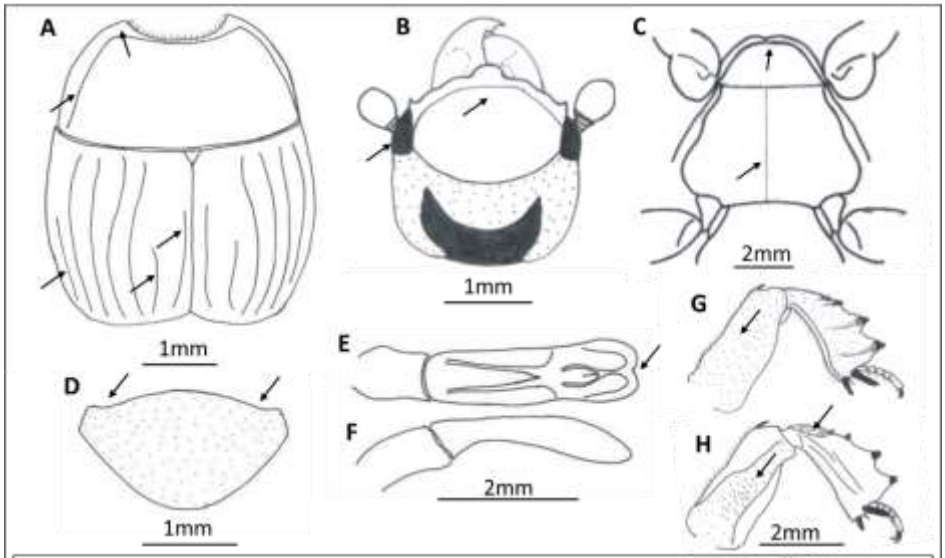

Figure 3.15: Diagrammatic drawings of *Hister nomas* Erichson, 1834 Adult A-H. A. Pronotum, arrows indicating acute pronotal angle and pronotal stria & elytra, arrows indicating subhumeral stria, sutural stria and dorsal stria 5 (dorsal view). B. Head, arrows indicating flat compound eye and complete frontal stria (dorsal view). C. Mesosternum, arrows indicating rounded mesosternum apex and presence of longitudinal suture (ventral view). D. Pygidium, arrows indicating anterior depressions (dorsal view). E. Aedeagus, arrows indicating emerginate apex (dorsal view). F. Aedeagus (lateral view). G. Foreleg, arrow indicating punctation on femur (dorsal view). H. Foreleg, arrow indicating setae on protibia and setae on femur (ventral).

Saprinus (Saprinus) splendens (Paykull, 1811)

Larval description

Diagnosis: Larvae of this species recognised by soft body; all instars with soft bodies and whitish in colour; head length 0.6 – 0.9mm and head width 0.7 – 1.3mm, n=30.

Head: Head orientated prognathous (Fig. 3.17B&E) and yellowish in colour; antenna with three segments; labrum with fine hairs; mandibles very large; molar regions of both right and left mandibles dentate; penicillus present on each mandible; maxilla with four maxillary palpomeres and basal palpomere with maxillary palp setae on each side; labium with two labial palps (Figs. 3.16 & 3.17A&B)

Thorax: Pronotum with longitudinal pronotal striae and indentations on each side of pronotum (Fig. 3.17C); legs very small; tarsi fused to form single claw (Fig. 3.17D&E).

Abdomen: Urogomphus present in all instars; urogomphus segmented not fused (Fig. 3.17F).

Remarks: Larvae of *Saprinus splendens* has a soft body and head which is orientated prognathous like those of *Philonthus* sp*.* and *Necrobia rufipes,* but the larvae differ from *Philonthus* sp. and *Necrobia rufipes* by having the body that is more rugose. *Saprinus splendens* larvae can be differentiated from the larvae of *Philonthus* sp. by a rounded urogomphus at the apex rather than pointy urogomphus. *Saprinus splendens* larvae can be differentiated from the larvae of *N. rufipes* by absences of sclerotised plate on the last abdominal segment.

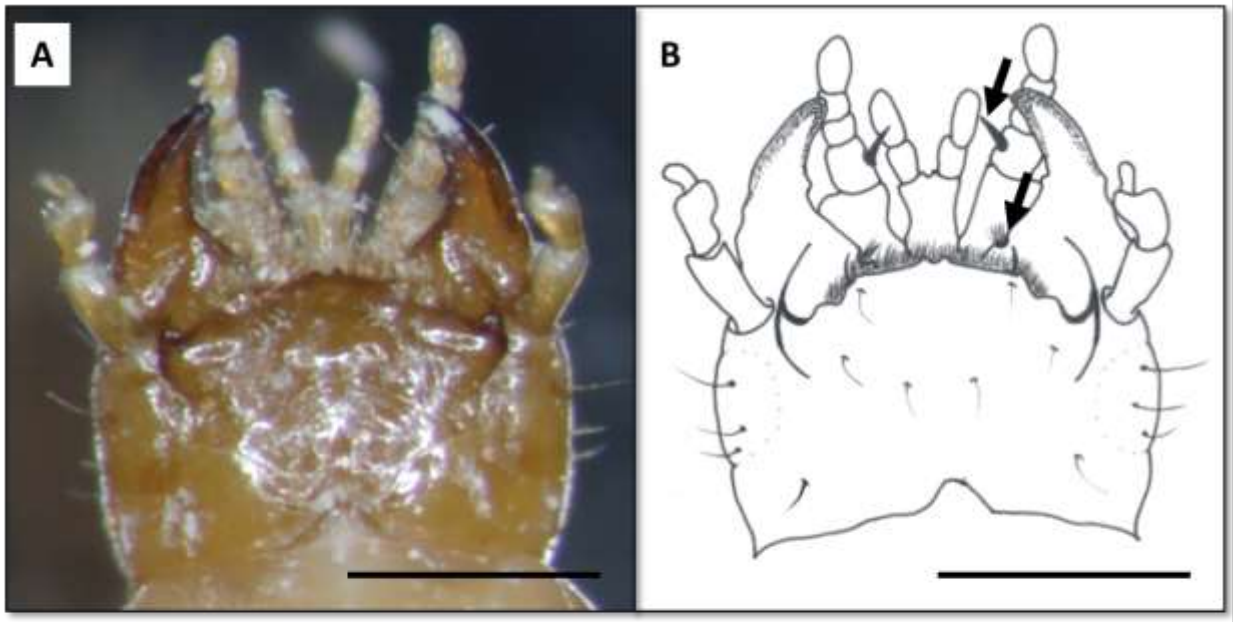

Figure 3.16: Stereomicrograph (A) and diagrammatic drawing (B) of larvae of *Saprinus splendens* (Paykull, 1811) (dorsal view) (Scale bar = 0.5mm). A. Head of the larvae. B. Detail drawing showing setae on the head, arrows two setae on the maxillary palpomere and pancillus on the mandibles.

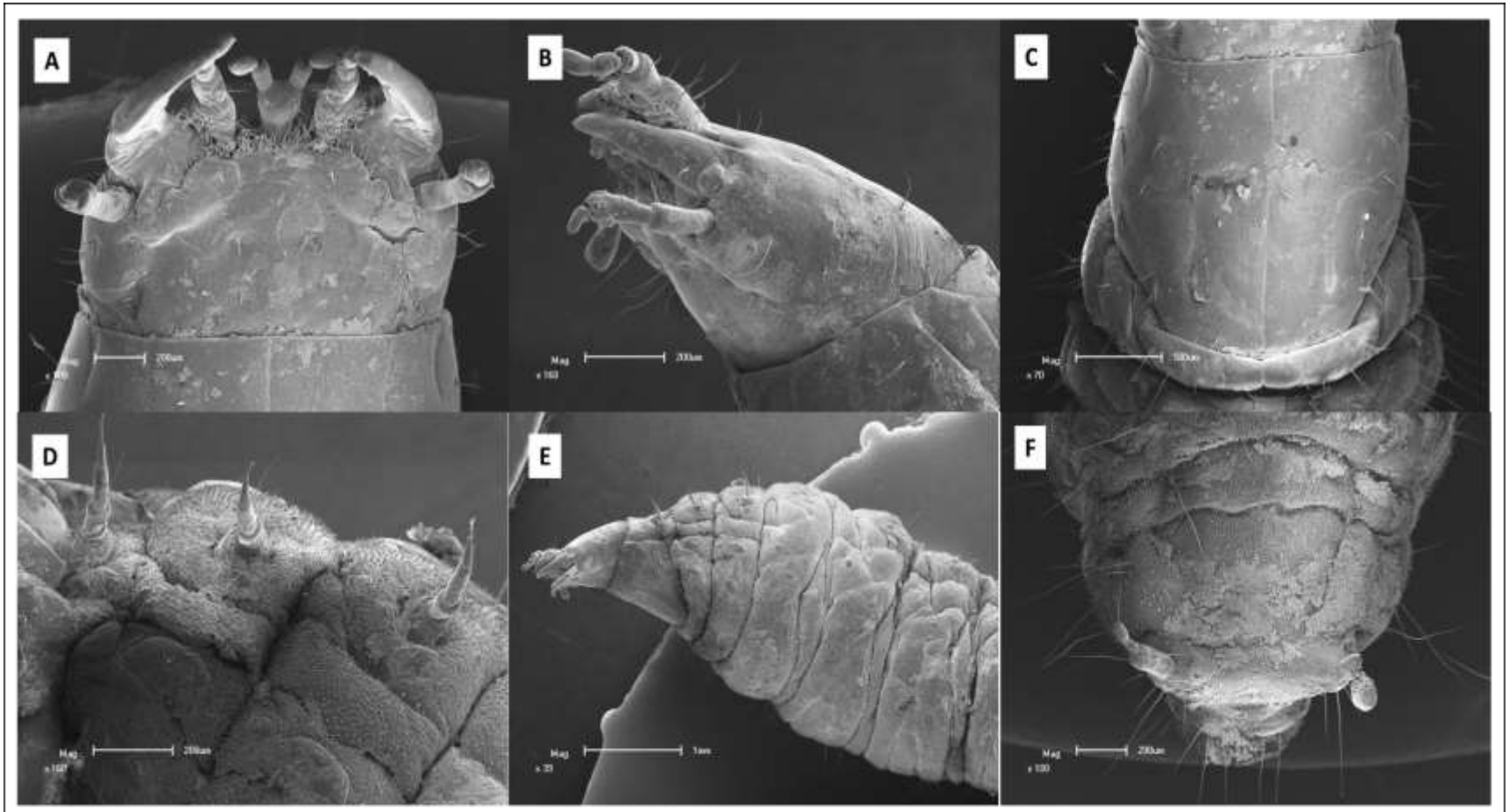

Figure 3.17: Scanning electron micrographs of *Saprinus (Saprinus) splendens* (Paykull, 1811) Larvae A-F. A. Head (dorsal view). B. Head (lateral view). C. Pronotum (dorsal view). D. Thorax (lateral view). E. Head, Thorax & Abdomen (lateral view). F. Abdomen (dorsal view).

Pupal description

Diagnosis: Pupa with pale to whitish colouration (Fig. 3.18A, B&C).

Head: Compound eyes red; frontal disc with setae (Fig. 3.18A&B).

Thorax: Wings with series of setae; front tibia fossorial (Fig. 3.18A).

Abdomen: Abdomen exposed when wings folded towards ventral side of pupa; abdomen with series of setae on lateral side; each abdominal tergite with row of setae; pygidium with distinct longitudinal line in middle from anterior to posterior and setae forming carinate shape (Fig. 3.18A, B&C).

Remarks: Preliminary descriptions of this pupa provided in the current study are based on only one pupal specimen. Only one pupa of this species was found in all trials. The development was terminated before any colour change was observed. Termination of the pupal development was done because of high mortalities of larval specimen that were not reaching pupal stage. The aim of the study was to describe life stages. Other species showed colour on the last day of their pupal stage. It is assumed that the pupa would have changed colour on its last day of the development.

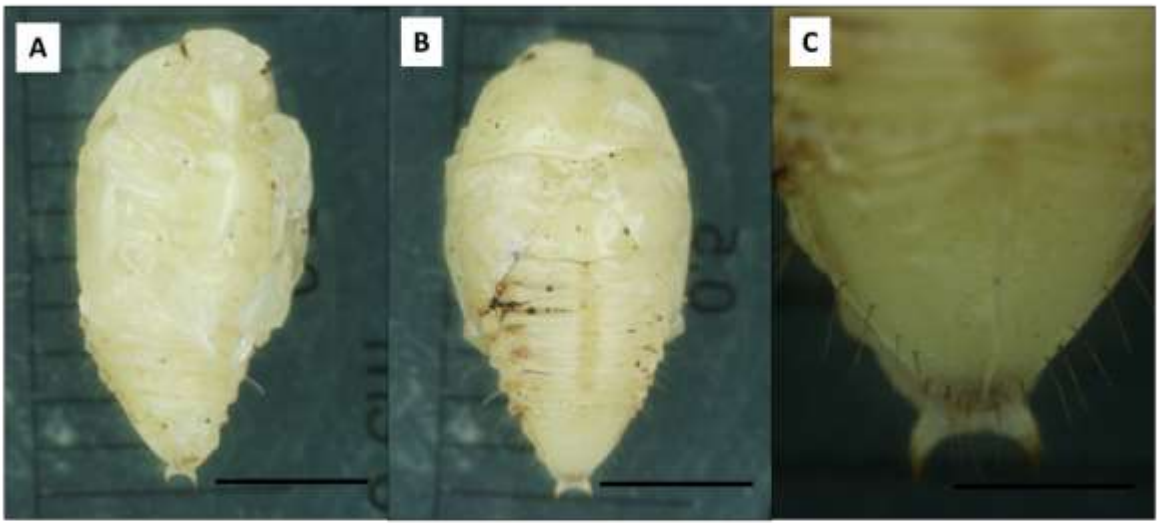

Figure 3.18: Stereomicrographs of *Saprinus (Sparinus) splendens* (Paykull, 1811) pupa (A-C). A. Ventral view (Scale bar = 3mm). B. Dorsal view (Scale bar = 3mm). C. Pygidium (dorsal view) (Scale bar = 1mm).

Adult description

Diagnosis: Adults body oval and with metallic blue colouration on elytra; pronotum with metallic green colouration; occasionally entire body with black colouration (Fig. 3.19). Body length: APW 1.6-1.9mm; EL 3.0-3.9mm; EW 4.1-5.0mm; PEL 4.9-6.1mm; PPW 3.9-4.9mm, n=30.

Head: Head visible from above and not completely concealed by pronotum (Fig. 3.19); compound eyes convex; compound eyes slightly extending laterally on each side when viewed from dorsal side; compound eyes undivided by clypeus into lower and upper parts; clypeus truncated at apex; labrum emerginate and visible from above; mandibles robust and punctured dorsally and on lateral side. Occiput more densely punctate than frontal disc and clypeus; occipital sutures carinate from posterior of occiput to occipital stria (Fig. 3.20B).

Thorax: Pronotum's lateral borders coarsely punctured and pronotal disc impunctate; pronotal stria present but does not reach base of pronotum; pronotal depressions present on each side of pronotum anteriorly; scutellum small but visible and triangular-shaped; elytra with four dorsal and one outer-humeral striae; sutural stria present and punctation on elytra does not reach sutural stria; apical elytral stria present (Fig. 3.20A); mesosternum concave at apex; longitudinal suture of mesosternum present but does not reach meso-meta sternal suture; tarsal segment 5-5-5; front tibia fossorial and serrated with up to 11 teeth; femur of foreleg coarsely punctate on ventral side (Fig. 3.20D) and finely punctate on dorsal side (Fig. 3.20E); cluster of setae present at apex of femur of foreleg (Fig. 3.20D).

Abdomen: Pygidium exposed and carinate with metallic blue colouration; pygidium punctation more coarsely anteriorly than posteriorly (Fig. 3.20C); five abdominal sternites visible when viewed ventrally; each abdominal sternites impunctate anteriorly and punctate posteriorly.

Male genitals: Basal piece one pieced; basal lobe hollow; slightly sclerotised medial and rounded apex (Fig. 3.20F); median lobe does not reach apex (Fig. 3.20F); tegmen slightly curved through medio-distal and angled (Fig. 3.20G); apical narrow and pointed (Fig. 3.20G); tegmen slightly broad medially (Fig. 3.20F&G) (Appendix 1, Fig. F).

Remarks: The description provided in this study supports the description given by Théry *et al*. (2009) and Lackner & Gomy (2016). This species has many variations depending on their geographic location (Théry *et al*. 2009; Lackner & Gomy 2016).

Figure 3.19: Stereomicrograph of dorsal view of the adult of *Saprinus splendens* (Paykull, 1811) (Scale bar = 2mm).

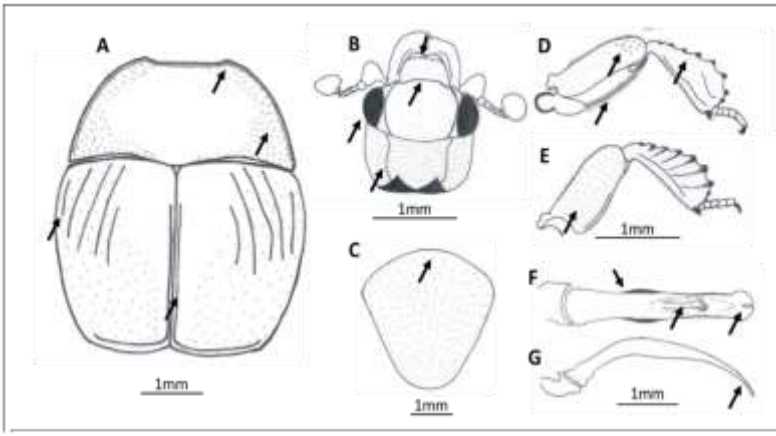

Figure 3.20: Diagrammatic drawings of *Saprinus splendens* (Paykull, 1834) *A*dult A-G. A. Pronotum, arrows indicating obtuse pronotal angle and pronotal punctation & elytra, arrows indicating outer-humeral stria and sutural stria (dorsal view). B. Head, arrows indicating convex compound eye, complete frontal stria and emerginate labrum (dorsal view). C. Pygidium, arrows indicating convex anterior (dorsal view). D. Foreleg, arrow indicating punctation on femur, cluster of apical setae (dorsal view). E. Foreleg, arrow indicating punctation on femur (ventral). F. Aedeagus, arrows indicating emerginate apex, medial lobe not reaching apex, and sclerosation on the lateral sides (dorsal view). G. Aedeagus, arrow indicating pointy apex (lateral view).

Saprinus (Saprinus) cupreus Erichson, 1834

Adult description

Diagnosis: Smallest species of histerid found in area; adult body oval with brownish colouration on elytra; pronotum with brownish to black colouration (Fig. 3.21B); at low magnification entire body with black colouration (Fig. 3.21A). body length: APW 0.6 – 0.8mm; EL 1.2 – 1.6mm; EW 1.9 – 2.5mm; PEL 1.7 – 2.1mm; PPW 1.5 – 1.9mm, n=30.

Head: Head visible from above and not completely concealed by pronotum (Fig. 3.21A); compound eyes convex; compound eyes extending slightly laterally on each side when viewed from dorsal side; compound eyes undivided by clypeus into lower and upper parts; clypeus truncated at apex; labrum emerginate and two setae visible dorsally; mandibles robust and punctured. Occiput densely punctate, same as frontal disc and clypeus (Fig. 3.22B); frontal stria incomplete.

