University of Reading

SOIL MITES AS FORENSIC MARKERS OF DECOMPOSITION OF CORPSES AND CARCASSES

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A thesis submitted for the degree of Doctor of Philosophy

School of Biological Sciences

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I confirm that this is my own work and the use of all material from other sources has been properly and fully acknowledged.

Naila Azma Che Kamaruzaman

Abstract

The use of forensic entomology in providing reliable death-time estimates using knowledge of carrion insect species' ecology and larval development has been used for many years. Aside from insects, mites (Acari) can act as reliable indicators of time of death. The use of forensic acarology is growing rapidly into a valuable additional input into forensic analysis. This field of forensic analysis has always been closely connected to forensic entomology. This study aimed to identify and qualitatively assess the mites of forensic importance within the outdoor environment. The outcomes from this study provide data that will enable a basic forensic acarology service to be provided in other research, as well as initiate further development of forensic acarology in crime scene investigations. The ubiquitous of mites in the soil beneath corpses adding valuable information on decomposition process and reconstruct the scene of death. Using the micro-habitat specific to mites, the abundance, species richness and composition of mite orders was examined and compared and the unique presence of certain mites would be uncovered. Two outdoor settings for forensic study were set up by using pigs as proxies for human cadavers: 1) seasonal study of temperate area with four different seasons in almost 2 years (November of 2013 – August 2015) 2) carcass position/condition over a year (Jun 2013 – September 2014). The majority of mites found were phoretic with the mesostigmatid families, Macrochelidae and Parasitidae the most abundant. There are few quantitative data available on the

carcass colonisation patterns of insects and other arthropods; however these data are forensically valuable. There may be differences in the taxa collected during this successional study on pigs and those that occur on human bodies from different habitats. Therefore, mites collected from three crime cases were used to prove the reliable approaches in using pig carcasses to the real cases. The mites present on the corpses were compared with those collected from the pig carcasses and there was extremely close agreement between the mite presence from bodies and carcasses.

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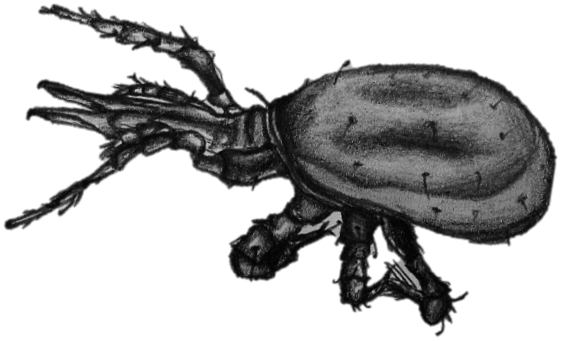
Photo of mite is credited to Jas



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1.1 OVERVIEW OF THE RESEARCH PROJECT

Forensic acarology is the term used to describe the use of mites, a group of arachnids, in forensic investigations. This approach is used mainly when other arthropods such as insects cannot reach the corpses because of the restricted conditions. Insects have been used to aid in forensic investigations by determining the minimum time since death. After the reports of forensic entomology by Brouardel (1879) that described the used of mites and caterpillars on the mummified body of a newborn child to estimate the time of death, acarology is being accepted and has played a major role in forensic investigations, and legal procedures in many countries (Wolffet al., 2001). The high diversity and abundance of mites that inhabit many microhabitats is the reason why mites are useful in forensic investigations. However, the term forensic acarology is rarely used. Instead, it is defined as one of the branches of forensic entomology. As in forensic entomology, the idea is to use the developmental rates and successional ecology of specific taxa that feeds on or colonises carcasses or corpses (Campobasso, Di Vella & Introna, 2001; Wolff et al., 2001; Goff, 2009; Turner, 2009). Using this concept of developmental rates and successional ecology, we can

estimate the time of death or post-mortem interval (PMI) of a corpse (Catts & Goff, 1992; Wolff et al., 2001; Amendt et al., 2007; Matuszewski, Barjelein, Konwerski & Szpila 2008).

The symbiotic relationship between mites and insects has become as important as using insects in forensic investigation and now, this relationship is being incorporated as trace evidence in investigation of crime cases. The relationship involves species of phoretic mites that attach themselves to insects of forensic importance (Campobasso, Di Vella & Introna, 2001; Perotti & Braig, 2009). The first use of mites in forensic investigations was started by army veterinarian, Jean Pierre Mégnin, a research assistant at the Museum of Natural History in Paris, who described the utility of mites in the case of the mummified body of a newborn child that was autopsied in 1878 (Perotti et al., 2009). He discovered a brownish layer that was composed of mite skins and faeces covering the whole body. A large number of single mite species was found inside the cranium. According to his calculations on the number of individual mites, he estimated that the corpse might have been abandoned for almost 7 to 8 months (Megnin, 1894; Benecke, 2001, 2008; Perotti, 2009; Perotti & Braig, 2009). This report linked phoresy, mites and the determination of a post-mortem interval (Perotti, Braig & Goff, 2010). Since then, mites have been reported in many cases involving human and animal remains (Goff, 1991; Perotti & Braig, 2009).

This research project is focused on a new field of research, aiming to investigate the effects of decomposition on mites (soil-mesofauna) underneath pig carcasses and analyse the fauna exposed to different experimental forensic settings. It is hoped that at the end of this study, a dataset of soil mite profiles of forensic importance can be produced for future forensic analysis.