Thorax: Pronotum's lateral borders densely punctured and pronotal disc finely punctate; pronotal stria present and reach base of pronotum; pronotal depressions present on each side of pronotum anteriorly; pronotal disc with scattered punctation; scutellum small but visible and triangular-shaped; elytra with four dorsal and one outer-humeral striae; sutural stria present and punctation on elytra does not reach sutural stria at base of wing, but does reach sutural stria at medial and wing apex; apical elytral stria present (Fig. 3.22A); mesosternum somehow concave at apex; mesosternum punctate; longitudinal suture of mesosternum present and does reach meso-meta sternal suture; posteriorly base of mesosternum with tubercles in males and without tubercles in females (Fig. 3.21C&D; Fig. 3.22C); tarsal segment 5-5-5; front tibia fossorial and serrated with up to 11 teeth; femur of foreleg with series of hairs on both anterior and posterior side (Fig. 3.22G&H).

Abdomen: Pygidium exposed and carinate, with brownish to black colouration; pygidium coarsely punctate anteriorly and less punctate posteriorly; pygidium posterior convex (Fig. 3.22F); five abdominal sternites visible when viewed ventrally; each abdominal sternites impunctate anteriorly and punctate posteriorly.

Male genitals: Basal piece one pieced; basal lobe hollow; tegmen apex forms square–shape and apex forms V-shape; angles of apex rounded (Fig. 3.22D); median lobe

does not reach apex (Fig. 3.22D); tegmen curved through medio-distal and slightly pointed; tegmen slightly broad medially and forms C–shape (Fig. 3.22E) (Appendix 1, Fig. I).

Remark: This species shows sexual dimorphism where males and females can be differentiated using external morphological characteristics (Fig. 3.21C&D). The posterior base of mesosternum with tubercles in males and without tubercles in females. The description provided in this study supports descriptions by Lackner & Gomy (2016).

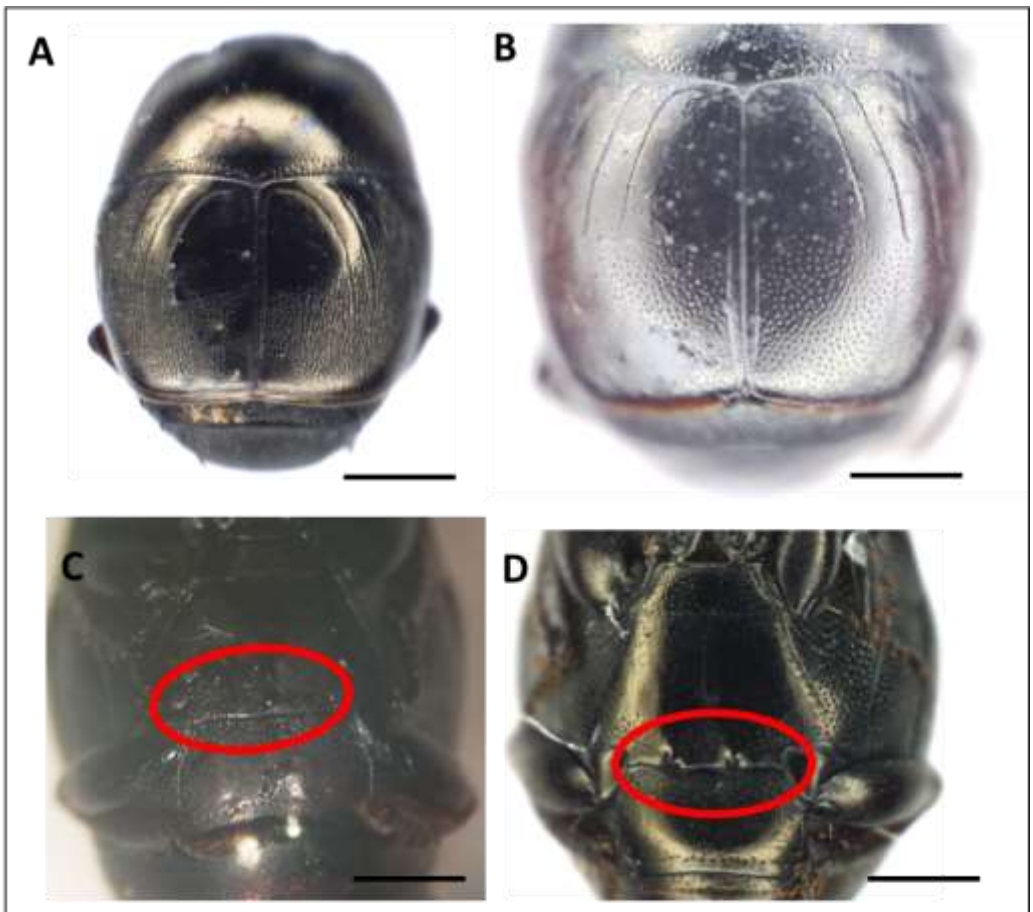

Figure 3.21: Stereomicrographs of *Saprinus (Saprinus) cupreus* Erichson, 1834 adults (A-D). A. Dorsal-ventral side (Scale bar = 1mm). B. Zoomed-in elytra showing brownish colour of the specimen (Scale bar = 0.5mm). C. Metasternum of a female specimen circle showing the absence of turbucles (Scale bar = 0.5mm). D. Metasternum of the male specimen circle showing the presence of the 2 turbucles (Scale bar = 0.5mm).

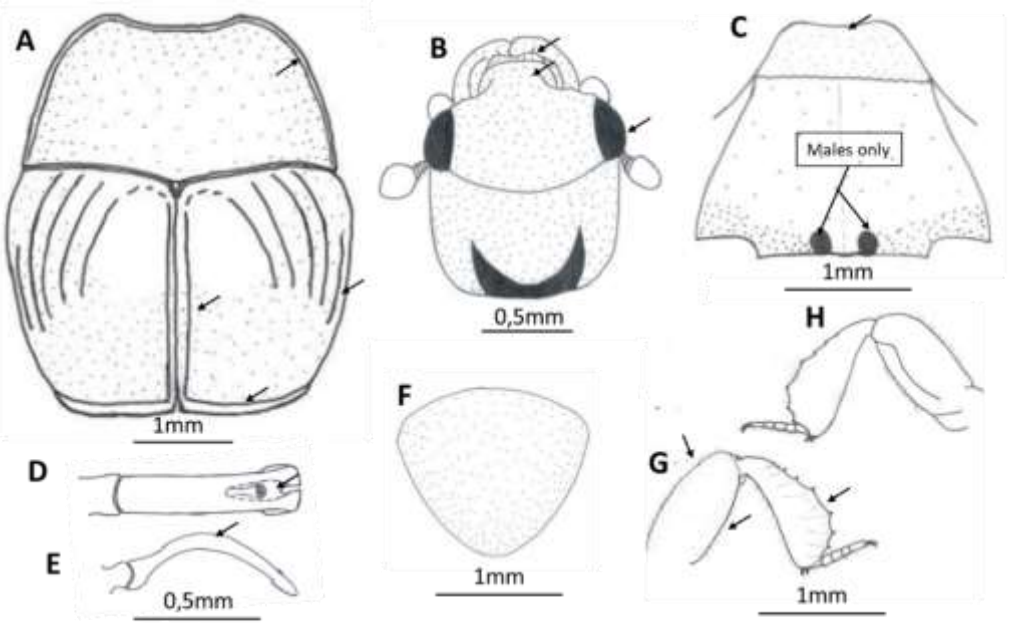

Figure 3.22: : Diagrammatic drawings of *Saprinus (Saprinus) cupreus* Erichson, 1834 adults A-G. A. Pronotum, arrow showing pronotal stria & elytra, arrows showing sutural stria, outer-humeral stria and apical stria (dorsal view). B. Head, arrows indicate emerginate labrum, incomplete frontal suture and convex compound eye (dorsal view). C. Meso-metasternum, arrow indicating concave apex (ventral view). D. Aedeagus, arrow indicating medial lobe not reaching the apex (dorsal view). E. Aedeagus, arrow indicating curve at the medial of tegmen (lateral view). F. Pygidium (dorsal view). G. Foreleg, arrows indicating setae (dorsal view). H. Foreleg (ventral).

Saprinus cruciatus flavipennis Péringuey, 1888

Adult description

Diagnosis: Adults black with yellow to reddish colouration on the elytra (Fig. 3.23A&B); Body length: APW 1.5-1.7mm; EL 3.1-3.7mm; EW 3.9-4.5mm; PEL 4.9-5.5mm; PPW 4.2-4.7mm, n=13.

Head: Head visible from above and not completely concealed by pronotum (Fig. 3.23A&B); compound eyes convex; compound eyes extending slightly laterally on each side when viewed from dorsal side; compound eyes undivided by clypeus into lower and upper parts; compound eyes convex; clypeus truncated at apex; labrum emerginate and two setae visible dorsally; mandibles punctate. Occiput densely punctate same as frontal disc and clypeus; frontal stria complete.

Thorax: Pronotum's lateral boarders densely punctured and pronotal disc finely punctate; pronotal stria present and reach base of pronotum; pronotal depressions

present on each side of pronotum anteriorly; scutellum small but visible and triangular-shaped; elytra with five dorsal striae; all dorsal striae do not complete elytra; sutural stria present and punctation on elytra does reach sutural stria; apical elytral stria present (Fig. 3.24A)

Abdomen: Pygidium exposed and carinate, with black colouration; pygidium punctation more coarsely anteriorly than posteriorly; pygidium posterior convex (Fig. 3.24D).

Male genitals: Basal piece one pieced; basal lobe hollow; tegmen curved through medio-distal and slightly pointed (Appendix 1, Fig. H).

Remarks: Stereomicrographs of this species were sent out to an expert for identification. No morphological comparisons were made from literature. This species is easily identified by its distinctive colouration on the elytra.

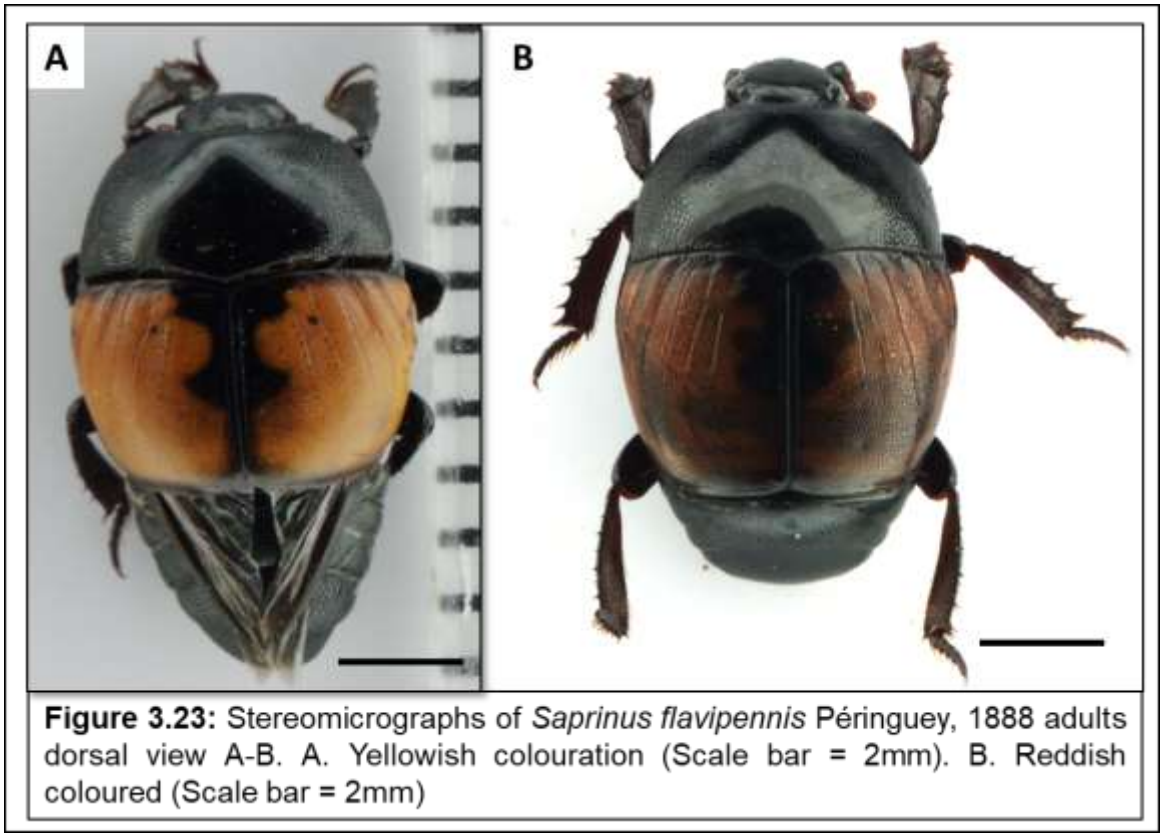

Figure 3.23: Stereomicrographs of *Saprinus flavipennis* Péringuey, 1888 adults dorsal view A-B. A. Yellowish colouration (Scale bar = 2mm). B. Reddish coloured (Scale bar = 2mm)

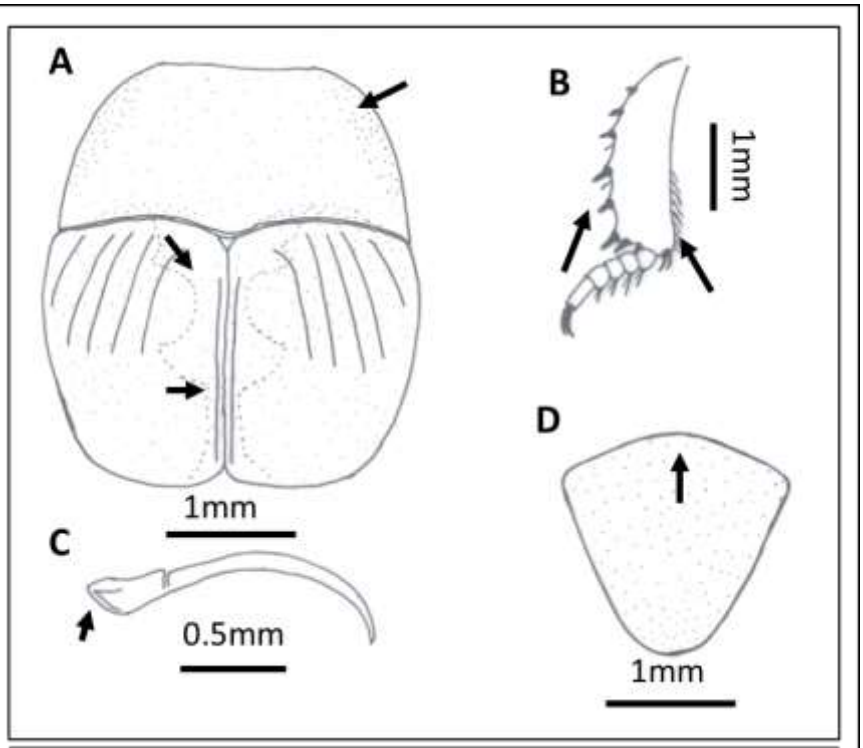

Figure 3.24: Diagrammatic drawings of adult of *Saprinus cruciatus flavipennis* Péringuey, 1888 (A-D). A. Pronotum, arrow indicating punctation and elytra, arrows indicating genuine leather sign and sutural stria (dorsal view). B. Left proleg, arrows indicating serrated teeh and setae (dorsal view). C. Male aedeagus, arrow indicating hollow basal lobe (lateral view). D Pygidium, arrow indicating convex anterior (dorsal view).

3.4 Dermestidae

Dermestids (hide beetles) are known to be a pest of various stored product foods (Proctor 1970; Rakowski & Cymboroskwi 1982; Veer *et al.* 1995; Richardson & Goff 2001; Schroeder *et al.* 2002; Zakka *et al.* 2013; Babarinde *et al.* 2018), as well as being associated with human and animal carcasses (Payne 1965; Braack 1981; Boucher 1997; Charabidze *et al.* 2013). The colonising of human and animal carcasses are of forensic significance and consequently they can be used to determine the PMI (Payne 1965).

The adults and the immatures of *D. maculatus* are both associated with decaying carcasses (Braack 1986, Villet 2011; Charabidze *et al.* 2013; Fontenot *et al.* 2015; Zanetti et al. 2015a). Adults of this species utilizes the carcass as both a breeding medium and food source (Von Hoermann *et al.* 2011; Zanetti *et al.* 2015a). They are necrophagous feeding on dry tissues and skin. According to Braack (1986), *D. maculatus* is a consistent dermatophage (feeds on skin) and also saprophage (feeds on decaying matter). The larvae of this species are facultative cannibalistic on defenceless pupa (Archer & Elgar 1998; Van Laerhoven 2009; Fontenot *et al.* 2015) and the adults are facultative predaceous on fly maggots (Braack 1986, Villet 2011). *Dermestes maculatus* adults were observed in the field feeding on *Chrysomya albiceps* maggots and they were also observed in the laboratory feeding on maggots (Fig. 3.25). *Dermestes maculatus* adults and larvae are considered as necrophages because predation and cannibalism are not observed consistently in nature.

Dermestes maculatus adults usually colonise the decomposing carcass in high numbers during wet stages of decay (active decay to dry stage). According to Von Hoermann *et al.* (2011) during these wet stages of decay a high concentration of benzyl butyrate is released which attracts *D. maculatus* adults. Cammack *et al.* (2015) stated that chemicals signals released by carrion and animals that are associated with carrion itself plays a very important role in mate location, resource quality and rearing of offspring.

Figure 3.25: Photographs of *Dermestes maculatus* DeGeer, 1774 (A-B) adults feeding on fly maggots. A. Adult in the field feeding on (Calliphoridae: *Chrysomya albiceps*) 22/03/2016. B. Adult in the lab feeding on blow fly maggot.

Of the three genera from the family Dermestidae recorded in Africa (Villet 2011) only one species (i.e. *Dermestes maculatus*) has been recorded at the study site during the current trial period as well as for previous years (Table 3.2). *Dermestes maculatus* adults were abundant (14%) on carcasses, also during previous succession studies at the study site (Table 3.2).

High numbers of *D. maculatus* larvae and pupae were collected from soil samples. The high incidence of immature stages on carrion is due to the fact that adults of this species not only utilise the carcass as a food source but also as a breeding medium (Von Hoermann *et al.* 2011; Zanetti *et al.* 2015). Breeding of these species was successful under artificial laboratory conditions and seven larval instars and pupae were produced at 28ºC. Due to this a description could be generated for the larvae and pupae as well as a key to distinguish between the seven larval instars.

Dermestes maculatus DeGeer, 1774

Description of the larvae

Diagnosis: Larvae with hairy appearance; display broad longitudinal line on pigmented sclerotised tergites (Fig. 3.26A).

Head: Head well developed in all instars and orientated hypognathous (Fig. 3.26A&B); antennae flagellated with three segments; epicranial and frontal sutures visible; frons bituberculate (Fig. 3.26B; Fig. 3.31F); up to six ocelli present on each side depending on larval instar; labrum laterally rounded and anteriorly emerginate (Fig. 3.31F).

Thorax: Well-developed legs adapted for running, with single claw; tarsus and claw fused into single claw-shape (Fig. 3.26B).

Abdomen: Urogomphus on second last abdominal segment depending on larval instar (early instar without urogomphus and late instars with urogomphus) (Fig. 3.26A; Fig. 3.27B, C &D; Fig. 3.31E); retrose turbecles with one seta present in matured larval instars (Fig. 3.27D; Fig. 3.31D).

Remarks: Larvae descriptions provided in the current study supports the descriptions of the larvae of this species provided by Prins (1984b).

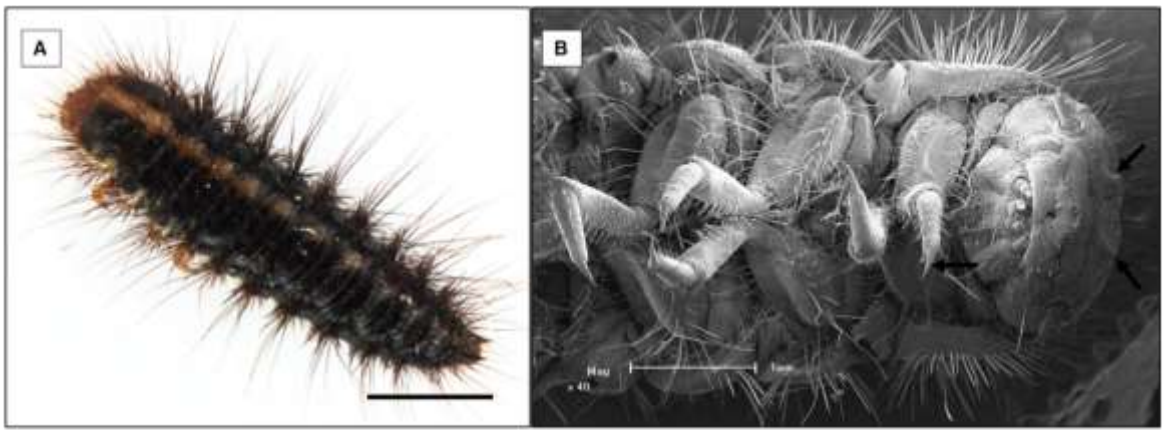

Figure 3.26: Stereomicrograph and scanning electron micrograph of *Dermestes maculatus* DeGeer, 1774 (A-B) larvae. A. Dorsal view showing a longitudinal line on tergites (Scale bar = 1mm). B. Ventral view arrows indicating a well-developed head with two tubercles and the legs tarsus and claws fused into a single claw-shaped at x40 magnification.

The morphological characteristics used to differentiate between of larval instars of *Dermestes maculatus.* The scanning electron micrographs shown in Fig. 3.27 are for instars 1, 3, 5 and 7 because of their distinctive characteristics used to differentiate between instars.

1. Spinulate setae arranged in pairs with one small seta in the middle; retrorse turbucle and urogomphus not present in second last abdominal segment; (Fig. 3.27A) …….……..…… ***Instar 1***

1' Spinulate setae with more than one seta in the middle and urogomphus is present.…….…… **(2)**

2. Spinulate setae arranged in pairs with two small setae in the middle; urogomphus present but small and not pointy, forms a knob-like structure; retrorse turbucle not present ……..……..…… ***Instar 2***

2' Urogomphus present and pointy (Fig. 3.27B, C&D) ……..……. **(3)**

3. Urogomphus present and pointy but not curved towards the anterior end of the body; retrorse tubercles are absent (Fig. 3.27B) ……..…….... ***Instar 3 & Instar 4***

3' Urogomphus present but curved towards the anterior end of the body…….…… **(4)**

4. Urogomphus slightly curved upwards and retrorse tubercles are absent (Fig. 3.27C) …….……..…….. ***Instar 5 & Instar 6***

4' Urogomphus robust, curved towards the anterior end of the body and retrorse tubercles are present (Fig. 3.27D) ……..……..……..…….... ***Instar 7***

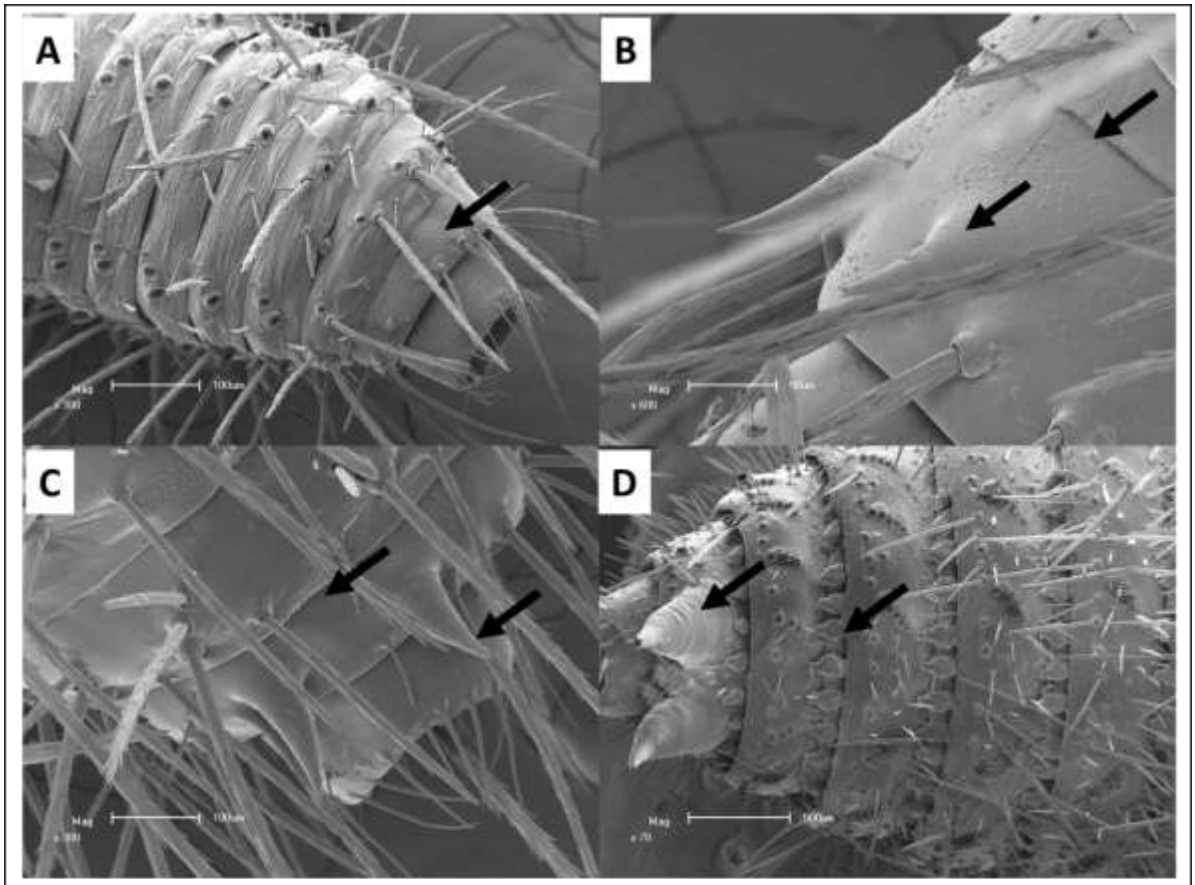

Figure 3.27: Scanning electron micrographs of *Dermestes maculatus* DeGeer, 1774 (A-D) larvae at different magnifications showing morphological characteristics that can be used to differentiate between larval instars. A. First instar larvae (arrow shows urogumphus absent). B. Third instar larvae (arrows shows urogomphus present and retrose turbucles absent). C. Fifth instar larvae (arrows shows urogomphus present and curved but retrose turbucles absent). D. Seventh instar larvae (arrows shows urogomphus present robust and retrose turbucle present).

Pupal description

Diagnosis: Pupa of *Dermestes maculatus* pale in colour, recognisable by exarate form; body appendages (legs and wings) not glued to body; unlike in larval form spinnulate setae absent and series of hairs present on both body and forewing (Fig. 3.28A&B).

Head: During pupal stage mouthparts orientated opisthognathous; compound eyes brown; head and pronotum fused.

Thorax: Forewing and hindwing not glued to body; setae on forewing form longitudinal lines starting from base to apex (requires high magnification).

Abdomen: Urogomphus robust and curved towards anterior end of body; five gin traps visible when viewed dorsally (Fig. 3.28B).

Remarks: The pupa remains pale yellow until the last day when it is ready to moult into adult stage. When ready to moult the fore-wing starts being sclerotised and move to the dorsal side. Tarsal development as shown in Fig. 3.29 single fused claw is now dividing to form tarsal claws and also the tarsus is divided to form tarsal segments; sex determination for pupa is given by Boucher (1997); descriptions provided in the current study supports the pupal description given by Prins (1984a).

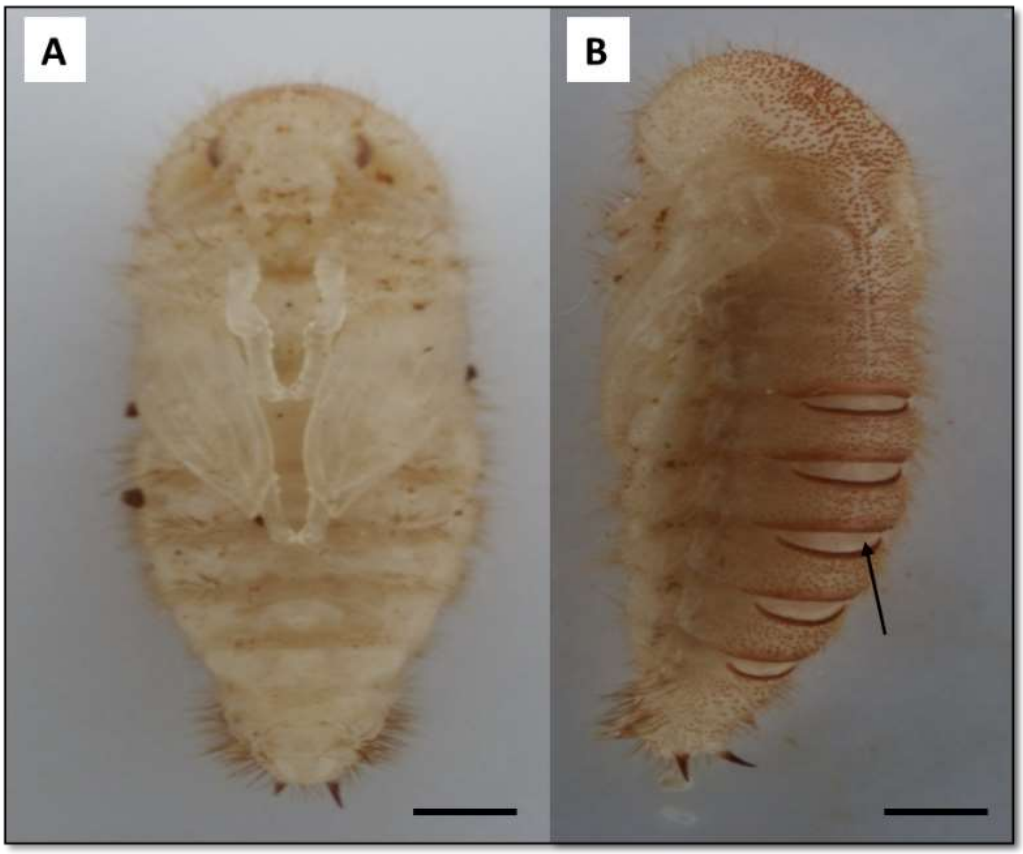

Figure 3.28: Stereomicrographs of *Dermestes maculatus* De Geer, 1774 (A-B) pupa. A. Ventral view. B. Dorsal-ventral view, arrow indicates gin trap (Scale bar 1mm).

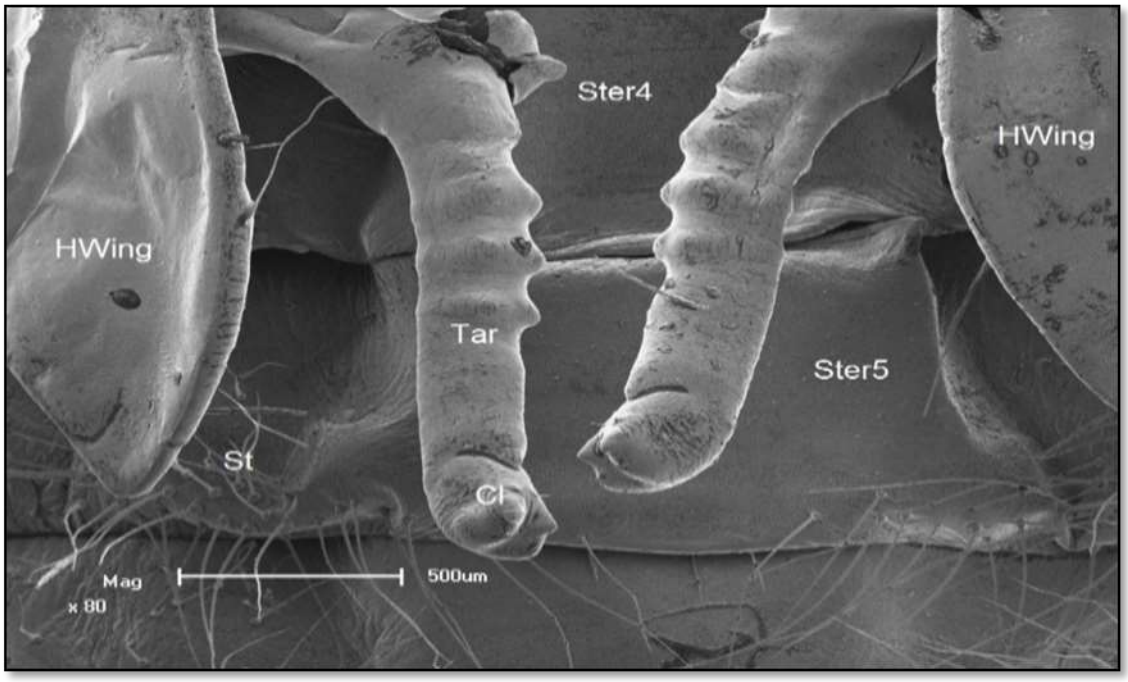

Figure 3.29: Scanning electron micrograph of *Dermestes maculatus* DeGeer, 1774. Pupa ventral view at 80x magnification showing the development of tarsus in pupal stage and glabrous hindwing.

Description of the adult

Diagnosis: Adults black with white and yellow hairs; head orientated hypognathous; body length: APW 1.5mm – 1.9mm; EL 5.2mm – 7.0mm; EW 3.1mm – 4.1mm; PEL 7.1mm – 9.8mm; PPW 2.9mm – 3.3mm, n=30.

Head: Head not completely concealed by pronotum when viewed from above; compound eyes visible from above; compound eyes extending laterally on each side when head viewed from above; compound eyes not divided by clypeus into lower and upper parts (Fig. 3.30A); antennae clubbed and greyish at apex and yellowish hairs at base; frons with white and yellow hairs; labrum not covered by clypeus and visible from above; frontoclypeal suture present; labrum emarginate and slightly sclerotised with hairs at labral apex; mandibles sclerotised and visible from above; mandibular

apex pointy with fine hairs on lateral side and mandibles divided into lobes; lacinia with teeth; maxillary palp with three palpomeres; apical maxillary palpomere longer than preapical palpomere and the basal palpomere; articulation of maxillary palpomeres not visible from below; labium with three labial palpomeres; articulation of labial palpomeres not visible from below.

Thorax: Pronotum black in colouration, pronotum's lateral boarders with white hairs forming U-shape (Fig. 3.30A); anterior angles of pronotum obtuse, does not form solid rounded curve with pronotal carinae (Fig. 3.31A); pronotum's lateral carinae separate pronotal disc and hypomeron; pronotum's lateral carinae smooth; posterior angles of pronotum acute; hypomeron setose; scutellum visible between bases of elytra; elytra covers most part of abdomen; elytra black with black and white hairs; elytron humerus with white and yellow hair (Fig. 3.30A); elytra apices meet at midline; prolegs cursorial, adapted for running; protibia with series of small spines on anterior and posterior side; two apical protibial spurs present; apical tarsi segment longer than tarsi 1-4; mesotibia and metatibia with one spur and five tarsi segments; basal and apical tarsi segments longer than tarsus 2-4; mesopretarsal and metapretarsal claws simple.

Abdomen: Five abdominal sternites visible when viewed ventrally (Fig. 3.31B); pygidium not usually exposed; elytra sometimes covers abdomen completely (Fig. 3.30A).

Male genitals: Trilobate type; median lobe same length as parameres with slightly pointy apex; parameres with hairs; paramere extensions fused at posterior end of medial lobe; paramere extensions heavily sclerotised at lateral sides; medial lobe forms V–shape at posterior end (Fig. 3.31C) (Appendix 1, Fig. J).

Remarks: This species shows great sexual dimorphism. Males have puncture and packed setae on their fourth abdominal sternite and females do not have puncture and packed setae (Fig. 3.30B). Sexual dimorphism of this species found in current study supports the findings of Halstead (1963) and Boucher (1997). A description of an adult of this species and its congeneric species are given by Prins (1984b) and also supports the description provided in the current study.

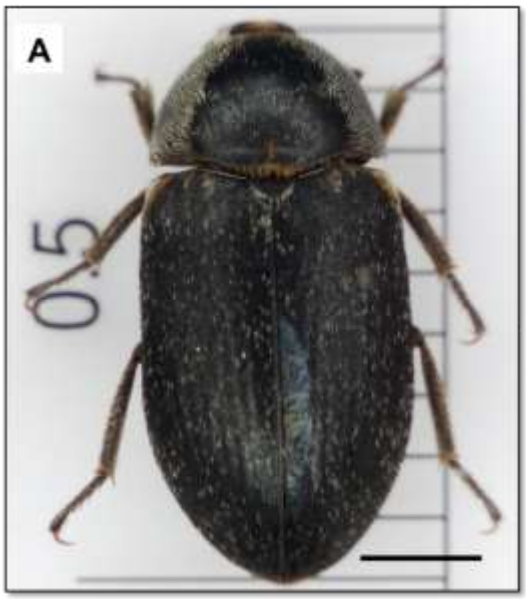

Figure 3.30: Stereomicrograph of *Dermestes maculatus* DeGeer, 1774 (A-B) adult. A. Dorsal view showing black and white scales on the elytra and the pronotum (Scale bar 2mm). B. Female on the right, arrow indicating absence of puncture and packed setae and the male on the left, arrow indicating puncture and packed setae on the fourth abdominal sternite (Scale bar 2mm).

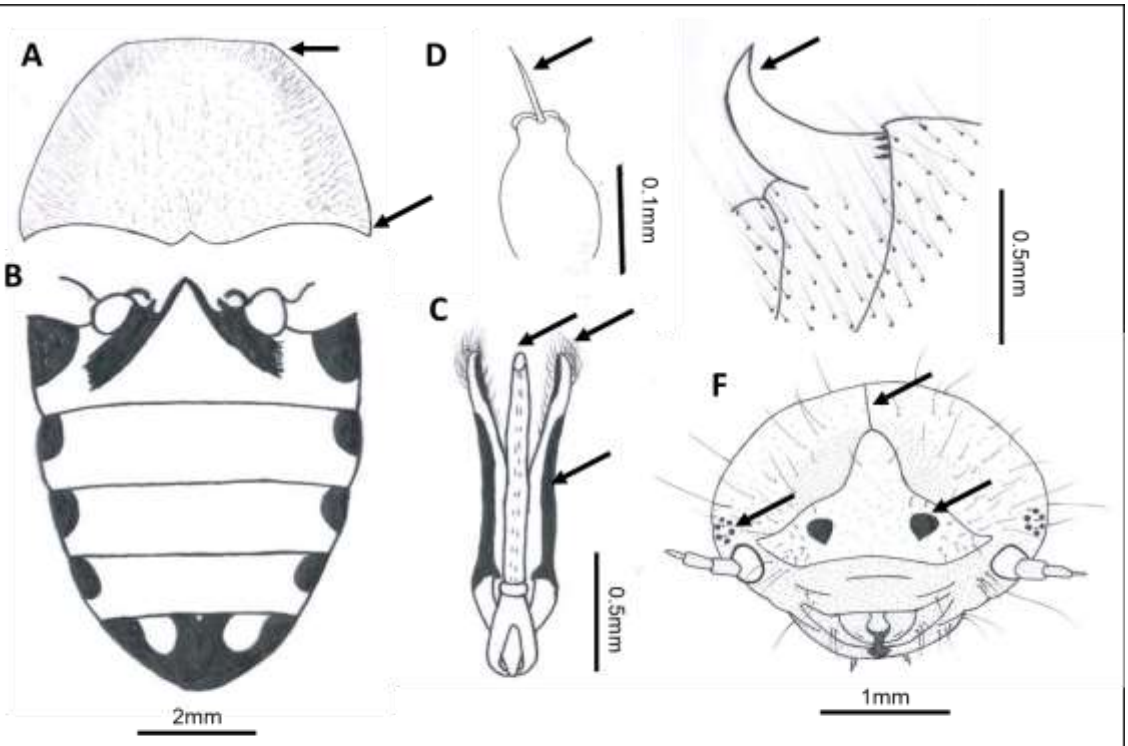

Figure 3.31: Diagrammatic drawing of *Dermestes maculatus* De Geer, 1774; A-C (Adult): A. Pronotum, arrows indicate obtuse anterior and acute posterior angles (dorsal view). B. Abdomen (ventral view). C. Male aedeagus, arrows indicate sclerotise paramere, paramere hairs and medial lobe (dorsal view). D-F (Matured Larvae): D. Retrose turbucle, arrow indicate one seta (high magnification required). E. Last three abdominal segments, arrow indicate curved urogomphi (dorsal-ventral view). F. Head, arrows indicates epicrinial suture, ocelli and turbucle (dorsal view).