1.2 THE INTRODUCTION OF MITES.

The Acari, comprising mites and ticks, are a group of arthropods in the Subphylum Chelicerata or Cheliceriformes. It forms one of the largest and most diverse groups of Arachnida. A large number of acarines have developed intimate associations with other animals while the free-living mites occur in a great variety of habitats but they are especially numerous where organic detritus is abundant. Most mites inhabit the organic strata of soil where they form a numerically important dominant component of the arthropod mesofauna. The mite associations range from commensalism to parasitism while many species living in temporary habitats practise phoresy, using a variety of other arthropods as vehicles for dispersal (Brown & Wilson, 1992). This group is named accordingly to their body plan which is different from other arthropods. Chelicerates do not have a head, thorax and abdomen but their bodies are divided into two parts; prosoma and opisthosoma. The prosoma holds all the appendages; the chelicerae or mouthparts are the first pair, followed by the palps or pedipalps and four pairs of walking legs.

1.2.1 Reproductive system of acari

One of the major reasons for the success of mites in diversity is their reproductive strategies. Three modes of reproduction exist in the Acari: diplodiploidy, haplodiploidy and thelytoky. Mites develop through the basic pattern of life cycle from the egg to a hexapod larva stage and nymphal stages (1, 2 or 3) to adult (Figure 1.1). However, the life cycle length varies between species, and within the same species under different environmental conditions (Schuster & Murphy, 1991). The adult is the productive stage. In 1961, Wade and Rodriguez managed to document the range of egg production by fertilized female mites. They found fertilized female mites produced an average of four to five eggs per day for about 22 days and a maximum of 25 eggs per day (Wade & Rodriguez, 1961). Parthenogenesis, the phenomenon of development of an organism from an unfertilized egg, is quite common among mites (Bloszyk, Klimczak & Lesniewska, 2006) and has various types such as arrhenotoky, thelytoky, deuterotoky, artificial parthenogenesis, gynogenesis. Parthenogenetic reproduction requires a mechanism to circumvent the normal halving of ploidy that results from gametogenesis. Mites are a promising group for investigating the evolution of haplodiploidy or arrhenotoky.

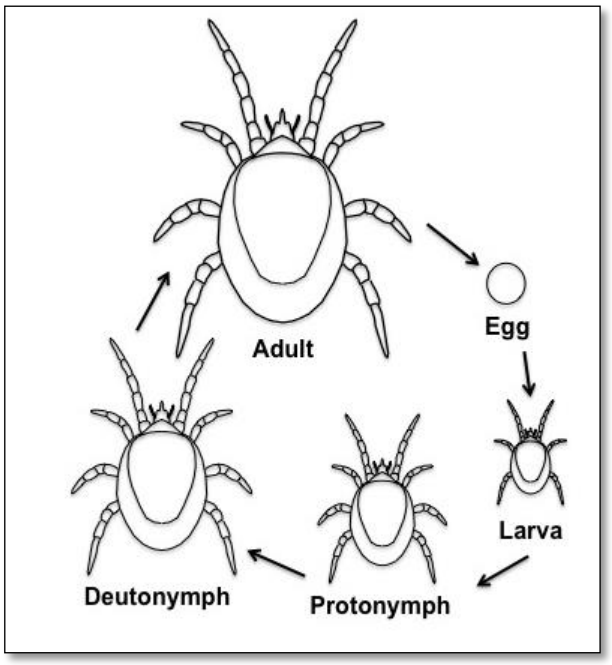

Figure 1.1: Life cycle of mite from *Symbiotic mites* by University of Nebraska – Lincoln, 1989, https://entomology.unl.edu/scilit/symbiotic-mites

1.2.2 The classification of Acari

Mites are classified into three major taxa: Opilioacariformes, Parasitiformes and Acariformes. The Opilioacariformes resemble small harvestmen while Parasitiformes that include orders Ixodida, Mesostigmata and Holothyrida. The Acariformes are all small mites encompassing three orders; the Sarcoptiformes, the Trombidiformes and the Endeostigmata. Almost all Parasitiformes are in the Mesostigmata, which includes ∽80 families and > 12,000 spp. (Krantz & Walter, 2009). Acari is a very large and diverse group which is well represented in the soil, has colonised a variety of terrestrial situations such as the aerial parts of herbaceous and woody vegetation, rock crevices, the nests of various invertebrates, birds and mammals, human habitations and decaying refuse.

1.3 INTRODUCTION TO MESOSTIGMATA

The Mesostigmata (Gamasida) are large, cosmopolitan assemblages of parasitiform mites and are known perform in unusually diverse variety life styles and habitats (Krantz, 1990). They are known from a wide range of habitats. Through their high diversity, often in great numbers, they are integrally involved in many ecological interactions. Most of them are free living predators (Karg, 1993; Krantz, 1998) in soil and litter, on the soil surface or on plants; while others are parasites or symbionts of mammals, birds, reptiles and arthropods (Walter & Proctor, 1999). Some are able to disperse rapidly by phoresy and it is relatively common in Mesostigmata (Costa, 1969; Lundqvist, 1974). Only a few members live in freshwater habitat. Mesostigmatid mites range in size from 200μm to 4,500μm. Many of the smaller forms are weakly sclerotized and pale in colour but generally the idiosoma is covered by a number of chestnut-brown shields separated by a whitish striated cuticle. The idiosoma of mesostigmatid mites carries several distinctive and recurrently diagnostic characters that serve to distinguish them from other parasitiform mites (Krantz, 1998). Distinct external features of these mites are shown in Figure 1.2.