3.5 Cleridae

Clerids are also known as ham beetles and are pests for several stored product foods as well as being colonisers of decomposing carcasses (Braack 1981; Hassan & Phillips 2010; Zannetti *et al.* 2015; Fakoorziba *et al.* 2017). *Necrobia rufipes* adults can be seen as early as bloat stage of decay feeding on both the carcass and the arthropods that are associated with the carcass (Braack 1984, Zannetti *et al.* 2015b). The larvae are seen during dry stage of decay, feeding on the dried-out carcass.

Two forensically important Cleridae genera (*Gyponyx* and *Necrobia*) have been recorded by Collett (2015) in South Africa. Villet (2011) stated that three species from the genus *Necrobia* are associated with carrion. *Necrobia rufipes* was the only species that has been recorded in this study and during previous studies (Table 3.2) at the study site. *Necrobia rufipes* adults were abundant (13%) on the carcasses set out during the current trial period.

Necrobia rufipes larvae were collected through active sampling from the carcass. Larvae of this species prefer moist areas such as the inside of vertebral foramen, layers of the skin and they were also observed digging into *D. maculatus* frass. This behaviour of digging into frass can be linked to the fact that *N. rufipes* larvae was predating on early larval stages of *D. maculatus* which were found in frass during the day when it is hot in the current study. Pupae were collected from soil samples. This species could be bred successfully under laboratory conditions; therefore, a description could be generated for the three larval instar stages and a pupal stage that was produced at 28ºC.

Necrobia rufipes (DeGeer, 1775)

Larval description

Diagnosis: Campodeiform; larvae easily recognised by soft bodies and brown colouration, except after moulting; body with fewer long hairs (Fig. 3.32); head orientated prognathous (Fig. 3.33B); head length 0.8 – 1.4mm and head width 0.4 – 1.1mm, n=30.

Head: Antenna with three antennomeres; second antennomere with setae at apex; labrum emarginate; mandible robust, molar region with teeth (Fig. 3.33A); lacina with series of setae at apex; maxillary palpi with three palpomeres.

Thorax: Thorax soft; legs well developed for running with tarsal segments fused to form single claw (Fig. 3.33C).

Abdomen: Urogomphus present on last abdominal segment and abdominal plate heavily sclerotised (Fig. 3.33D).

Remarks: Larvae of *Necrobia rufipes* has a soft body, and a sclerotised head which is orientated prognathous like those of *Saprinus splendens* and *Philonthus caffer,* but this species differs from these two by having heavily sclerotised plate and a non-segmented urogomphus on its last abdominal segment. All three instars of *N. rufipes* were indistinguishable using morphological characteristics they only differ in size.

Figure 3.32: Stereomicrograph of *Necrobia rufipes* (De Geer, 1775) dorsal view (Scale bar = 2mm).

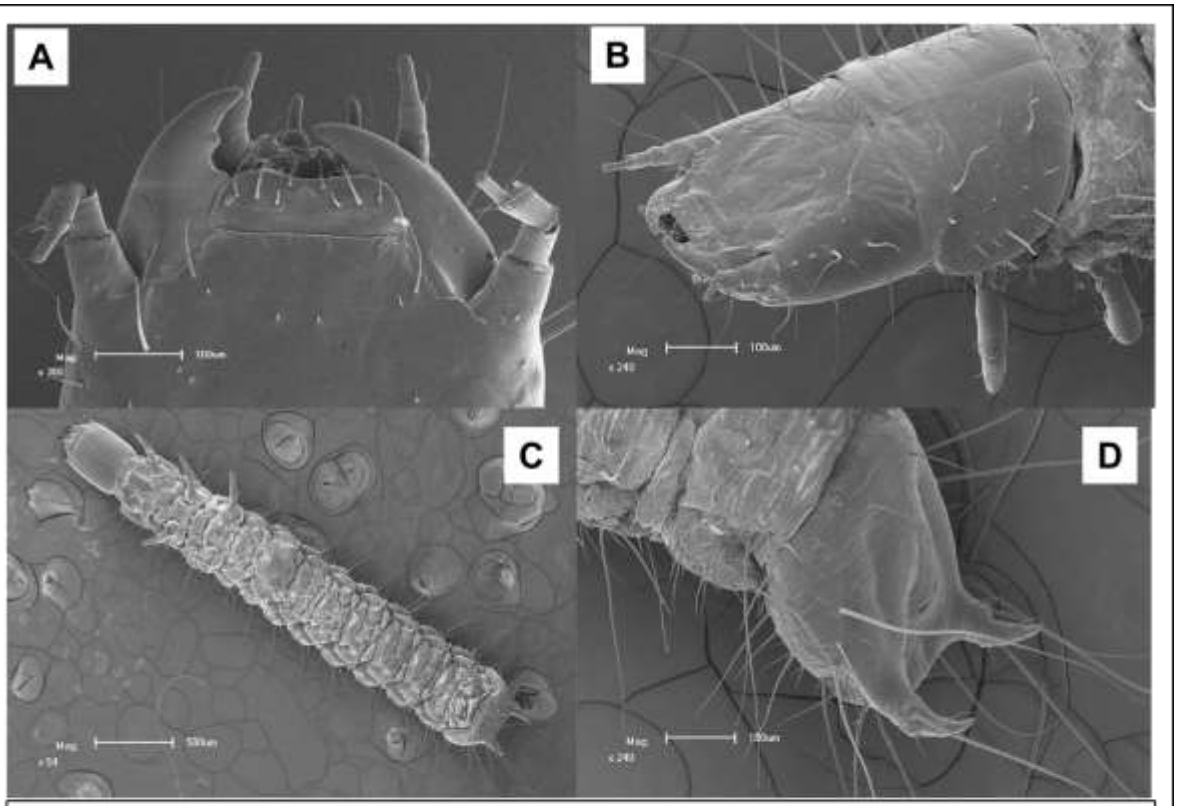

Figure 3.33: Scanning electron micrographs of larvae of *Necrobia rufipes* (De Geer, 1775) (A-D). A. Head close up showing emarginate labrum and setae at 300X magnification. B. Head and pronotum 240X magnification. C. Body ventral view at 54X magnification. D. Abdominal plate close-up showing the non-segmented urogomphus orientated towards the anterior side of the body at 240X magnification.

Pupal description

Diagnosis: Pupa of *Necrobia rufipes* pale in colour, recognisable by exarate form; body appendages such as legs and wings not glued to body (Fig. 3.34).

Head: Head orientated episthognathous; compound eyes brown (Fig. 3.47); head and pronotum fused; scattered setae on head and pronotum.

Thorax: Wings folded towards ventral side

Abdomen: Urogomphus present and pointy (Fig. 3.34).

Remark: The micrograph shown in Fig. 3.34 have been in 75% ethanol for some time and colour change on the abdomen can be seen. Please refer to (Fig. 3.47) showing a pupa in pupal chamber for fresh live specimen. In Fig. 3.47 a fresh live specimen was used to show the difference between fixed and nonfixed specimen.

Figure 3.34: Stereomicrograph of *Necrobia rufipes* (De Geer, 1775) ventral view.

Adult description

Diagnosis: Adults metallic green with heads orientated prognathous (Fig. 3.35A); body length: EL 4.2mm – 2.4mm; EW 1.4mm – 3.0mm; PEL 3.3mm – 7.1mm; PPW 1.1mm – 2.0mm; head width 0.9mm – 1.4mm; n=30.

Head: Head not completely concealed by pronotum when viewed from above; compound eyes visible from above; compound eyes extending laterally on each side when head viewed from above; compound eyes not divided by clypeus into lower and upper parts; antenna capitate; antennomeres reddish at base and black apically (Fig. 3.35A); clypeus and labrum not covered by frons labrum emarginate and slightly sclerotised with hairs at labral apex; mandibles sclerotised and visible from above; mandibles robust with teeth on molar region, mandible with hairs on lateral side; frontoclypeal suture present; maxillary palp with three palpomeres; apical maxillary palpomere longer than preapical palpomere and the basal palpomere; articulation of maxillary palpomeres not visible from below; labium with two labial palpomeres; articulation of labial palpomeres visible from below.

Thorax: Pronotum metallic green in colouration; pronotum's lateral carinae separates pronotal disc and hypomeron; pronotum's lateral carinae smooth; posterior angles of pronotum rounded; hypomeron asetose; scutellum visible between bases of elytra; elytra covers most part of abdomen; elytra metallic green with hairs; elytron humerus truncate; elytra apices meet at midline; prolegs cursorial, adapted for running; all three pairs of legs reddish in colour (Fig. 3.35A) plantulae distal; all legs with two spurs; tarsal segment 5-5-5.

Abdomen: Five abdominal sternites visible when viewed ventrally; pygidium not usually exposed.

Male genitals: Tegmen triangular in shape at anterior; phallus almost twice as long as tegmen; ventral phallobases longer than phallus (Fig. 3.35B) (Appendix 1, Fig. A).

Remarks: Adult males and females of this species are distinguishable by the setae on the elytra. Males setae lean towards the posterior-end/backward of an individual and females have setae that lean towards the anterior-end/forward of an individual.

Figure 3.35: Stereomicrograph (A) and diagrammatic drawing (B) of *Necrobia rufipes* (De Geer, 1775) adult. A. Dorsal view (Scale bar = 2mm). B. dorsal view male aedeagus (Scale bar = 2mm).

3.6 Trogidae

Trogidae it is a very small family with just more than 330 species described. For Africa about 100 species are described (Scholtz 1982, 1986a, b, 1990; Strumpher *et al.* 2014) of which 50 are endemic to southern Africa (Scholtz & Holm 2008). Traditionally three genera *Trox* Fabricius 1775, *Polynoncus* Burmeister 1876, *Omorgus* Erichson, 1847 are recognized (Scholtz 1982, 1986a, b, 1990; van der Merwe 2008; Strumpher & Scholtz 2009; Strumpher & Scholtz 2011; Strumpher *et al.* 2014; Strumpher *et al.* 2016).

Both the adults and the larvae trogids are associated with carcasses (Braack 1987; Scholtz & Holm 2008). Trogids are found at the later stages when the corpse is dried and have the ability to digest keratin (van der Merwe & Scholtz 2005; Gennard 2006; Scholtz & Holm 2008; Villet 2011; Strumpher *et al.* 2014). Trogids can also be opportunistic feeders of insect eggs as well as immobile larvae (van der Merwe & Scholtz 2005). Braack (1986) noted trogids feeding on hair, moist or dry muscle tissue, shreds of skin, carcass fluids and dried blood mixed with soil, fly maggots partially eaten by histerids and other organic materials.

It was unclear which of the Trogidae species found in the study site are firmly associated with carrion. Except for the Kolver (2003), Kelly (2006) and Hoffman (2014) studies, Trogidae species were recorded in all previous studies (Table 3.2) conducted at the study site. It was only Botham (2016) who recorded this family to the species level. Botham (2016) recorded *Phoberus* (*Trox) sulcatus*) being present at the carcass in very low numbers (< 5). Two Trogidae species, *Omorgus (Afromorgus) squalidus* and *Phoberus strigosus,* were collected from carcasses during the present study. Both of these species were present in low number (< 1%) of the total number of beetles at the carcasses). Adult Trogidae beetles were collected from soil samples. Braack (1986) observed them digging into the soil to avoid unfavorable conditions, pitfall traps and through active sampling.

Scholtz & Holm (2008) reported Trogidae larvae forming vertical tunnels in the soil under the carcass. No immature stages were collected from soil samples or pitfall traps during the course of the current study. Furthermore, immature stages could not be sourced through breeding since not enough adults of the species could be collected (the two Trogidae species comprised less than 1% of the adults collected during the current trial period) for the establishment of a breeding colony.

Afromorgus squalidus (Olivier, 1789)

Adult description

Diagnosis: Adults black with fine hairs; bodies mostly covered with mud (Fig. 3.36); head orientated hypognathous; body length: APW 3.8mm – 4.0mm; EL 12mm – 13.1mm; EW 9.0mm – 10mm; PEL 14.9mm – 16.2mm; PPW 6.7mm – 7.1mm; n=21.

Head: Mouthparts covered by clypeus when viewed from above; head concealed by pronotum when viewed from above; compound eyes not visible from above; compound eyes large and convex; compound eyes extending laterally on each side when head viewed from above; compound eyes not divided by clypeus into lower and upper parts; antennae lamellate; clypeus and frons fused; frons bituberculate (Fig. 3.37A); frontoclypeal suture absent; labrum covered by clypeus and not visible from above; labrum sclerotised and punctured; labrum emarginate and posterior angles of labrum acute (Fig. 3.37B); mandibles robust, with hairs on lateral sides; right mandible with molar area (Fig. 3.37C); stipes and galea with hairs; lacinia armed with four teeth and fine hairs; maxillary palp with three palpomeres; basal and apical palpomeres bigger than preapical palpomere (Fig. 3.37D); articulation of maxillary palpomeres not visible from below.

Thorax: Pronotum black and brown in colouration; pronotal disc with ridges; anterior angles of pronotum acute and not forming solid curve with pronotal carinae (Fig. 3.36); pronotum's lateral carinae separate pronotal disc and hypomeron; pronotum lateral carinae with depressions; posterior angles of pronotum obtuse; hypomeron setose and setae extending to pronotal sides and visible from above; scutellum visible between bases of elytra; elytra covers abdomen completely; elytra disc with large longitudinal impressions and small impressions in middle of larger impression; elytra apices meet

at midline; hindwing venation illustrated in Fig. 3.37F; front tibia fossorial with one apical spur; meso and metatibia with two apical spurs at apex; apical tarsi longer than preapical tarsus and metapretarsal claws simple.

Abdomen: Four abdominal sternites visible when viewed ventrally; pygidium not exposed.

Male genitals: Trilobate type; parameres elongate and curved apically; median lobe same length as parameres; basal lobe forming concave-shape at anterior site (Fig. 3.37E) (Appendix 1, Fig. L).

Remarks: The descriptions provided in current study supports the descriptions provided by Kral & Kuban (2012).

Figure 3.36: Stereomicrograph of *Afromorgus squalidus* (Olivier, 1789) adult dorsal view (Scale bar = 3mm).

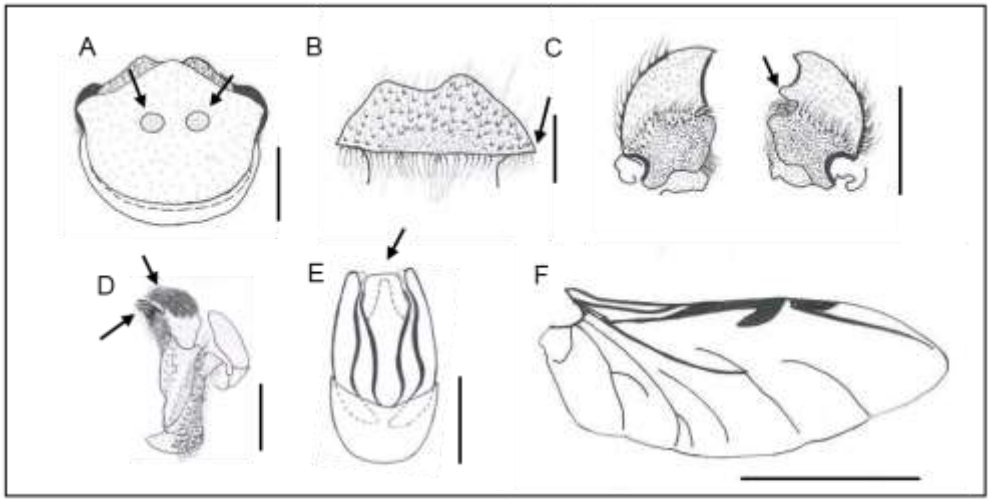

Figure 3.37: Diagrammatic drawings of *Omorgus* (*Afromorgus*) *squalidus* (Olivier, 1789) adult (A-F) dorsal view. A. Frons, arrows indicating the turbucles (scale bar = 1mm). B. Labrum, arrow indicating acute posterior angle (scale bar 0.5mm). C. Mandibles, arrow indicating tooth (scale bar = 1mm). D. Right maxilla, arrows indicating lacina teeth and fine hairs (scale bar = 1mm). E. Male aedeagus, arrow indicating medial lobe (scale bar = 2mm). F. Right hind wing (scale bar = 5mm).

Phoberus strigosus (Haaf, 1953)

Adult description

Diagnosis: Adults brown to black with head orientated hypognathous (Fig. 3.38); body length: APW 1.6mm – 1.9mm; EL 4.9mm – 5.4mm; EW 3.7mm – 4.3mm; PEL 6.2mm – 7.0mm; PPW 3.0mm – 3.4mm, n=30.

Head: Head concealed by pronotum when viewed from above; mouthparts covered by clypeus when viewed from above; compound eyes visible when viewed from above; compound eyes large and convex; compound eyes extending laterally on each side when viewed from above; compound eyes not divided by clypeus into lower and upper parts; setae on sides almost reaching clypeal apex; antennae lamellate; clypeus and frons fused (Fig. 3.39A); frontoclypeal suture absent; labrum covered by clypeus and not visible from above; labrum sclerotised and punctured; labrum round laterally and emarginate at apex; labrum surface punctured and hairy and covers mouthparts (Fig.

3.39B); mandibles robust, with hairs on lateral sides; right mandible with molar area (Fig. 3.39C); stipes and galea with hairs; lacinia armed with four teeth and fine hairs; maxillary palp with three palpomeres; basal and apical palpomeres longer than preapical palpomere (Fig. 3.39D); articulation of maxillary palpomeres not visible from below.

Thorax: Pronotum surface punctured with lateral boarders covered with setae; pronotum's lateral boarders flattened; scutellum visible and U-shaped; elytra covers abdomen completely; elytra with longitudinal serrated turbucles with setae; elytra surface with ridges; series of setae at elytra margin; hindwing venation illustrated in Fig. 3.39F; all legs clawed; tarsal segment 5-5-5; front tibia fossorial with one apical spur; meso and metatibia armed with teeth and two apical spurs.

Abdomen: Four abdominal sternites visible when viewed ventrally; pygidium not exposed.

Male genitals: Trilobate type; parameres slender and flattened, recurved apically; anterior edge of median lobe with shallow u-shaped notch, not projecting beyond parameres (Appendix 1, Fig. N).