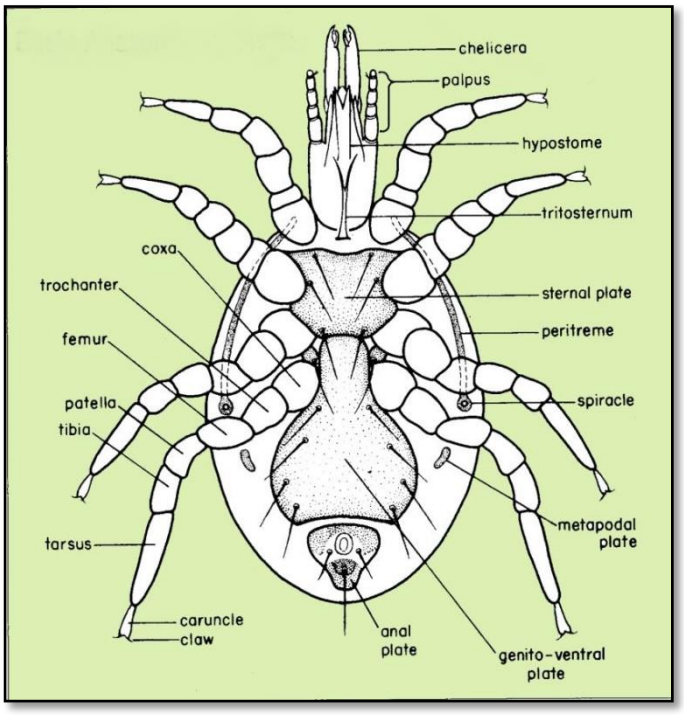

Figure 1.2: External morphology of Mesostigmata (Krantz, 2009)

Ontogenic development in the Mesostigmata is limited to a larval and two nymphal (proto and deutonymph) instars prior to appearance to the adult. Arrhenotoky is a reproductive system that produces diploid females from fertilized eggs while haploid males are produced parthenogenetically, as illustrated in Figure 1.3. This mechanism of sex-determining is common among the Mesostigmata and predominant in at least several families; Macrochelidae, Dermanyssidae, Macronysidae and Phytoseiidae. (Oliver Jr, 1977). Facultative parthenogenesis is a relatively common phenomenon in phoretic females of the Mesostigmata.

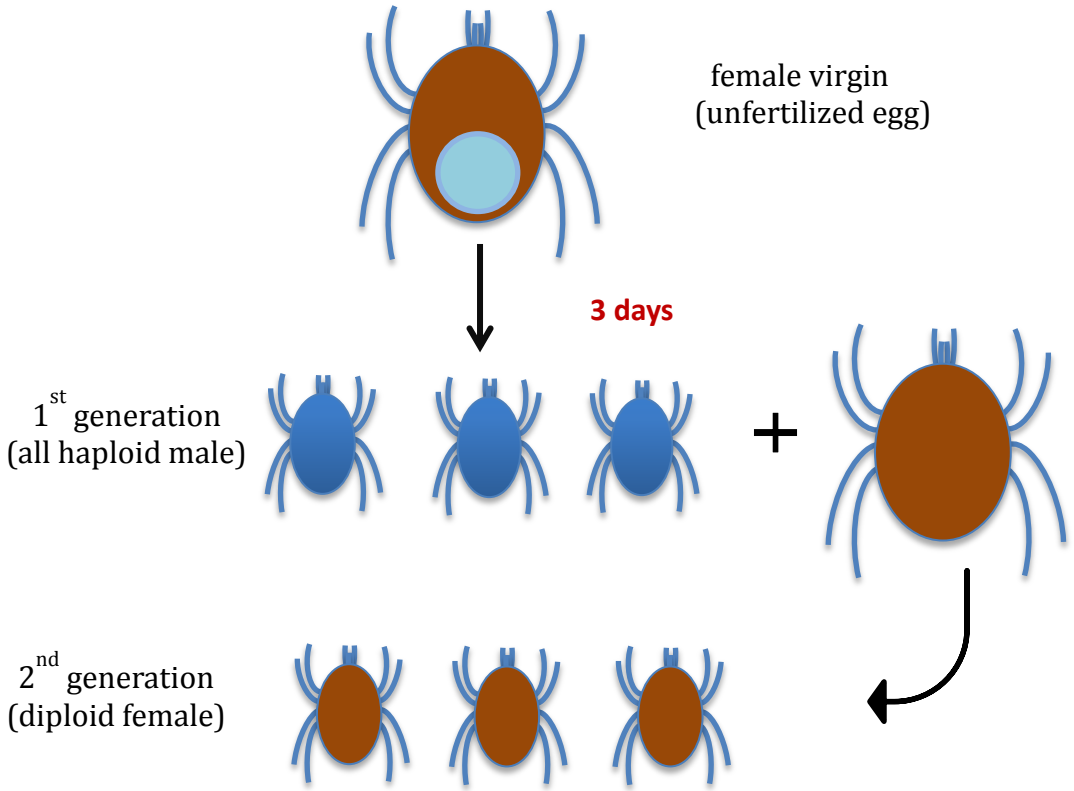

Figure 1.3: Mesostigmata reproductive strategies.

1.4 THE SOIL ENVIRONMENT

1.4.1 Soil chemistry

Soil is a mixture of organic and inorganic components which are present in different combinations of chemical composition and physical attributes such as particle size (Marumo, 2001). The soil environment is complex and heterogenous. Sampling soil can be challenging considering the range of conditions that can affect decomposition of organic materials (Cragg & Bardgett, 2001; Ducarme, & Lebrun, 2004). In the past century, scientific innovations have increased our understanding

of soil and our ability to analyse it for forensic applications (Dawson & Hillier, 2010). Various types of analysis using soil properties in terms of both its chemical composition and physical attributes such as particle size (Sugita & Marumo, 2001) can reveal a great deal of information in forensic investigation (Dawson & Hillier, 2010).

The process of decomposition on the soil releases the cadaver materials that enter associated soil beneath or surrounding the cadaver, which results in the formation of a concentrated island of fertility. A Cadaver decomposition island (CDI) is defined as a highly concentrated island of fertility (Carter, Yellowlees & Tibbett, 2007) which is the region that below and around decomposing cadaver. These changes in soil chemistry alter the structure of insect communities, which are typically associated with the degrees in richness (insect communities) over time (Bornemissza, 1957; Anderson & Vanlaerhoven, 1996). The soil chemistry on the decomposition products may have an effect on post-mortem interval (PMI) predictions using CDI soil models (Aitkenhead-Peterson et al., 2015).

1.4.2 Soil arthropods and decomposition

Soil is one of the most complex habitat systems that provides a living place for many arthropods, either for their entire life cycle or at least for a part of their life cycle. Small arthropods, including several groups of mites, contribute to the humus fraction and permit complexes of other soil organisms, such as micro-organisms, nematodes and Collembola to exist. The most important group of arthropods that is

affected by the formation and maintenance of soil structure is mites. Even though the role of mites in soil mixing is small in comparison with that of larger invertebrates such as earthworms, insects, crustaceans and millipedes, nevertheless mites exercise an important function in mineral turnover, vegetation succession and as decomposers of organic matter (Peterson & Luxton, 1982). The analysis of soil associated with decomposed remains can provide useful forensic information, particularly for estimating time since death (Vass, et al., 1992) or deposition (Forbes, 2004). The carcass itself acts as host for hundreds of different species of microbes and invertebrates that are attracted to the corpse by different chemicals produced during cadaver decomposition.

1.5 MITES AND FORENSIC INVESTIGATIONS

1.5.1 Mites in the soil

Soil mites are one of the most abundant micro-arthropod types in the upper soil layers and form a large and functionally important part of the mesofauna in the soil. They are particularly abundant not only within the soil, but also in forest litter, as important decomposers and nutrient-cycling of organic materials. (Badejo, 1990; Stork & Eggleton, 1992). Mite communities are extremely sensitive to all types of soil disturbance. Water content in soil influences mite population. This was proved and stated in the findings of Badejo (1990) who compared mites from two contrasting soil environments. High soil temperature will cause mortality of the sperm of soil mites, as well as reducing egg-laying in mites (Butcher, Snider, &

Snider, 1971). This makes them suitable bioindicators for soil system (Gulvik, 2007). A nation-wide study of soil biota in Great Britain suggested that mites are the most frequently recorded group, occurring in 94% of all soil samples (Gulvik, 2007).

Mites are the most abundant invertebrates beneath the carrion (Behan-Pelletier, 1998) and Goff and Catts (1990) recovered several significant mite groups. The abundance of Macrochelidae (Parasitiformes: Mesostigmata, Gamasina) in soil was observed to increase over time in the presence of carrion (Reed, 1958; Anderson & Vanlaerhoven, 1996). Population development of Gamasina is very much influenced by microclimate (Koehler, 1999). The differences in the microclimate influenced the rate of decay (Bornesmissza, 1956). Bunch (2009), in his observational study on pigs at Oswego, USA, came out with the result observed that there were varying rates of decomposition between three specimens with different microclimates.

1.5.2 Phoretic mites relation with insects

Phoresy is a phenomenon in which an animal actively attaches to another animal in order to disperse (Athias-Binche, 1994). Phoresy by mites occurs typically via transmission on associated insects. This association is only transitory and general with the mite using a variety of insect species for transport. In many cases, however, the mites have specialized on a restricted range of host species and have established permanent associations in which their non-phoretic stages also live in

close proximity to the hosts (Schwarz, 1997). This is the most common method by which mites disperse from and colonize temporary accumulations of organic matters. These associations occur among mites species that inhabit temporary and discontinuous habitats such as dung, carrion, fungi or plant materials (Hunter & Rosario, 1988). These associations can be influenced by ecological conditions such as host availability, climate and soil conditions. Phoresy results in dispersal from one habitat to the new ones which have better conditions for the development of phoretic mites or their offspring (Farish & Axtell, 1971; Athias-Biche, 1994).

A majority of larger species of phoretic mites belong to the suborder Mesostigmata (Takaku, Katakura, & Yoshida, 1994). Studies came out with lot of carrier records of mesostigmatid mites (Takaku, 1994). In many species, the life cycle of the mite is synchronized to the host (phoront) species that may accelerate or suspended development of phoretic instar (Evans & Hyatt, 1963). Generally, only one developmental stage of each mite species is phoretic (a second stage only occurs in rare cases) and most *Macrocheles* species associated with insects are arrhenotokous (Cicolani 1992; Norton et al., 1993; Schwarz, Starrach & Koulianos, 1998). Only the female is phoretic in the Macrochelidae and she is arrhenotokous which enables the establishment of the species within the new habitat by a single or few individuals (Filipponi, 1955). Takaku revealed that phoretic mesostigmata are also known as important control agents of pest flies (Takaku, 1994). He also investigated the carrier specificity of some species in Mesostigmata (Takaku, 1994).