Remarks: Specimen images were sent out to an expert for identification. No morphological comparisons were made from literature.

Figure 3.38: Stereomicrograph of *Phoberus strigosus* (Haaf, 1953) adult dorsal view (Scale bar = 3mm). .

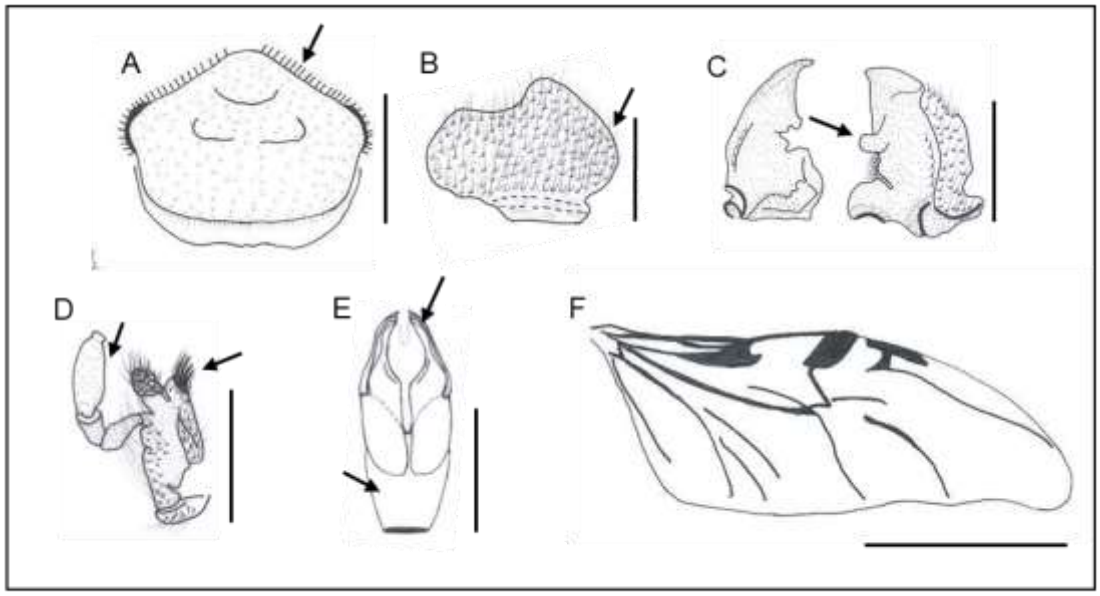

Figure 3.39: Diagrammatic drawings of *Phoberus strigosus* (Haaf, 1953) adult (A-F) dorsal view. A. Frons, arrow indicating marginal setae (scale bar 1mm). B. Labrum, arrow indicating rounded posterior angle (scale bar 0.5mm). C. Mandibles, arrow indicating tooth (scale bar 1mm). D. Left maxilla, arrows indicating apical palpomere and teeth on lacina (scale bar 1mm). E. Male aedeagus, arrows indicating curved parameres (scale bar 2mm). F. Right hind wing (scale bar 5mm).

3.7 Scarabaeidae

Scarabaeidae is a very large family consisting of small to very large beetles (Braack 1986; Braack 1987; Browne & Scholtz 1999; Midgley 2012). These beetles have different feeding habits such as fresh and decaying plant material, fungi, dung, and nectar. Carrion associated scarabs from the genera *Onthophagus* and *Scarabaeus* are coprophages. According to Scholtz *et al.* (2009) dung beetles uses dung for breeding, as a food source and as a cue to attract mates. Species representing both genera were found during wet and dry stages of the decomposition. The abundance of dung beetles can be associated with the rupture of the carcass exposing the gut contents of the carcass. Species of the genus *Onthophagus* are dung tunnellers meaning they construct a tunnel underneath the carcass/dung and this can be the reason why they are observed closer to a carcass. *Scarabaeus* species are dung-rollers as they construct a brood ball or dung ball and move it far from where it was constructed (Harrison & Philips 2003; Scholtz & Holm 2008).

Villet (2011) indicated that certain species of the Scarabaeidae genera (i.e. *Anachalcos, Epirinus, Onthophagus, Phaeochrous and Sarophorus*) are associated with carrion. During the current study specimens of three of that genera were recorded. Members of the subfamily Cetoniinae were recorded from the study site by Boucher (1997) and Kolver (2003). However, this species was not recorded by other researcher working at the same site in previous years (Table 3.2) and only one specimen belonging to the predatory chafer tribe (Cremastocheilini: *Scaptobius* sp.) was found on the carcass during the current trial period. Relatively high numbers of individuals of *Onthophagus* sp. (7%) and *Scarabaeus* sp. (6%) were found associated with the carcasses during the current trial period. *Onthophagus* species were observed under/around the carcasses before the rupture of the carcass torso. This genus was also recorded by Boucher (1997) at the study site. The species of *Scarabaeus* were observed rolling balls of soil combined with decomposition fluids away from the carcass. Sheep carcasses supported a high number of scarabs compared to the pigs and the baboon. This is can be explained by the fact that the sheep gut content was composed of mainly grass.

No immature stages of this beetle family were collected from pitfall traps or soil samples. The immatures are not considered as forensically important because immature stages would likely not be found closely associated with carrion, therefore no attempt was made to source immature stages for our description through a breeding program.

Dung beetles were only identified to family or genus level by the researchers working at the study site during previous years (Table 3.2). This might be because that although comprehensive keys are available for genera, there are no consolidated resource available for species identification (Villet, 2011).

Onthophagus sp. Latreille, 1802

Adult description

Diagnosis: Adults black to brown in colouration and body completely punctured and covered with fine hairs (Fig. 3.40); body length: APW 1.1mm – 2.5mm; EL 1.9mm – 3.0mm; EW 2.1 – 4.6mm; PEL 3.0mm – 6.1mm; PPW 2.0mm – 4.0mm; n=30.

Head: Head not completely concealed by pronotum when viewed from above; mouthparts concealed by large clypeus; compound eyes visible from above; compound eyes not divided by clypeus into lower and upper parts; antennae lamellate; labrum covered by clypeus and not visible from above; clypeus emerginate; clypeus and frons fused and both punctate; frontoclypeal suture absent; males with horn on clypeus; horns can be very short or very long in some male specimen (Fig. 3.40A); females without horn on clypeus (Fig. 3.40B) labrum slightly emarginate and completely membranous with hairs at labral apex; mandibles membranous and not visible from above; mandibular apex broadly rounded with fine hairs at margin and not divided into lobes or teeth; lacinia without teeth but with fine hairs; maxillary palp with four maxillary palpomeres; apical maxillary palpomere longer than first three palpomeres; labium with four labial palpomeres; apical palpomeres shorter and smaller than preapical palpomere; labial articulation of labial palps visible from below.

Thorax: Pronotum completely black in colouration with dense setose punctation; anterior angles of pronotum acute, not forming solid curve with pronotal carinae; pronotum lateral carinae separates pronotal disc and hypomeron; pronotum's lateral carinae not smooth; posterior angles of pronotum rounded; hypomeron setose and setae visible from above; scutellum not visible from above; scutellum covered by elytra; anterior edges of scutellum elevated; posteriorly scutellum acute; elytra covers abdomen and only exposing pygidium; punctation on elytra same as pronotal punctation and elytral punctures with series of hairs; elytral hairs aligned longitudinally; elytra apices meet at midline; tarsal segment 5-5-5 in both males and females; protibia with four large denticles or teeth; one apical protibial spur present; mesotibia and metatibia with two spurs and five tarsi segments; basal and apical tarsi segments longer than tarsus 2 – 4.

Abdomen: Four sternites visible from below; pygidium punctate and exposed.

Male genitals: Paramere smaller than tegmen; endophallus forming semicircular shape with paramere; parameres pointy at apex (Appendix 1, Fig. O).

Remarks: Sexual dimorphism can be seen in this species where by the presence and the absence of the horn on the clypeus is used to distinguish between male and females. Another characteristic used to distinguish is dense pronotal punctation in females and less pronotal punctation in males. Specimen were sent out to an expert and were only identified to genus level.

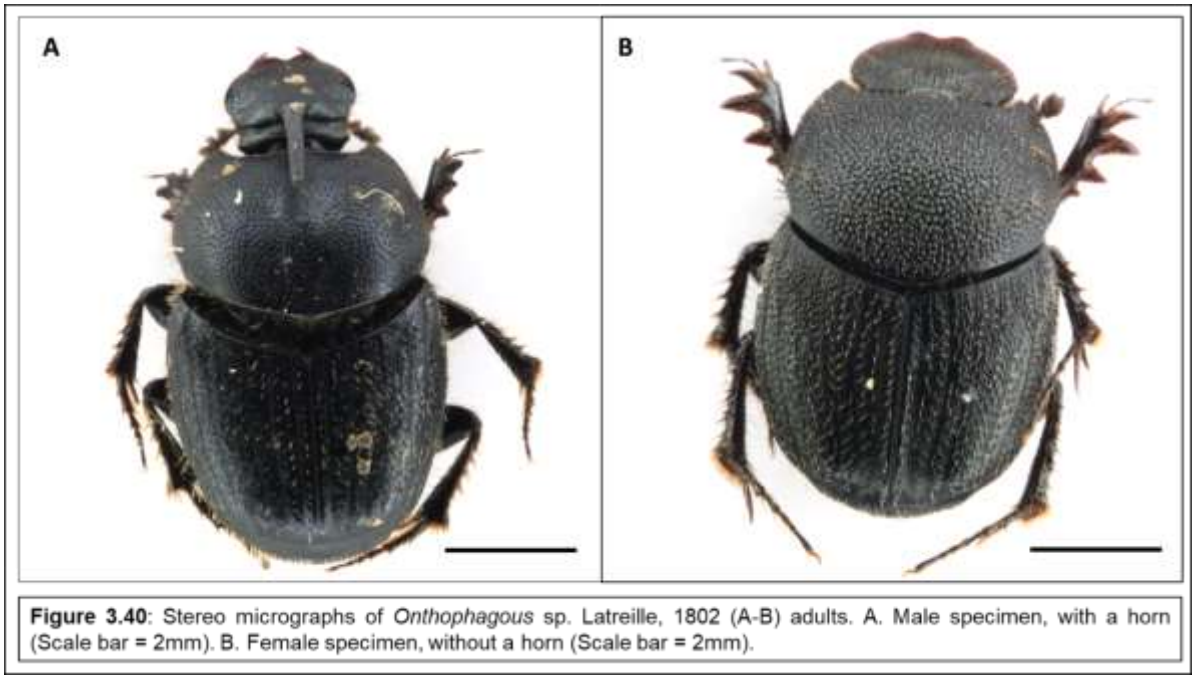

Figure 3.40: Stereo micrographs of *Onthophagous* sp. Latreille, 1802 (A-B) adults. A. Male specimen, with a horn (Scale bar = 2mm). B. Female specimen, without a horn (Scale bar = 2mm).

Scarabaeus sp. Linnaeus, 1758

Adult description

Diagnosis: Adults black and body completely punctured; head orientated hypognathous (Fig. 3.41); body length: APW 2.1mm – 3.0mm; EL 6.7mm – 7.1mm; EW 6.1 – 7.9mm; PEL 9.9mm – 10.6mm; PPW 6.9mm – 7.5mm; n=30.

Head: Head not completely concealed by pronotum when viewed from above (Fig. 3.41); mouthparts concealed by large clypeus; compound eyes visible from above; compound eyes divided by clypeus into lower and upper parts; antennae lamellate; labrum covered by clypeus and not visible from above; clypeus with six teeth; clypeus and frons fused and both punctate; frontoclypeal suture absent; labrum emarginate and completely membranous with hairs at labral apex; mandibles membranous and not visible when head viewed from above; mandibular apex broadly rounded with fine hairs at margin and not divided into lobes or teeth; lacinia without teeth but with fine hairs; maxillary palp with four maxillary palpomeres; apical maxillary palpomere longer than first three palpomeres; labium with three labial palpomeres; labial articulation of labial palps not visible from below.

Thorax: Pronotum completely black in colouration with scattered dense setose punctation; anterior angles of pronotum rounded, not forming solid curve with pronotal carinae; pronotum lateral carinae separates pronotal disc and hypomeron; pronotum lateral carinae not smooth but finely denticulate; posterior angles of pronotum rounded; hypomeron setose and setae can visible from above; scutellum not visible; elytra covers abdomen, only exposing pygidium; elytra disc coarsely punctate with punctation scattered forming elytral ridges; elytral punctation with hairs; elytra apices meet at midline; proleg without tarsi; protibia with four large denticles or teeth; protibia with series of small denticles on anterior and posterior side; one apical protibial spur present; mesotibia and metatibia with one spur and five tarsi segments; basal and apical tarsi segments longer than tarsus 2 – 4; mesopretarsal and metapretarsal claws simple.

Abdomen: Four sternites visible from below; pygidium punctate and exposed.

Male genitals: Basal piece two pieced; paramere and tegmen almost same length; paramere formes V-shape; paramere wide at posterior tegmen (Appendix 1, Fig. M).

Remark: Members of this species showed a reduced proleg tarsus while the tarsus was only observed in the meso and metalegs. Micrographs were sent out to an expert and were only identified to genus level.

Figure 3.41: Stereomicrograph of *Scarabeaus* sp. Linnaeus, 1758 dorsal view (Scale bar = 3mm).

Scaptobius sp. Schaum, 1841

Adult description

Diagnosis: Adults black and body covered with fine brown hairs; head orientated hypognathous (Fig. 3.42); body length; EL 7.1mm; EW 6.9mm; PEL 13.6mm; PPW 5.0mm; n=1.

Head: Mouthparts concealed by large clypeus; head not completely concealed by pronotum when viewed from above (Fig. 3.42); compound eyes visible from above; compound eyes not divided by clypeus into lower and upper parts; antennae lamellate; labrum covered by clypeus and not visible from above; clypeus emarginate; clypeus and frons fused and both punctate; frontoclypeal suture absent; labrum emarginate and completely membranous with hairs at labral apex; mandibles slightly sclerotised and not visible from above; mandibular apex curved and truncated; lacinia with teeth and fine hairs; maxillary palp with three palpomeres; apical and basal maxillary palpomere longer than preapical palpomeres.

Thorax: Pronotum completely black in colouration with fine setae and punctate (Fig. 42); anterior angles of pronotum rounded and form solid curve with pronotal carinae; anterior angles of pronotum rounded; pronotum lateral carinae separates pronotal disc and hypomeron; pronotum lateral carinae smooth; posterior angles of pronotum rounded; hypomeron setose; scutellum visible from bases of elytra; elytra covers abdomen completely; elytra disc with longitudinal depressions that forms elytral ridges; elytral with fine hairs; elytra apices meet at midline; proleg with tarsi; protibia with two large denticles or teeth; two apical protibial spurs present; mesotibia and metatibia with two apical spurs and five tarsi segments; tarsi segments almost same size; all pretarsal claws simple.

Abdomen: Four sternites visible from below; pygidium not exposed from above (Fig. 3.42).

Male genitals: Only one female specimen collected.

Remarks: Specimen micrograph was sent out to expert and were only identified to genus level. One specimen was found during the current study. The preliminary descriptions based on single female specimen.

Figure 3.42: Stereomicrograph of *Scaptobius* sp. Schaum, 1841 dorsal view (Scale bar = 3mm)

3.8 Nitidulidae

Nitidulidae is a very large family with more than 2000 species described (Byrd & Castner 2009; Ortloff *et al.* 2014). They have a variety of feeding guilds and this includes; mycophagy, saprophagy, phytophagy, and necrophagy. They have been recorded in both animals and human carcasses. Three most important genera in forensic investigations are *Nitidula, Omosita,* and *Carpophilus* (Ortloff *et al.* 2014; Keshavarzi *et al.* 2015). Byrd & Castner (2009) mentioned that the value of members of the family Nitidulidae that are associated with decomposing carcasses is unknown. However, in the past few years, species from this family have been used successfully in PMI estimations (Ortloff *et al.* 2014; Keshavarzi *et al.* 2015). Nitidulidae species are associated with advanced stages of decomposition. Ortloff *et al.* (2014) stated that Nitidulidae species are mostly found co-occurring with the species from the genus *Dermestes* in carrion ecosystem.

One species of this family has been recorded (Table 3.2) by Boucher (1997) but the particular species recorded by Boucher (1997) was not recorded in this study. However, in the current study, only one specimen of *Carpophilus obsoletus* was found. A description of this one adult specimen is provided.

Carpophilus obsoletus Erichson, 1843

Adult description

Diagnosis: Adults brown and black with head orientated prognathous; body elongated; body length; EL 1.1mm; EW 1.3mm; PEL 1.6mm; PPW 1.0mm; n=1.

Head: Head visible from above and not concealed by pronotum; compound eyes convex; compound eyes extending laterally on each side when viewed from dorsal side; compound eyes undivided by clypeus into lower and upper parts; antenna geniculate; antennomeres visible from above and not concealed by frontal ridges (Fi. 3.4); frons black and covered with hairs; labrum visible from above and not concealed by clypeus; labrum well sclerotised and labral apex emerginate; mandibles visible from

above; mandible molar region with teeth; maxillary palp with four palpomeres; basal maxillary palpomere smaller than apical palpomere; maxillary palpomeres 2 – 4 almost same size; articulation of maxillary palpomeres not visible when head viewed ventrally; galea with fine hairs at apex; labium with two labial palpomeres; apical labial palpomere longer than basal palpomeres; articulation of labial palpomeres visible when head viewed ventrally.

Thorax: Pronotum with fine hairs and brown; anterior angles of pronotum obtuse and do not form solid rounded curve with pronotal carinae; lateral carinae separates pronotal disc and hypomeron; pronotal carinae smooth; posterior angles of pronotum obtuse; pronotal hypomeron setose; scutellum visible between elytral bases and V-shaped; elytra expose more than two complete tergites; elytral disc brown and covered with hairs; elytra truncated and meet at midline when wings at rest; forelegs cursorial and adapted for running; mesotibia and metatibia widened; tarsal segment 5-5-5.

Abdomen: Abdomen black with hairs three abdominal tergites exposed when viewed from above; pygidium long exposed (Fig. 3.43).

Male genitals: No male specimen found.

Remarks: This is the first time this species is recorded in the area and only one female specimen was recorded and the preliminary description is based on single female specimen. The specimen is small, only reaching 4mm in length.

Figure 3.43: Stereomicrograph of *Carpophilus obsoletus* Erichson, 1843 adult dorsal view (Scale bar = 1mm)

3.9 Breeding, development and general observations

Breeding protocols of some of the beetle families of forensic importance are available in the literature. For the studies described, the breeding media that these necrophagous beetles were reared on were not muscle tissue. Catts & Haskell (1990) mentioned that when rearing insects for PMI estimations the breeding media for necrophage insects must be tissue by-products that have high protein content. They further mentioned that if the collected individuals are predatory on other insects, maggots must also be collected to served as food source. Two species of the eighteen collected from the field were successfully bred in the laboratory and all their life stages were recorded. Breeding was carried out to obtain life stages that were not found from the field and also to help with extra specimen for the descriptions.

3.9.1 Silphidae: *Thanatophilus micans*

Larvae and adults of *T. micans* collected from the study site were placed in three main breeding containers. The first container was used for general observations; adults and the larvae were kept together in this container. The second container was used for egg-laying, the adults were kept and in the last container only the larvae were kept. The third container was use for the development of larvae, only the larvae was placed in that container. Separate containers were used to prevent cannibalism.