1.6 DECOMPOSITION AND INSECTS SUCCESSION

Decomposition is a process that varies greatly from body to body, environment-to-environment, the circumstances of the death, the place where the body was found, and even the climate. It consists of a number of processes including the enzymatic liquefaction of cells, bacterial decomposition of tissue, dying of the skin and remaining soft tissue, followed by skeletonisation (Smith, 1986). The biological process of decaying of a carcass or a corpse will continuously produce a new biological process and consequently change the underlying structure of soil and its fauna under the remains (Saloña-Bordas et al., 2010). The exploration of different species associated with decomposition is part of creating a wider ecological understanding of the ecosystem (Lindgren et. al., 2015). As the bacteria begin the processes of cell breakdown, fermentation and putrefaction, the large scavenging animals will begin to play a significant role in the consumption of the soft tissues of the copses (Smith, 1986). The role of insects as the main invertebrate assemblage that associates with carrion has commonly been applied in homicide investigation (Morris & Dadour, 2005). However, the use of invertebrates other than carrion-dwelling insects, particularly the soil fauna, has received little attention in a forensic context. Soil fauna affects decomposition processes both directly, through fragmentation and comminution of litter material and indirectly by altering microbial biomass and through excretion of nutrient rich waste (Petersen & Luxton, 1982; Cole et al., 2006). Carrion-associated mites often disperse between

carcasses or corpses using phoresy, typically flies and beetles (Perotti et al., 2009; Perotti, Braig & Goff, 2010).

As the body progresses through the stages of decomposition, the odours emitted by the corpse will change (Anderson, 2001) and this will attract different insects. As the body decomposes and various resources are depleted, new insect types will colonize while being more suited to the current decomposition stage (Dadour & Harvey, 2008). These insect taxa reflect the physical changes in the body and are therefore, predictable and useful in the estimation of Post-mortem interval (PMI). According to Payne (1965), insects arrive at a corpse in a predictable manner specific to the location and environmental conditions under which the remains are found. In such cases, the composition of taxa found on a corpse (named corpse fauna) are usually compared with the composition the arthropod assemblage at a given period of time, derived from an animal model (baseline fauna) (Schoenly & Reid, 1987). Decomposition studies have shown that decomposing carcasses or tissues have an effect on soil chemistry such as soil nutrients, trace elements and pH (Aitkenhead-Peterson et al., 2012).

Organic body decomposition takes place in five distinct stages: i) fresh, ii) bloat, iii) active decay, iv) advanced decay and v) dry/skeleton (Wolff et al., 2001;; Amendt et a*l.,* 2004; 2007; Matuszewski et al.,2008; Goff, 2009). The process of decomposition primarily depends on environmental temperature, humidity, light, and wind.

Studies of decay rates of corpses in Tennessee by Rodriguez and Bass (Rodriguez & Bass, 1983) showed temperature, access by insects and depth of burial are the three most important environmental factors in corpse decay (Catts & Goff, 1992). Later, Campobasso (2001) revealed that other than ambient temperature, ventilation and air humidity are important factors that influence decomposition. Microclimatic factors such as light, temperature and atmospheric pressure, precipitation, humidity, wind and turbulence affect insect dispersal (Pasek, 1988). It is proposed that in a strong wind atmosphere, there is no carrier that can transport mites from a carcass to a new habitat. Mites will accumulate when the phoresy conditions are restrained. This situation may also change the mite host-specificity behaviour to non-specific phoretics. The switching of mites to non-specific hosts so that they can leave a carcass was observed in indoor cases and concealed bodies, which prevented insect dispersal (Perotti et al., 2010). Therefore, lack of dispersal due to windy conditions increases mite abundance. We need to understand the decomposition process because it affects forensic investigations in a variety of ways. There is successional activity by a community of arthropods along the decomposition process (Payne, 1965; Archer, 2003) which is continuous, starting at the point of death until the corpse or carcass reaches the skeletal stage. Mites, as well as other arthropods are typically understood to show a rapid invasion stage, a peak in abundance and richness, and a monotonic decline thereafter (Schoenly & Reid, 1987). Mites are hyperabundant, proliferate and complete many lifecycles leading to an increase in population abundance and density (Perotti, Braig & Goff, 2010); whilst a particular beetle species may colonise

a carcass, but then only complete one life cycle (Barton, Weaver & Manning, 2014). Specific mite families do occupy a carcass at specific stages of decay (Bornemissza, 1957) and this can be of potential value.

1.7 OBJECTIVES

Aims and objectives for each chapter:

Chapter 2: Seasonal changes in the soil mite fauna underneath pig carcasses in Reading, preliminary investigation. The aim was to provide the first comprehensive experiment investigation the mites of forensic importance using the concept of forensic entomology. The objectives for the experiment: 1) to describe the fauna of soil Acari associated with pig carcasses lying on the ground, in an outdoor environment in Reading (Berkshire, UK); and 2) gather information on potential mite markers of stages of decomposition.

Chapter 3: Mesostigmata mites of forensic importance in the soil beneath hanged and on-the-ground carcasses. The main aim was to gather information on potential mite markers of the body's position. The objectives are; 1) to compare the diversity and abundance of mites between 2 different treatments; pigs on the ground and the hanging with the empty plot as a control; 2) to compare the decomposition stages among the 3 treatments.

Chapter 4: The value of *Macrocheles* species (Acari: Macrochelidae) as trace evidence markers of location and time: three case studies from Europe. This chapter was aimed to demonstrate the used of biology features of phoretic mite in real crime cases in giving the additional information in forensic investigation.

Chapter 2：Seasonal changes in the soil mite fauna underneath pig carcasses in Reading, a preliminary investigation.

2.1 INTRODUCTION

During the process of decay in animals (including humans), bodies go through a series of decomposition stages (Smith, 1986) and characteristic assemblages of invertebrates colonise them as a medium for oviposition or feeding (Payne, 1965). This includes specialization of scavengers to particular stages of decomposition (Schoener, 1974; Braack, 1987). The composition of the invertebrate assemblage can be utilised forensically to estimate minimum time since death (Catts & Haskell, 1990) since the order of succession of carrion invertebrates and the arrival and departure times of taxa involved are potentially predictable (Smith, 1986). The sequence of the insect succession visiting carcasses is predictable at the family level (Early & Goff, 1986) however, at the genus and species level, the colonization relies on environmental and geographical characteristics (Payne, 1965; Early & Goff, 1986).

Seasonal variation has been documented as a factor influencing insect activity (Reed, 1958; Rodriguez & Bass, 1983; Goddard & Lago, 1985) and the pattern of

the succession differs greatly between seasons (Linhares & de Carvalho, 2001). The movement of Earth in an elliptical path around, and is tilted towards or away from, the sun causes the seasons. Astronomically, it is considered that the occurance of 2 annual solstices mark the beginning of summer and winter, while spring and autumn begin on the occurance of 2 annual equinoxes.The sun heats the Earth and changes the temperatures. Temperatures also depend on the heat that is absorbed and reflected by land and the oceans. The change of seasons has an effect on the weather. Temperatures ranging between 25^0C and 35^0C are optimal for the development of bacteria (Campobasso, Vella & Introna, 2001) that helps the decomposition process. Variability of ambient temperature (certainly the most important of extrinsic factors) among seasons is the most important factor influencing the rate of decomposition of carcasses (Horenstein, Rosso & Garcia, 2012). It is also influenced by variables of different nature concerning the corpse itself and the external environment that include temperature, humidity, precipitation (especially rainfall) and by the composition of the carrion-associated fauna (scavengers) and the circumstances related to death (Bornemissza, 1957; Smith, 1986; Linhares & de Carvalho, 2001). Archer (2004) in his study on the patterns of decomposition of exposed neonatal remains found that high temperatures increased the rates of body mass loss and progression of decomposition. Decomposition studies in the temperate regions must therefore examine insect activity and species composition in the four seasons; autumn, spring, summer and winter.

Few studies have been conducted to observe the relationship of insect succession in the decomposition process considering the effect of seasonality (Mann, Bass & Meadows, 1990; de Carvalho & Linhares, 2001). Some carrion-taxa may also be seasonally active (Anderson, 1982; Davies, 1999; Archer & Elgar, 2003), whilst others maybe active all year-round (Archer, 2004). Bass (1997), in his experiment on 150 cadavers exposed on the soil surface in Tennessee, concluded that the decay process was retarded at low temperature which caused severe reduction of insects colonising the corpses. Several groups of arthropods are known to visit the carcass of a vertebrate at its various stages of decay (Bornemissza, 1957; Arnaldos et al., 2005; Perotti et al., 2010;). They play a main role in the consumption of carcass by reducing them to the skeleton, depending on their biological preferences and the stage of body decay. This produces a faunal succession which varies between seasons and environmental conditions (Díaz-Martín & Saloña-Bordas, 2015). Two abundant groups of arthropods that are always found at carrion or carcasses are insects (beetles and flies) and mites (Bornemissza, 1957; Braack, 1987; Braig & Perotti, 2009). Many mite species found on carrion are phoretic and use flies and beetles as hosts for dispersal between carcasses. Many mite species prefer to attach to a specific host (Perotti & Braig, 2009). Aside from insects, mites are capable of providing a timescale of death based on their colonisation patterns in relation to different stages of decay. It is possible to analyse the time of arrival of the host of the phoretic mites to the carcass even if the host has already departed from the carcass. Therefore mite evidence can often complement or reinforce the

colonisation information provided by insects in time of death estimations(Perotti et al., 2009; Saloña-Bordas & Perotti, 2014).

In this work, the seasonal effects on the process of decomposition were investigated by studying the Mesostigmata (Acari: Parasitiformes), following Krantz & Walter (2009), mite fauna underneath carcasses with the main aim of uncovering the occurrence of certain mite species that can be used as markers of season or environmental conditions. A true picture of seasonal variation requires data on decomposition over more than one year at a single study site. This is because between years, as well as within the same year, seasonal differences may affect decomposition.

The aim of this research was to study carcass decay in relation to diversity and seasonal variation of mite species and their potential value as forensic indicators.

2.2 MATERIALS AND METHODS

2.2.1 Study area

The study site for this research project was situated within the main campus of University of Reading at the following geographic coordinates 51°26′31″N, 0°56′44″W (Fig. 2.1 a) & b)), located in Berkshire, South West England. A research environment

was set up near the small bushes close to the Greenhouse of the School of Biological Science. Predominant tree cover on the site is from deciduous trees, while on the ground mostly covered by shrubs. The soil is porous and dry and tree cover of the area is dense. This site is beside a road (Pepper Lane) that has heavy traffic during peak hours. Faunal native inhabitants of the area are foxes, squirrels, birds and small rodents such as mice.

Figure 2.1: a) & b); Location and study area of research. c); Cage used to keep the carcasses.

2.2.2 Experimental design for test subjects

Four domestic pigs (*Sus scrofa domesticus*), that were acquired from a private butcher located in Oxford, were used. The pigs were bred for the domestic meat markets and were killed on the farms by a licensed professional. Following DEFRA and Health and Safety regulations (University of Reading) to ensure biosecurity and prevent any inadvertent spread of disease during transportation from farm, each animal was placed into a body bag, which was sealed before being transported within 2-3 hours to the experimental site. One carcass was placed in each season, a total of 4 carcasses (Table 2.1). However, due to limitations regarding to availability of pigs, availability of study site and waiting for permissions, the experiments were not being able to start in the exact date of particular season but attempted to carry out as close to start of each season as it was possible. The carcasses were always placed on the site between 1300 to 1400hrs, were enclosed in scavenger-proof cages (Figure 2.1 c)) which prevented vertebrate disturbance while allowing the access to invertebrates. The cages used were 100cm long, 80cm wide and 60cm high with a 25-mm mesh and were secured to the ground with four 45cm long steel spikes. One part of the cage was hinged to open for easy access while doing the soil sampling underneath. All the carcasses were placed in a similar position; laid laterally and with the same environmental setting; under trees giving canopy cover, to standardise microclimate as far as possible.