The breeding of *T. micans* was unsuccessful under laboratory conditions, i.e. not all life stages were reached. The adults deposited their eggs in the soil; Prins (1984a) also observed this. Boucher (1997) used damp cotton wool and Midgley (2007) used damp sand as oviposition substrates, illustrating that this species has a variety of oviposition substrates. The eggs removed from the main adult colony were placed in different petri dishes with chicken liver as a food source. The eggs emerged as first instar larvae on the second day. These emerged first instar larvae fed on chicken liver provided as a food source. It took 3 to 4 days for the larvae to transition from the first to the second instar stage and 7 to 9 days from the second instar to the third stage.

No instars reared under laboratory conditions reached the pupal stage. Midgley (2007) indicated that pupation did not occur at temperatures above 25°C. This might have been the cause why the third instar larvae of the current experiment did not transition into pupae. Midgley (2007) also found high mortalities at temperatures above 25°C and between 15°C and 17°C. Prins (1984a) and Boucher (1997) mentioned that third instar larvae were dormant for 3 to 4 days before pupation. In addition to the high temperature of the current experiment another possible reason why pupae were not found might have been due to cannibalism of dormant larvae by active larvae and adults.

No pupae were found in the soil samples collected from around the carcass. Soil samples were only collected from under the carcass and from the immediate area around the carcass. Boucher (1997) observed that the third instar *T. micans* larvae migrated up to 2 m away from the carcass for pupation. Boucher (1997) further observed that newly emerged adults will climb the stems of the surrounding vegetation after pupation; this was not observed during the current field observations although there were a high number of this species during the autumn trial (March – April 2017).

During the laboratory breeding experiment, the matured larvae were observed feeding on the early instar larvae (Fig. 3.33). It was also observed that the adults will wait for mature larvae to initiate cannibalism before they joined feeding on early instar larvae.

Figure 3.44: Photograph showing two third instar larvae (indicated by a blue circle) of *Thanatophilus micans* (Fabricius, 1794) cannibalising on early instar larva. Larva of *Piophila casei* (Diptera: Piophilidae) which was provided as food source, circled in red.

3.9.2 Dermestidae: *Dermestes maculatus*

To determine the development, 10 eggs were placed in three different breeding containers, i.e. a total of 30 eggs were tracked for development. The eggs were placed with moisten tissue paper and pig muscle tissue. Styrofoam was placed in all *Dermestes maculatus* experimental breeding containers (Fig. 3.45) for the larvae to burrow into for pupation. The developmental data was recorded daily and old exoskeletons were used to monitor instar changes.

Breeding for *D. maculatus* was successful under laboratory conditions. All developmental stages were observed and recorded. Total immature development

from egg hatching to emergence of the adults was 48 to 55 days at 28±2 ºC. A total of 7 larval instars were produced during the experiment.

It was observed that adults collected from the field, which were not directly placed in the insectarium within a day were not mating when temperatures were lower than 18°C. As soon as they were transported to the insectarium where the temperature was maintained at 28±2°C they became active and started mating. This could also have been due to the insects being under stress from being removed from their natural environment to captivity or because there was no food source for the adults to initiate reproduction. Cymborowski (1987); Zakka *et al.* (2013) stated that adults of this species produce sex pheromones that initiate copulation within 30 minutes when the conditions are favourable and when enough food were available.

Dermestes maculatus adults laid their eggs on and under moist tissue paper a few hours after the food source and egg laying substrate were introduced into the breeding medium. Boucher (1997) found that this species can lay their eggs on and in the soil, Robinson (2005) only supplied dried tissue, Zakka *et al.* (2013) used different fish species, Fontenot *et al.* (2015) used 100 % polyester synthetic fur and (Martin-Vega *et al*. (2017) used pig skin as an egg laying substrate. From this it is evident that *D. maculatus* can utilise different egg laying substrates.

Eggs hatched between 24 and 36 hours after it was laid. The first instar took 1 day to moult to the second instar; second instar to the third instar took 2 days; third instar to the fourth instar took 5 to 6 days; fourth instar to the fifth instar took 8 to 10 days; fifth instar to the sixth instar took 12 to 13 days and from the sixth instar to the last instar took 13 to 15 days. Larvae burrowed into the styrofoam (Fig. 3.45) which was introduced as pupation media. Larvae were dormant before pupation occurred; the dormant stage lasted for 2 days. The pupa development took 5 days. The pupa remained whitish for the first 4 days of pupation and on their last day there was a change in the wings; the wing colour changed from whitish to black and also moved

from the ventral side to the dorsal site. The overall colour of newly emerged adults only changed a few hours after emergence to brown.

From field collected soil samples it was evident that the last instar larvae burrow into the soil and formed pupal chambers. This species unlike *T. micans* pupates closer to the carcass. During the dry stages of decomposition high numbers of larvae and pupae were collected.

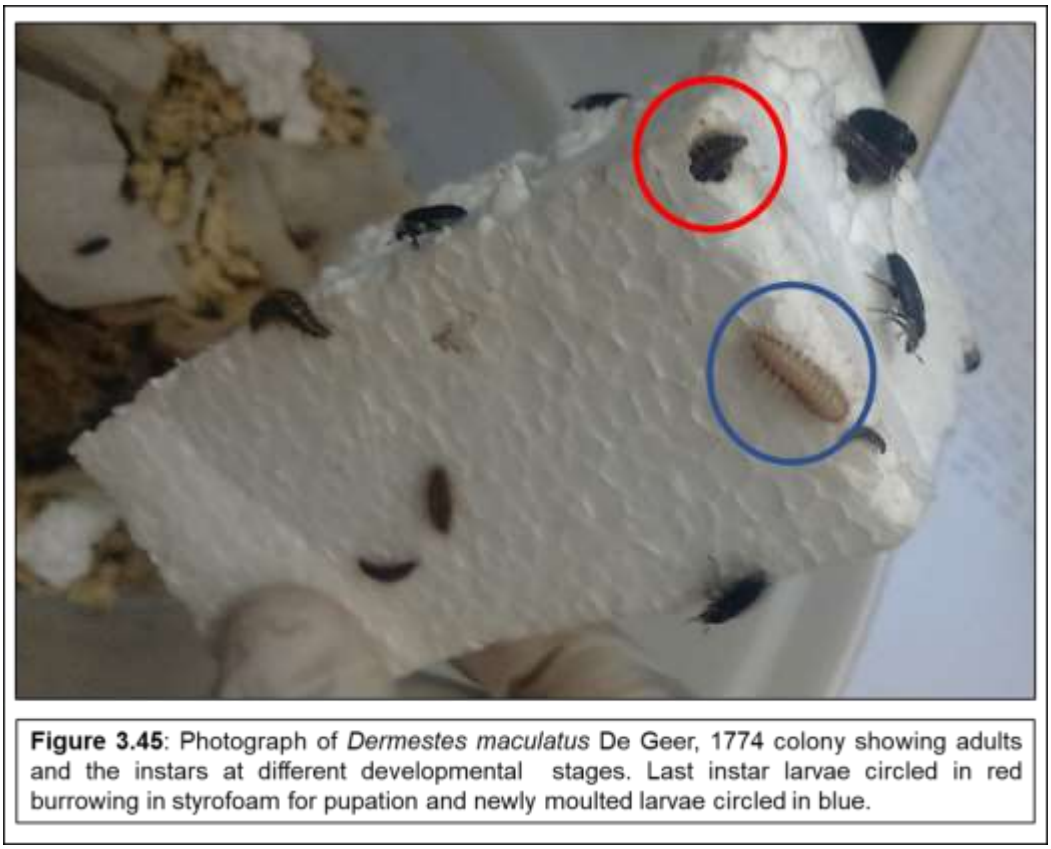

Figure 3.45: Photograph of *Dermestes maculatus* De Geer, 1774 colony showing adults and the instars at different developmental stages. Last instar larvae circled in red burrowing in styrofoam for pupation and newly moulted larvae circled in blue.

Robinson (2005) found that larval development took about 50 days and that 7–9 instars was produced at 21°C and 75% relative humidity on a diet of dried tissue; larval development took 34 days and 6–9 instars was produced at 55% relative humidity and 27°C and it took 19 days for 6 instars to be produced at 35°C. Zakka *et al.* (2013) did their experiment on four fish species as a food source at 30ºC and a relative humidity of 65±5 %; five larval instars were produced from three of the fish species and six larval instars from the other fish species. They concluded that the differences in larval

instar number might be influenced by the nutritional composition of the food source. Martin-Vega *et al*. (2017) found that at different temperatures the species has different number of instars. Their results showed that at 15ºC, 9 instars were produced; at 20ºC and 30ºC, 6 instars were produced; at 25ºC, 5 instars and at 35ºC, 8 instars were produced. It can be concluded that both temperature and food source should be considered when breeding this species.

3.9.3 Cleridae: *Necrobia rufipes*

Three main breeding containers were used to breed the immatures of *Necrobia rufipes*. The first container was used for adult colony maintenance. To maintain the colony of *Necrobia rufipes,* the adults were placed in a single breeding container along with the adults of *Dermestes maculatus*. This strategy was followed because *N. rufipes* is a facultative predator that feed on other arthropods. *D. maculatus* was chosen as a prey species because it oviposited a high number of eggs within a few hours when the food source and the temperature are favourable (pers. obsv). Some of the emerged *D. maculatus* larvae were removed each day to avoid overcrowding.

The second container was used for egg-laying. The adults were placed in a container for egg-laying and the muscle tissue were moved to a different container. The pig muscles tissue from a colony of adults (Fig. 3.46) containing the eggs was placed in a different container the following day to avoid cannibalism by adults. Cannibalism by adults and mature larvae on newly emerged larvae was also observed by Hasan & Phillips (2010).

Other containers were used for the development of the larva until they reach pupal stage then moved to different container to further their pupal development. This was done because during pilot studies on the breeding of *N. rufipes* that was performed in 2015 high mortality rates (i.e. no larvae reached the pupal stage) were experienced when the larvae were bred in colonies of less than ten larvae per breeding container.

The breeding conditions were adjusted in order to obtain all life stages required for descriptions.

During the 2015 breeding trial, pig muscle tissue was provided as food source. Eggs were deposited amongst the pig muscles fibers (seen under a dissecting microscope). High mortalities of immature stages were encountered due to cannibalism when the eggs and newly hatched larvae were left in the same colony with the adults. During the current breeding trial, both pig muscle tissue and chicken livers were introduced to the mixed colony of *N. rufipes* and *D. maculatus* for egg laying.

Figure 3.46: Photograph of unsexed adults of *Necrobia rufipes* De Geer, 1775 colony with pig muscle meat use for egg-laying.

Necrobia rufipes successfully completed its development under laboratory conditions. Total immature development from egg hatching to emergence of the adults was 34 to 43 days at 28 ±2ºC. A total of 3 larval instars were produced. The development of this species was shorter than that of *D. maculatus*. Eggs hatched between 1 to 2 days after they were laid; the first instar took 2 to 4 days to moult to the second instar; the

second instar took 10 to 13 days to moult to the third instar and the third instar took 13 to 16 days before they dug into the soil to form pupal chambers. Larvae were dormant for 2 to 3 days before pupation. Pupa development took 4 to 5 days. The adults emerged with a brown colouration; the colour changed to a shiny metallic blue-green a few hours after emergence.

There are several studies that have been done on the development of *N. rufipes*. Ashman (1963) mentioned that there are differences in the development of this species when it is bred on different food substrates. *Necrobia rufipes* develop faster when they are fed squashed larvae of beetles (37.9 days at 30ºC) and developed slower when they were fed fishmeal (42.6 days at 30ºC) (Ashman 1963). Hasan & Phillips (2010) mentioned that the development was 57.9 to 65.5 days when they were reared on dried fish, dog food and ham at 27ºC. Zannetti *et al.* (2015b) mentioned that it took 32 days from when the adults were placed together for breeding to the emergence of the young adults at 21 ±3ºC on pig trotters as food source. Boucher (1997) introduced chicken liver and mice carcasses during breeding. Initially, she did not notice any eggs but she later noticed the larvae. During the current breeding experiment, it was noted that the eggs are laid inside the food source and this was also supported by the findings of Zanetti *et al.* (2015b).

The immature stage of this species was collected from soil samples. The larvae of this species were observed co-occurring with *D. maculatus* larvae during the dry stage of decomposition. The pupa was found inside the pupal chamber in the soil (Fig. 3.47). This species pupates in close proximity to the carcass. Zannetti *et al.* (2015b) showed that they can also form pupal chamber in cotton wool and it was also observed during the current breeding experiment that they can also use styrofoam as pupal refugia.

Figure 3.47: Photograph of pupa of *Necrobia rufipes* De Geer, 1775 inside the soil pupal chamber also showing the larval exoskeleton of the last larval instar circled in red.

Other species

The immature stages of other forensically important species that were collected from the field could not complete their development in the laboratory. The larvae of *Philonthus sp.* and *Saprinus splendens* predated on each other. Larvae of these two species were subsequently separated. Despite this effort, only one dormant instar larvae of *S. splendens* that was collected from the field reached the pupal stage and none of *Philonthus sp.* larvae reached the pupal stage in the laboratory.

Chapter 4: Summary, Limitations, Recommendations, Concluding remarks, and Keys

4.1 Summary

Forensic entomology research at the UFS was initiated approximately 30 years ago; in 1993 Louw and van der Linde gave an account of the insects associated with decomposing remains in central South Africa. Throughout the years many excellent succession-based carrion ecology studies were produced by students at the UFS (Table 3.2). With hindsight, the only deficiency of these studies is that for the majority of these studies beetles were only identified to the family level. Furthermore, these succession studies did not include sampling methods such as pitfall traps and soil sampling to record the immature stages of beetles associated with carrion. This is understandable; for succession studies, disturbances should be avoided. In this study, beetles were collected through manual sampling, pitfall traps, and soil sampling. Consequently, there was a disturbance to the carcasses because it was moved every day when pitfall traps were emptied and also during soil sample collections from underneath the carcass. The aim of the study was to collect a representative sample of beetle species of forensic importance at the study site and to describe these beetles using morphological characteristics. Factors such as carcass disturbance were not considered in this study. However, by not sampling by means of pitfall traps or soil samples many beetle species of forensic importance will be missed.

4.2 Limitations of this study

All beetle families previously recorded at the study site were also logged during the current study period. However, not all species recorded during previous studies were collected during the current trial period. A deficiency of the current study was that it did not include a winter trial and some of the species collected in winter trials during previous studies were not collected in this study. Furthermore, no reference collection

samples from previous studies were kept, therefore an identification, specifically a species identification, could not be adressed.

When we look at both the adults and immature stages of the beetles that were found in the field, we found that the situations regarding the immature stages was worse off than for the adult stages. In some instances, it was expected that the immature stages of some beetle species would be found, but it was not found and could not be described. It is possible that the sampling sites around the body was not sufficient. To circumvent the sampling deficiencies, breeding and rearing was attempted but this was only successful for a few beetle species or only successful for certain immature stages.

4.3 Recommendations for future studies

Future studies for both succession and developmental studies on beetles of forensic importance should consider to expand sampling for all seasons and conditions. They should also consider to increase the number of soil sample collections and pitfall traps to collect all beetles of forensic importance in central Free State.

Breeding protocols need to be established taking into account suitable feeding media and refugia. The elimination of physical stressors as well as figuring out which species should be reared individually and why others should be reared as colonies.

Furthermore, although some of the species collected during the course of this study were only identified to genus level the morphological characteristics provided in this study can possibly be used to identify them to species level in the future. In order to keep up with developments in the field of forensic entomology, future succession studies to be conducted at the UFS should rectify the situation regarding species identification and the collection of beetles.

4.4 Conclusion

Diptera (true flies) and Coleoptera (beetles) are the two most important orders of insects that are used in forensic investigation; generally, flies are used during early stages of decomposition and beetles are used in later stages of decomposition. Before any of these insects can be used in forensic investigations they must be identified. They can be identified either by using molecular identification, descriptive taxonomy or both in some cases. Before molecular identification of arthropods was instigated the only method that was used to identify species was descriptive taxonomy. Descriptive taxonomy is the use of morphological characteristics to identify specimens to a specific level of classification.

The disadvantage of using descriptive taxonomy is that in some cases difficulty is experienced when working with sister species, cryptic species or species group. In these instances, molecular identification is necessary. However, identifying species by morphological means are still of practical use. It is important to simplify morphological identification to enable people who do not have a solid competency in insect identification to identify beetle specimens found at a crime scene.

The information on morphological characteristics for most of the adult beetle species and some of the immature stages found in central Free State is available scattered in the literature. The focus of this study was to organize descriptions of the forensically important beetle species found in the central Free State region in keys. The results provided in this study can not only be used for medicolegal forensic entomology, but also for other forensic entomology sub-disciplines (i.e. stored product forensic entomology) when these beetles are found.

4.5 Key to Families of forensic importance in central Free State

1. Elytra is exposing more than two abdominal segments ……..…... **(2)**

1’. Elytra exposing two abdominal segments or covering the abdomen completely ..... **(5)**

2. Elytra exposing six complete abdominal segments .....…. **Staphylinidae**

2’. Elytra exposing less than six abdominal segments …..……..…… **(3)**

3. Antennae clavate ……..……...……...……... **Silphidae**

3’. Antenna geniculate……..……..…… **(4)**

4. Elytra with dorsal striae……...…….. **Histeridae**

4’. Elytra plain without striae…… **Nitidulidae**

5. Clypeus covering mouthparts from above; protibia fossorial ……..... **(6)**

5’. Mouthparts visible from above; protibia cursorial …… **(7)**

6. Head conceal by pronotum from above ……..……. **Trogidae**

6’. Head visible from above and not concealed by pronotum ..... **Scarabaeidae**

7. Posterior angles of pronotum acute ……..... **Dermestidae**

7’. Posterior angles of pronotum rounded ……..…… **Cleridae**

4.6 Key to species of Silphidae

1. Body flattened; antennal socket visible from the above; compound eyes large and convex; compound eyes are extending laterally on each side when viewed from the dorsal side; scutellum is visible between elytral bases and is large; scutellum is anteriorly plain and posteriorly acute; pronotum with fine hairs and has a greenish to black colouration…… ….. ……...***Thanatophilus micans*** **(Fabricius, 1794)**

4.7 Key to species of Staphylinidae

1. Head and pronotum metallic shiny and punctate ……..……... **(2)**

1'. Head and pronotum covered with hairs ……..…… **(3)**

2. Body completely black …..……. ***Philonthus longicornis*** **Stephens, 1832**

2'. Elytra apices brown and basal black; legs brown and exposed abdominal tergites black and brown……..... ***Philonthus caffer*** **Boheman, 1848**

3. Antennal sockets before compound eyes; head, pronotum and elytra brown; legs black and brown; abdomen black with white marking on lateral sides ……. ***Platydracus hottentotus*** **(Nordman, 1837)**

3'. Antennal sockets between compound eyes; pronotum with fine hairs and brownish to black colouration …… ***Aleochara sp.*** **Stephens, 1832**

4.8 Key to species of Histeridae

1. Dorsal striae on elytra disc completes more than half or 80% of elytra; apical elytral stria absent ……. **(2)**

1'. Dorsal striae complete only half or 50% of elytra disc from base to apex; elytra and pronotum punctured ……..... **(3)**

2. Compound eye flat; clypeus truncated at apex; pronotum with pronotal stria and pronotal stria reaches posterior angles of pronotum; depressions on each side of pronotum; elytra with five dorsal striae; stria 1 – 4 almost completing elytra disc and stria 5 shorter than stria 1 – 4; inner sub-humeral stria present; sutural striae present and shorter than stria 5 ……
…….. ***Pachylister heros*** **(Erichson, 1843)**

2'. Compound eyes are flat; frontal disc and the clypeus are impunctate Pronotum is impunctate; pronotal stria present and reach the base of the pronotum; dorsal stria five shorter than dorsal striae 1-4 and the sutural stria……
…… ***Hister nomas*** **Erichson, 1834**

3. Compound eyes convex; clypeus truncated at the apex; labrum is emerginate and two setae visible dorsally; elytra black and orange or occasionally black and red; sutural stria present and punctation on the elytra does reach the sutural stria; apical elytral stria present……..……
…….…… ***Saprinus cruciatus flavipennis*** **Péringuey, 1888**

3'. Elytra metallic blue or copper black…….……. **(4)**

4. Elytra metallic blue; compound eyes convex; elytra with four dorsal and one outer-humeral striae; sutural stria present and punctation on elytra does not reach sutural stria……..…… ***Saprinus (Saprinus) splendens*** **(Paykull, 1811)**

4'. Elytra copper black; small beetle 3 to 5mm; compound eyes convex; occiput densely punctate same as frontal disc and clypeus; elytra with four dorsal and one outer-humeral striae; sutural stria present……..……
…… ***Saprinus (Saprinus) cupreus*** **Erichson, 1834**

4.9 Key to species of Dermestidae

1. Pronotum lateral boarders are have white scales; elytral humerus is yellow and white……. ***Dermestes maculatus*** **De Geer, 1774**

4.10 Key to species of Cleridae

1. Compound eyes extending laterally on each side when viewed from above; posterior angles pronotum rounded and both pronotum and elytra metallic green in colour; legs red…… ***Necrobia rufipes*** **(De Geer, 1775)**

4.11 Key to species of Trogidae

1. Elytra and pronotum longitudinal with tubercles……..…. ……. ***Phoberus strigosus*** **(Haaf, 1953)**

1'. Elytra with longitudinal ridges; clypeus and frons fused; frons bituberculate ……..…… ***Afromorgus squalidus*** **(Olivier, 1789)**

4.12 Keys to species of Scarabaeidae

1. Body metallic black; eyes separated by clypeus into lower and upper parts; clypeus with six teeth…… ***Scarabaeus*** **sp. Linnaeus, 1758**

1' Clypeus is emerginate……. **(2)**

2. Elytra with longitudinal ridges…….. ***Scaptobius*** **sp. Schaum, 1841**

2'. Elytra with longitudinal series of setae; males with horn on clypeus and females without horns……..… ***Onthophagus*** **sp. Latreille, 1802**

4.13 Key to species of Nitidulidae

1. Elytra and pronotum truncated; elytra expose three complete tergites; small specimen of about 4-5mm ……. ***Carpophilus obsoletus*** **Erichson, 1843**

4.14 Keys to larval instars found in central Free State

1. Urogomphus present on the second last abdominal segment ..……..…… **(2)**

1'. Urogomphus not present on the second last abdominal segment; mall hairy larvae; cephalic width 0.4 to 0.5mm; head with two tubercles;(early instar larvae) ……..…..…… ***Dermestes maculatus*** **De Geer, 1774**

2. Head orientated hypognathous to the body when viewed from the dorsal-ventral side……..……..…. **(4)**
2'. Head orientated prognathous to the body when viewed from the dorsal-ventral side……..... **(5)**

3. Urogomphus with three segments; body sclerotised not soft (Fig. 3.4.); mesothoracic spiracle with two spiracular setae; mandible with two ventral mandibular setae and one apical seta ……... ***Thanatophilus micans*** **(Fabricius, 1794)**
3'. Urogomphi single sclerotized spine; body hairy (if present retrose turbucle with one setae); broad median longitudinal line on tergites; frons with two distinct tubercles……..……..… ***Dermestes maculatus*** **De Geer, 1774**

4. Mandibles slender with no tooth; retinaculum present; head flattened…….
…….. ……..……. ***Philonthus*** **sp. Stephens, 1829**
4'. Mandibles thick with tooth…… **(5)**

5. Mandibles with penicillus; maxillary palpomeres with one seta each ; body soft urogomphi with two segments …….
…….....…… ***Saprinus (Saprinus) splendens*** **(Paykull, 1811)**

5'. Mandibles without penicillus; labrum with eight setae arranged 2-3-3 form; urogomphi single sclerotised spine and orientated towards anterior end of body ..…….. ***Necrobia rufipes*** **De Geer, 1774**

4.15 Identification using male genitalia

The male genitalia of beetles are heavily sclerotised and are species specific. In the cases that bodies have been complete decomposed and the only insect fragments such as the abdomen are found, male genitalia can be dissected and prepared to make species identification. Some species are known to appear during the certain season (cold or warm). Knowing seasonal appearance of a specific species combine with the knowledge of stage of decomposition the species appear at can give clue on when (seasonally) the person died.

The method of using insects to estimate the season which the insect has colonized the body has been recently conducted on fly puparia. Sharma *et al.* (2015) wrote a review on how the hydrocarbons found in pupal cases can be used to estimate minimum post mortem interval. It is suggested that the combination of such technique as well as the correct identification of species can give a clear understanding of the carrion community played a role in decomposition and post mortem interval range when the body is recovered and there is no insect activity and only insect fragments are found.

The male genitalia terminologies used in this study follows the ones used by Matuda (1976); D'Hotman & Scholtz (1990) and Ozdemir & Sert (2008).

Aedeagus is the median lobe and the tegmen. Synonyms: oedeagus, phallus.

Tegmen is the basal lobe connected to the parameres.

Basal lobe is the unpaired region of the aedeagus which is connected to the genital segment by membranes and muscles. Synonyms: basal piece, tambour, phallobase, gonocoxite.

Parameres are posterior extensions of tegmen and can be fused to single lobe. Synonyms: lateral lobe, valves, gonostyli.

Paramere extensions are ventral or dorsal prolongation of paramere which can be either fused to joined to the paramere.

Median lobe is the sclerotized or membraneous central part of the aedeagus which is connected to the basal piece. Synonyms: penis, aedeagus, oedeagus, phallosome.

Internal sac is a membranous structure within the median lobe and basal piece which forms part of ejaculation system during copulation. Synonyms: endophallus, endophallic chamber.

According to Matsuda (1976); D'Hotman & Scholtz (1990) and Ozdemir & Sert (2008) there are four types of structures in which beetles can be classified into; articulate, annulate, trilobate, and veginate. Articulate type true articulate condyle connects the lateral parameres to the penis. Annulate type the paramere are reduced and fused to the basal piece and they are connected to the penis. Trilobate type; parameres are connected to the sclerotised basal piece and the penis is flanked by parameres on each side. Veginate type; tegmen is responsible for copulation because of the short penis and parameres elongate the basal piece; two lateral paramere forms a tube and the penis slides in it.

4.16 Keys the male genitals of the species found in central Free State

1. Aedeagus veginate; tegmen triangular shaped at anterior; phallus almost two times as long as tegmen; ventral phallobases are slender and longer than phallus (Appendix 1A) ……..……..…….…..... **Cleridae** (*Necrobia rufipes*)

1'. Aedeagus is not veginate but aedeagus is articulate, annulate or trilobate ……. **(2)**

2. Aedeagus is articulate ……..…… **Staphylinidae (3)**

2'. Aedeagus is not articulate but annulate or trilobate ……..……... **(5)**

3. One paramere present; ventrally the paramere is skewed to the right; paramere with peg setae at the apex (Appendix 1D) ……..……. *Philonthus longicornis*

3'. Two parameres present at each side……..…… **(4)**

4. Parameres forming a spade-like shape; medial lobe broad; basal lobe heavily sclerotised; parameres extent below the basal lobe; two apical setae and a series of peg setae on each parameres (appendix 1B) ……..……..... *Philonthus caffer*

4'. Parameres crossing at the apex; medial lobe curved and shorter than the parameres (Appendix 1C) ……..…..……...…… *Aleochara sp.* **(4)**

5. Aedeagus annulate……..... **Histeridae (6)**

5'. Aedeagus trilobate…..… **Silphidae, Dermestidae, Trogidae, Scarabaeidae (10)**

6. Tegmen medial is straight and heavily sclerotised; tegmen hollow; apex is slightly curved laterally; apex emerginate and has bumps (Appendix 1G) ……
…….……..……..……... *Pachylister heros*

6'. Tegmen medial is curved ……..……..……...…... **(7)**

7. Tegmen slightly curved at the medial; tegmen thick towards the apex; medial lobe almost reaching the apex (Appendix 1E) ……..……..… *Hister nomas*

7'. Tegmen strongly curved forming a C-shape……. **(8)**

8. Curving at the apex decline less than 45-degrees angle; basal lobe hollow (Appendix 1H) ……..…… *Saprinus flavipennis*

8'. Curving at the apex incline at least 45-degree angle ……..…… **(9)**

9. Apex retuse; basal piece one pieced; basal lobe hollow; slightly sclerotised medial; median lobe does not reach the apex; tegmen slightly curved through medio-distal and angled apical narrow and pointed; tegmen slightly broad medial (Appendix 1F) ……..…….… *Saprinus splendens*

9'. Apex truncated; basal lobe hollow; tegmen is curved through medio-distal and slightly pointed (Appendix 1I)……..…..…… *Saprinus cupreus*

10. Parameres are connected to the tegmen by a membrane…… ……... **Scarabaeidae (11)**

10'. Parameres are connected directly to basal lobe or the tegmen……..…..… **(12)**

11. Parameres almost the same size as the tegmen; endophallus forming V-shape; paramere curved inward at the apex (Appendix 1M) ……..……. *Scarabaeus sp.*

11'. Parameres shorter than the tegmen; ventrally parameres are curved and pointy at the apex (Appendix 1O) ……..……..…. *Onthophagus sp.*

12. Parameres are attached to the medial lobe on the lateral sides; parameres do not extend in to the basal lobe or the tegmen…….…….... **(13)**

12'. Paramere extend into the basal lobe or tegmen……..…... **Trogidae (14)**

13. Medial lobe slender; parameres almost same length as the medial lobe; paramere with hairs at the apex (Appendix 1J) .....…….... ……..…… **Dermestidae** (*Dermestes maculatus*)

13'. Paramere curved towards the medial lobe; medial lobe broad; inner sac present at the base (Appendix 1K) …….…… **Silphidae** (*Thanatophilus micans*)

14. Parameres elongate and curved apically; median lobe same length as the parameres; basal lobe forming a concave shape at the anterior site (Appendix 1L) ..……*Afromorgus squalidus*

14’. Parameres slender flattened and recurved apically; anterior edge of median lobe with shallow u-shaped notch, not projecting beyond parameres (Appendix 1N) ……..…… *Phoberus strigosus*

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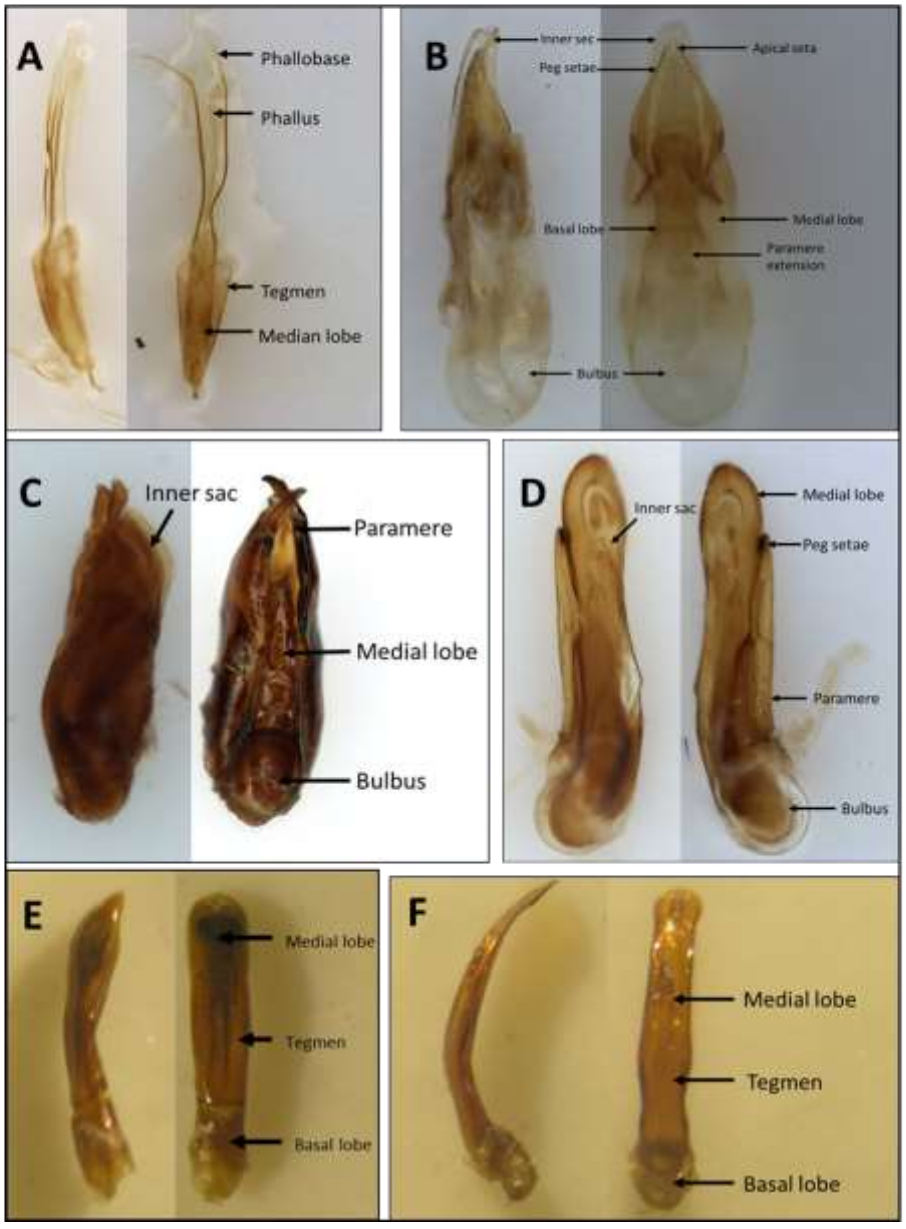
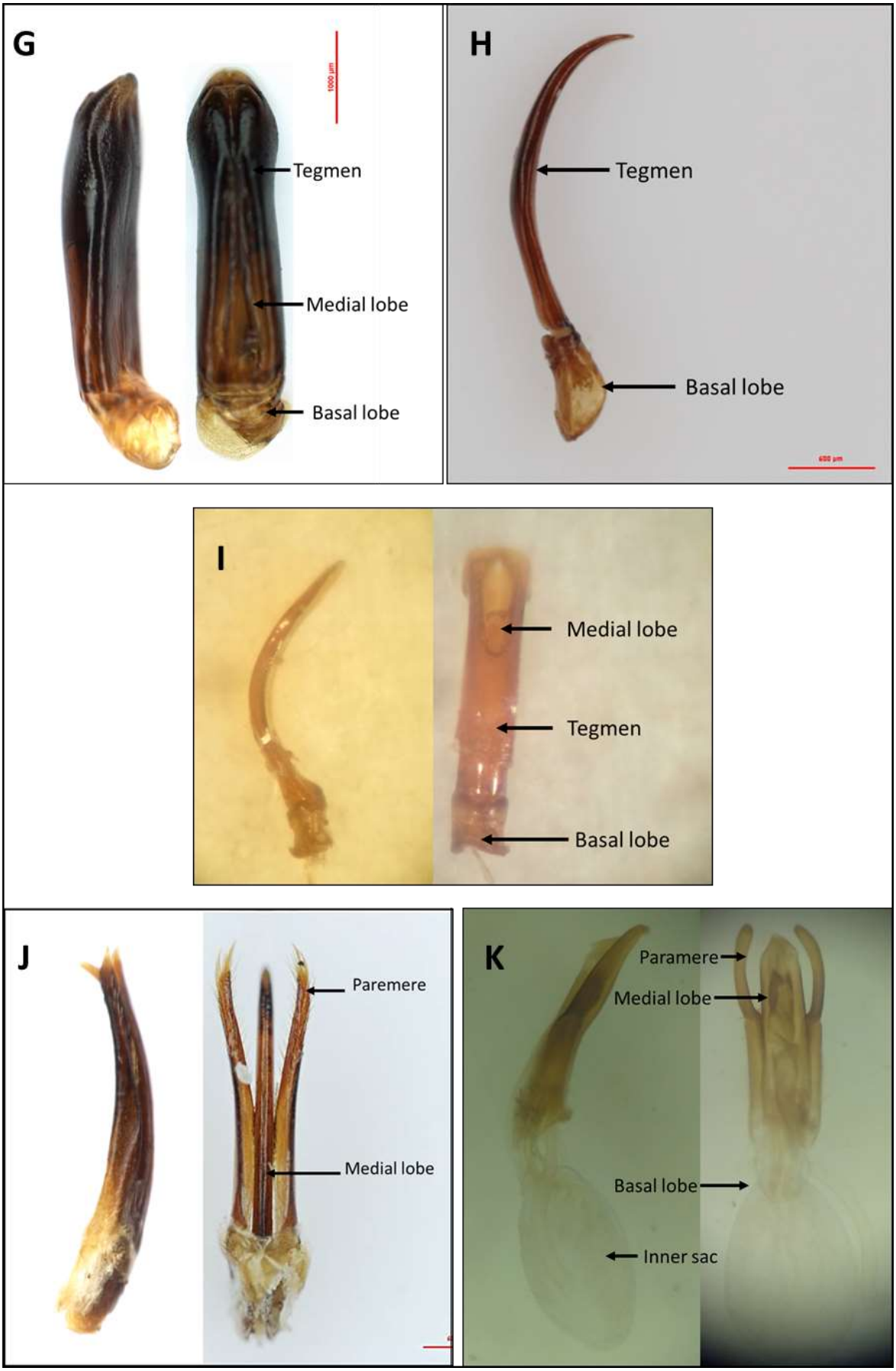
Appendices

Appendix 1: Male aedeagus of the beetle species found in central Free State

Appendix 1: Dorsal and Ventral view of the males aedeagus found in central Free State A-O. A. *Necrobia rufipes*. B. *Philonthus caffer*. C. *Aleochara sp*. D. *Philonthus longicornis*. E. *Hister nomas*. F. *Saprinus splendens*. G. *Patchylister heros*. H. *Saprinus flavipennis* (ventral only). I. *Saprinus cupreus*. J. *Dermestes maculatus*. K. *Thanatophilus micans*. L. *Afromorgus squalidus*. M. *Scarabaeus sp*. N. *Phoberus strigosus*. O. *Onthophagus sp*.