Table 2-1: Date of placement.

Season	Periods of observation
Autumn	From 20th Oct 2013 until 20th March 2014
Winter	From 9th Dec 2014 until 3rd August 2015
Spring	From 14th April 2014 until 17th July 2014
Summer	From 19th August 2014 until 2nd Dec 2014

2.2.3 Data collection protocol

In each season, carcasses were visited daily for the first 2 weeks after placement, every third day for the following week and once a week thereafter until they reached skeletal remains. At each visit, carcasses were photographed from each direction around the enclosure with a digital camera (Canon EOS500D with EF-S 18-55mm lens), and odour and appearance were also described. Detailed notes were taken for any changes during the decomposition process as well as arthropod abundance and activity on carcasses was recorded over time. Classification of the stages of decomposition followed the definitions of Payne (1965) and Anderson (1978).

A Fourtec Microlite USB Temperature and Humidity data logger was placed attached to the cage with the carcass. The same type and brand of data logger was also placed underneath the carcass to record the body's temperature. The data loggers were attached to the cage and were programmed to take the daily maximum and minimum temperature as well as temperature readings at 30-minutes intervals from 0000-2330hrs. The temperature readings were compared with the meteorological data from

the Department of Meteorology, University Reading. Other environmental factors that were carried out during sampling are detailed in Table 2.2.

Table 2-2: The environmental factors that were taken *in situ*

Instrument	Method and function
Infrared Digital Gun with Laser Sight by Mazoom (-50°C ~380°C)	Pointing the laser beam to the body surface – body temperature
Digital Sound level meter (A & C Measuring functions) (30 ~130dB) (31.5Hz ~ 8kHz)	Using *in situ* while sampling - record the surrounding noise
Waterproof pH and temperature meter tester model 8685 by Easypet	pH of soil was measured in the lab with an electronic pH meter. for pH by using the pH meter – soil pH
Light meter LX1330B Lux Luxmeter - Mastech (0.1 ~200,000 Lux)	Using *in situ* while sampling – read the intensity of light that reach the carcass

For soil sampling, two body parts of the carcass; head and lower abdomen (bottom), were raised briefly and the soil beneath was collected. Soil samples were collected between 0900 to 1200hrs every 2 days for the first 15 days of decomposition or while decay was between fresh to active. As decay entered an advanced stage, the collection of samples was reduced to collection once in five days for 2 weeks and afterward once a week until the entire carcass flesh has been consumed (dry).

Soil samples were then separated by using adapted apparatus based on the Berlese-Tullgren method (Stork & Eggleton, 1992; Behan-Pelletier, 1998). Large clean plastic bottles (~2L) were used to make disposable funnels to separate small arthropods from soils (André, Ducarme & Lebrun, 2002) (Fig. 2.2). Table lamps with 15W incandescent light bulbs were placed 8-10cm above the funnels to provide heat and light for up to 7 days after the beginning of the extraction process.

Figure 2.2: Funnels to separate the soil samples.

2.2.4 Isolation and identification of invertebrates

The contents of collection-jars from separating funnels were sorted under a stereo microscope (MOTIC magnification up to 40x). Taxa separation included mites, insects and other invertebrates. Mites were collected in separate vials for each

sample, labelled and preserved for further identification. All samples were kept preserved in 70% ethanol until further processing for identification. Only mites from order Mesostigmata were identified up to species level for this study while other taxa were identified to family level and kept preserved for future research studies.

2.2.4 Clearing and mounting

For each individual mite the procedure used for permanent mounting for identification was as follows:

- Mites from ethanol were placed in distilled water in a watch glass, using a flat-tip needle or fine brush, and were kept in water for one hour. The mites were transferred again into a second watch glass containing fresh distilled water and kept for an hour.
- The mites were then transferred to an Eppendorf centrifuge tube with 50% lactic acid to clear the well-sclerotized mites.
- The tubes containing lactic acid and mites were then heated on a hot plate (40^0C) for about one hour or kept overnight at room temperature (give temperature (or range)).
- Most specimens were ready to mount the next day but mounting can be postponed for up a week if the specimens have a hard cuticle (Faraji & Bakker, 2008). Mite specimens were mounted dorso-ventrally on

microscope slides in Hoyer's medium (Faraji & Bakker, 2008) for permanent mounting.

- The mounted slides were sealed along the edge of the coverslip with a layer of Glyptal paint by using a fine artists' brush. Glyptal prevents moisture entering and fracturing the specimen (Travis, 1968).

The mounted mites were then added to the lab mite collection.

2.2.6 Identification

The mounted mites were identified under a phase contrast microscope (Leica DMLS) (objectives used 10x to 100x). Mites were identified at the Order and Family level using the keys of Krantz et al., (2009). Mesostigmata species were identified using the keys of Evans (1956), Hyatt (1980) Hyatt & Embersom (1988) Hyatt (1990) and Masan (2003).