G
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Medial lobe
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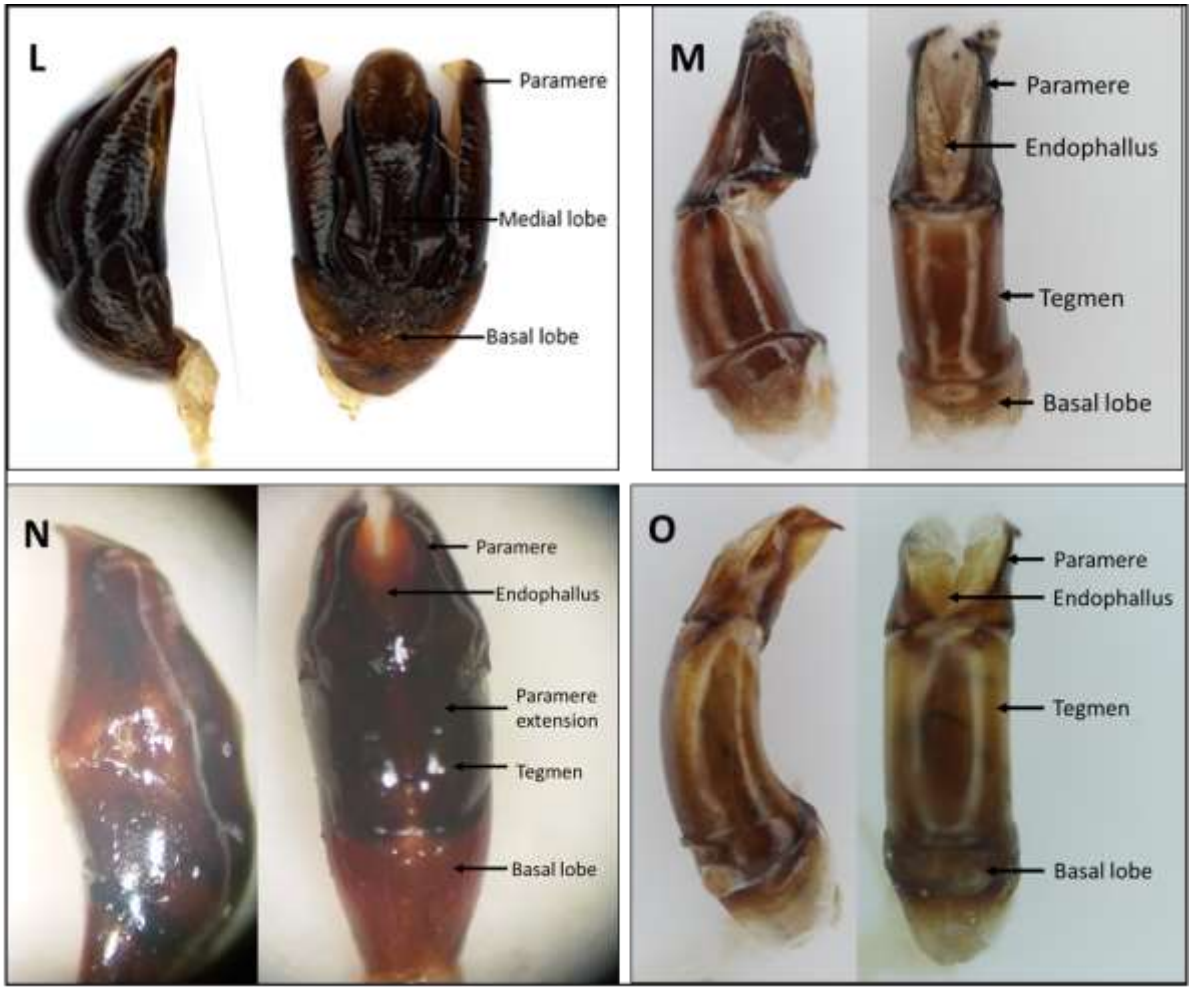
Medial lobe
Basal lobe
Inner sac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Endophallus
Paramere extension
Tegmen
Basal lobe
O
Paramere
Endophallus
Tegmen
Basal lobe

Appendix 2: Weather data

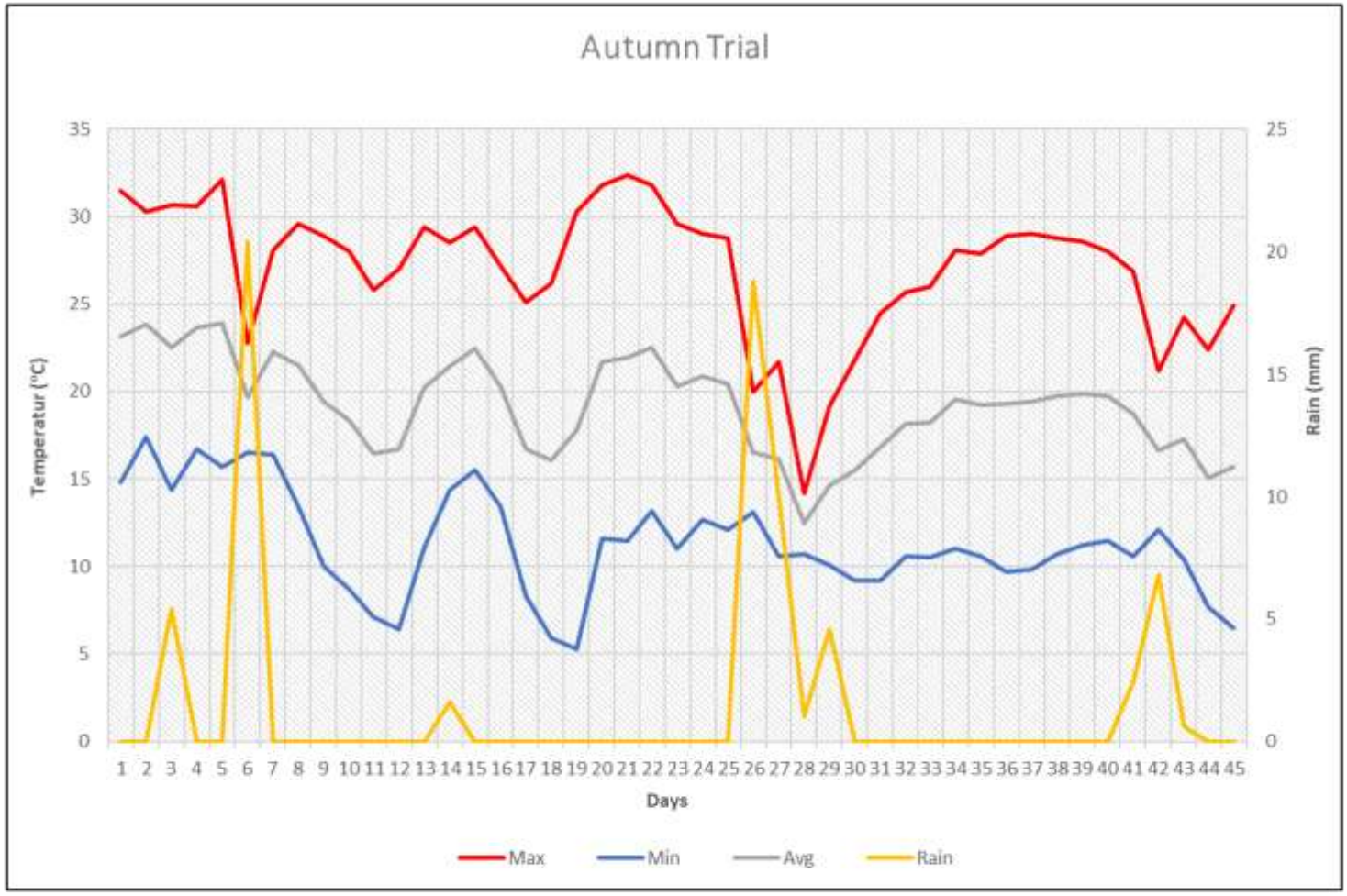
The trials were conducted for different number of days with autumn trial was ran for 45 days, summer for 39 days and spring for 38 days. The maximum temperature range for autumn 14.2 ˚C (day 28) and 32.4 ˚C (day 21) (Appendix 2A). The minimum temperatures ranged from 5.3 ˚C (day 19) and 17.4 ˚C (day 2). Rain was 20.4 mm (day 6) and this was the highest rainfall for autumn trial.

Summer trial maximum temperatures ranged from 19.1 ˚C (day 16) and 31.6 ˚C (day 32). The minimum summer temperatures ranged from 9.1 ˚C (day 35). Summer trial showed several peaks of rain with day 37 having the highest rain of 38.8 mm and day 2 showed the lowest rain of 3.2 mm (Appendix 2B).

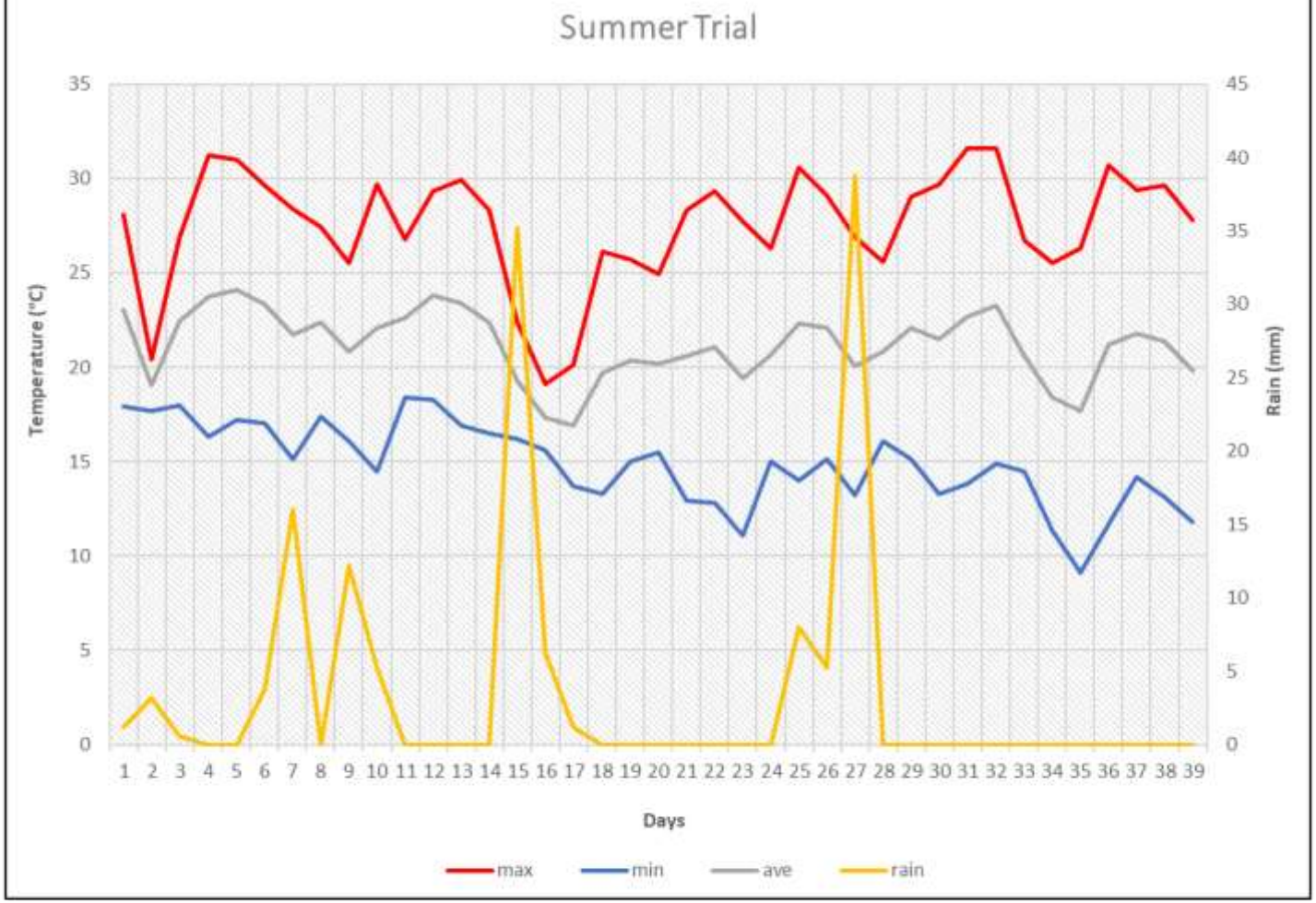
Spring maximum temperature ranged from 17.8 ˚C (day 20) and 32 ˚C (day 34). The minimum spring temperatures ranged from -2.1 ˚C (day 7) and 15.7 ˚C (day 33). Spring has few rain peaks and the highest rain was 27.8 on day 15 (Appendix 2C).

Winter trial was not conducted for this study. It was mentioned by Boucher (1997); Kolver (2003); Kelly (2006); Kolver (2009); Hoffman (2014); Botham (2016) that the minimum temperatures during winter studies were below zero degrees Celsius.

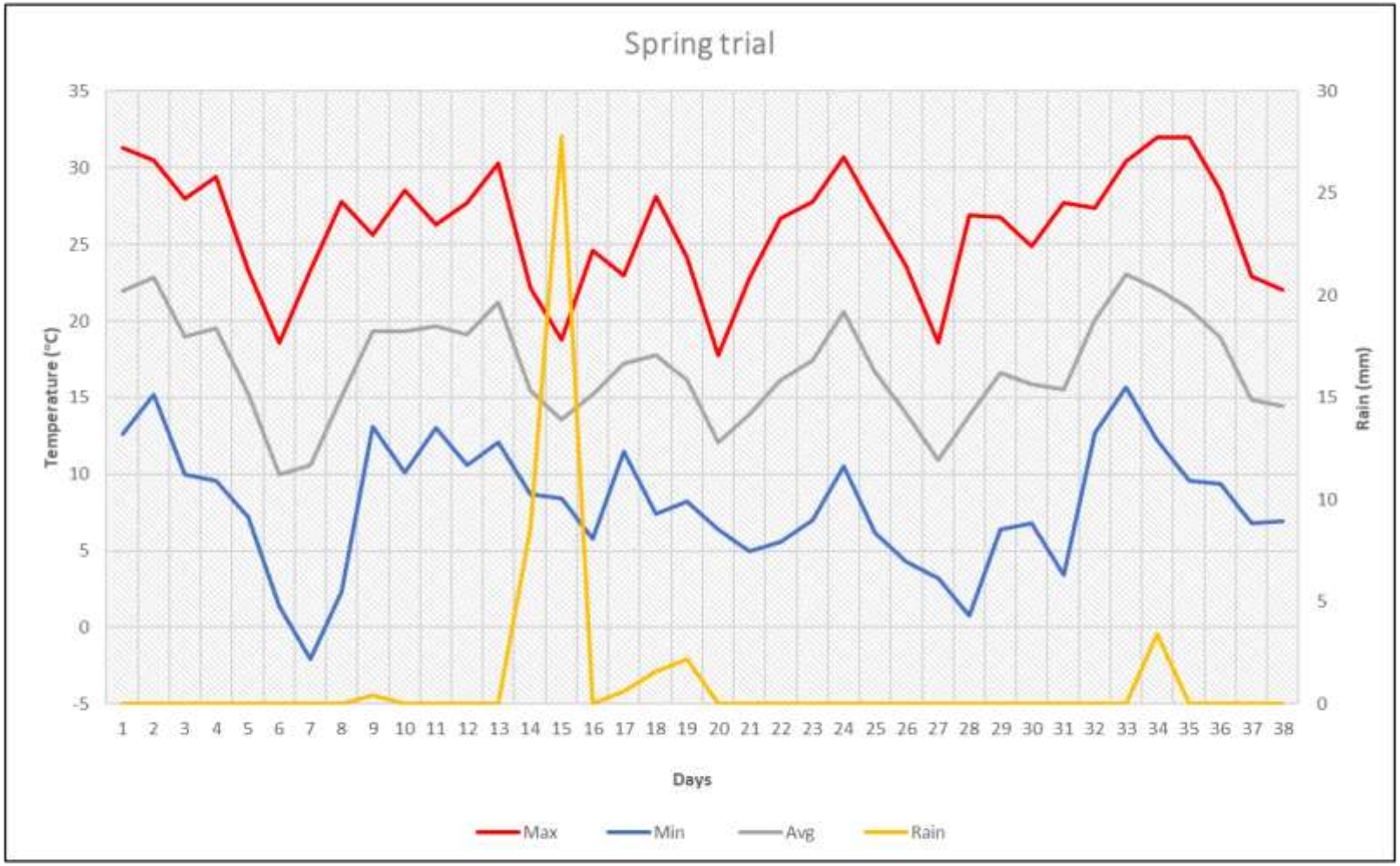
When we look at the rainfall in all three trials it was found that the rainfall was more in autumn but reached the highest peak in spring. The autumn trial can be described as the moist train because of the many peaks throughout the trial. Spring trial the carcasses were exposed to mostly dry conditions even though there was the highest peak on day 15 the carcasses were exposed to dry conditions for many days as compared to other two trials (autumn and summer).



Appendix 2A: Weather data for autumn trial. The red line indicates maximum temperature, grey line indicates the average daily temperature, blue line indicates minimum temperatures and the yellow line indicates the daily rainfall.



Appendix 2B: Weather data for summer trial. The red line indicates maximum temperature, grey line indicates the average daily temperature, blue line indicates minimum temperatures and the yellow line indicates the daily rainfall.



Appendix 2C: Weather data for summer trial. The red line indicates maximum temperature, grey line indicates the average daily temperature, blue line indicates minimum temperatures and the yellow line indicates the daily rainfall.

Appendix 2 References

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. MSc Dissertation. University of the Free State. RSA.

BOUCHER, J. 1997 *unpublished*. Succession and life traits of carrion-feeding Coleoptera associated with decomposing carcasses in the central Free State. MSc Dissertation. University of the Orange Free State. RSA.

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.

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KOLVER, J.H. 2003 *unpublished*. Decomposition and insect succession in hanging and prone carcasses, with special reference to *Chrysomya chloropyga* (Diptera: Calliphoridae). MSc, Dissertation. University of the Free State, Bloemfontein, RSA.

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Appendix 3: Ethical query

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

UFS·UV
HEALTH SCIENCES
GESONDHEIDSWETENSKAPPE

22 August 2018

Dear Dr. S Brink

ENQUIRY WITH REGARD TO ETHICAL APPROVAL FOR TWO COMPLETED RESEARCH PROJECTS

Background:
Both the research projects were registered in 2016 as part of MSc qualifications. These projects were approved by a Research evaluation committee.Ethical approval for these projects were not requested. Both projects was entomological (using insects). Carcasses (sheep and pig) were used. The carcasses were obtained from the animal research unit. The carcasses were from other approved research projects in the Faculty of Health Sciences. Furthermore, chicken liver and offal obtained from a butcher as bait to catch flies were used. Maggots used for the one experiment were reared on chicken livers.

Regarding ethical approval:
The Interfaculty Animal Ethics Committee (IAEC) of the University of the Free State (UFS) do not issue retrospective ethical approval for research studies. Since these studies commenced in 2016 and is almost completed in 2018, **the IAEC cannot give Ethical Approval for these studies**.

Insects are not defined as animals according to the South African National Standards (SANS10386:2008). Research projects on insects do not require ethical approval from an Animal Ethics Committee (AEC) according to National (NHREC), SANS10386:2008, and UFS regulations. **These studies therefore did not, and still don't, need ethical approval from and AEC.**

When a research study utilize carcasses and organs from dead animals, it is the responsibility of the IAEC to ensure these animal tissue were obtained according to ethical research practices. The carcasses and animal organs used in this study was from ethical approved research projects (UFS), and from well known, ethically approved sources (registered butcher). The IAEC are satisfied that the **carcasses and animal organs used were sourced from ethically acceptable suppliers.**

In 2016 the UFS did not require ethical approval for studies that did not involve humans or animals as defined by the SANS10386:2008. **These studies therefore did not require ethical approval according to the UFS regulations** of 2016.

Implications:
The IAEC cannot issue Ethical Approval for these studies.
Both these studies were performed according to the National and UFS guidelines and regulations with regard to research and ethical approval.
The IAEC were aware of these studies and as far as the IAEC could determine, these studies were performed according to ethical acceptable standards.

Recommendation:
The IAEC recommend that the research projects, results from these research projects, and publications from these research (including MSc-thesis) be accepted as ethically performed research.

Gerhard van Zyl
CHAIR: Interfaculty Animal ethics Committee

Yours Sincerely

Mr. Gerhard Johannes van Zyl
Chair: Animal Research Ethics Committee

Conferences

Combined Congress of the Entomological and Zoological Societies of Southern Africa, held in Pretoria, 03 July 2017 – 07 July 2017.

Poster Presentation: Moeti, A.T., Brink, S.L. & Louw S.VdM. Description of the life stages of beetles in the carrion ecosystem of the central Free State.