2.2.7 Data and statistical analysis

The total of individuals counted for each species was used for analyses. The analysis of variance (ANOVA) is performed to primarily test whether the 2 factors; decomposition stage and season, are significantly different from each other. This test is implemented using the statistical software PAST (Hammer, 2001). Faunistic indexes (FI) for the species of the major families of Mesostigmata collected were calculated to determine the diversity of species found in the study area for each

season sampled. The three faunistic indexes calculated for the collected species were the Evenness, Richness and Simpson's index (D) (Help, Herman & Soetaert, 1998).

The relationship of Mesostigmata abundance among the different decomposition stages and seasons was analysed using a Generalised Linear mixed-effects model with stages and seasons as fixed effects and 'individuals' count of mesostigmatid' as random effect. Model fitting and estimates were obtained with the linear mixed-effect with a specified 'Poisson' error family using R package (R studio version 3.2.5, 2016). Wald's Z statistic and probability 'P' values of best-fit models were quoted throughout. Environmental data were averaged over each day and were used for the analyses. The average data of environmental variables were compared as these were the main factors that affect the stages of decomposition. A regression analysis provided by Generalised Linear Model ANOVA with Fisher's protected least significant differences (R studio version 3.2.5, 2016) is used to test the environmental variables and decomposition stages as factors with the effect on the Mesostigmata abundance.

2.3 RESULTS

2.3.1 Decomposition factors

Seasonal variation of decomposition stages was observed from year 2013 to 2015 and corresponded with factors of decomposition variation such as; ambient temperature, soil temperature, soil pH, rainfall, wind speed, light intensity, air humidity and carcass temperature. Reading, United Kingdom is located in the temperate zone and experiences four distinct seasons. The fresh stage of each carcass began approximately at the beginning of each season and each carcass was studied until it reached the skeletal stage. As the duration of decomposition is affected by various seasonal factors; mainly temperature, the time it took for each carcass to reach the skelatal stage during each season took more than the 3 months associated with each season.Therefore, although there were a total four experiments, one for each season, the experiments, in reality, overlapped by a few months. For example, the duration for the whole body to be fully decomposed and reach the skeletal (dry/remain) stage was approximately 150 days during autumn, 94 days during spring, 98 days during summer and 223 days during winter. As noted, the decomposition process for summer is longer than spring since spring attracts pollinating insects that carry phoretic mites along which expedites the decomposition process of the carcass.

The mean temperature is shown in (Fig. 2.3(a) – 2.3(d)). Throughout the study, spring (April-July) had mild to warm weather with low rainfall, summer (July-

September) had warm to mild weather with moderate to high rainfall, autumn (October-December) had mild to cool weather with moderate rainfall, and winter (January-March) was cool with low rainfall. Temperature is one of the important factors in determining the rate of decomposition (Mann, Bass & Meadows, 1990) then it is critical that accurate temperature data is collected. The averages of other factors such as soil temperature, wind speed at 2m high from soil surface, ambient humidity, soil pH, body temperature of carcasses, ambient noise and light intensity that reached the bodies are shown in Table 2.3.

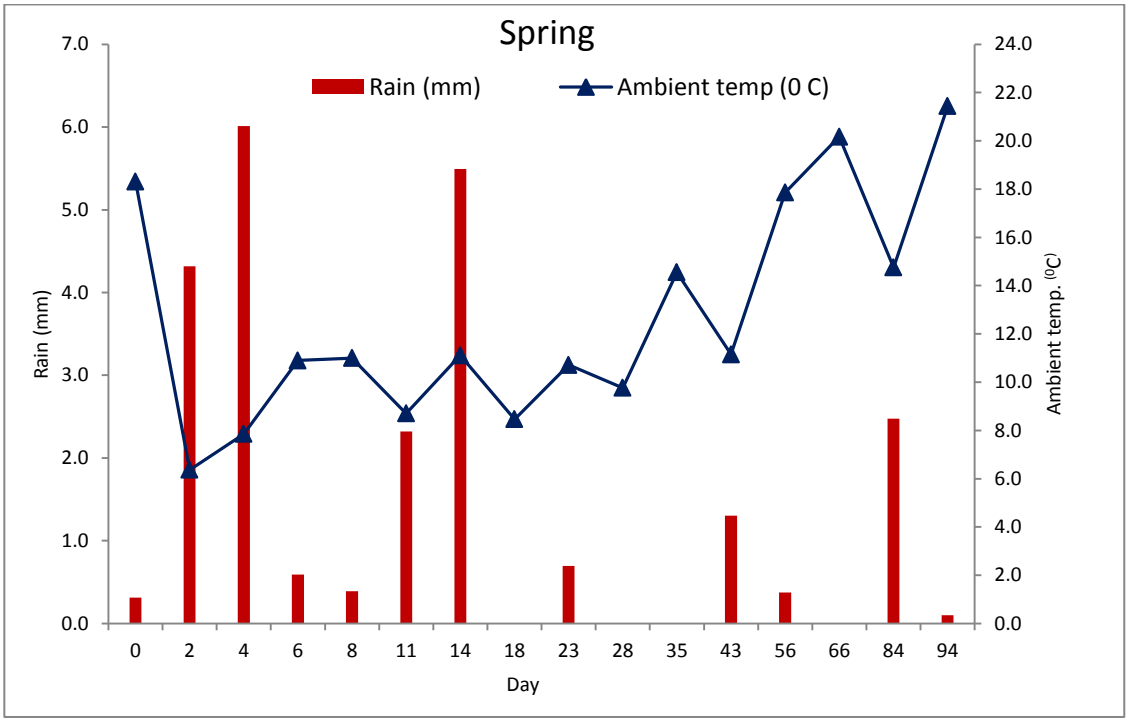

Figure 2.3: Mean temperature and cumulative rainfall (mm) per sampling throughout decay for pig placed in spring.

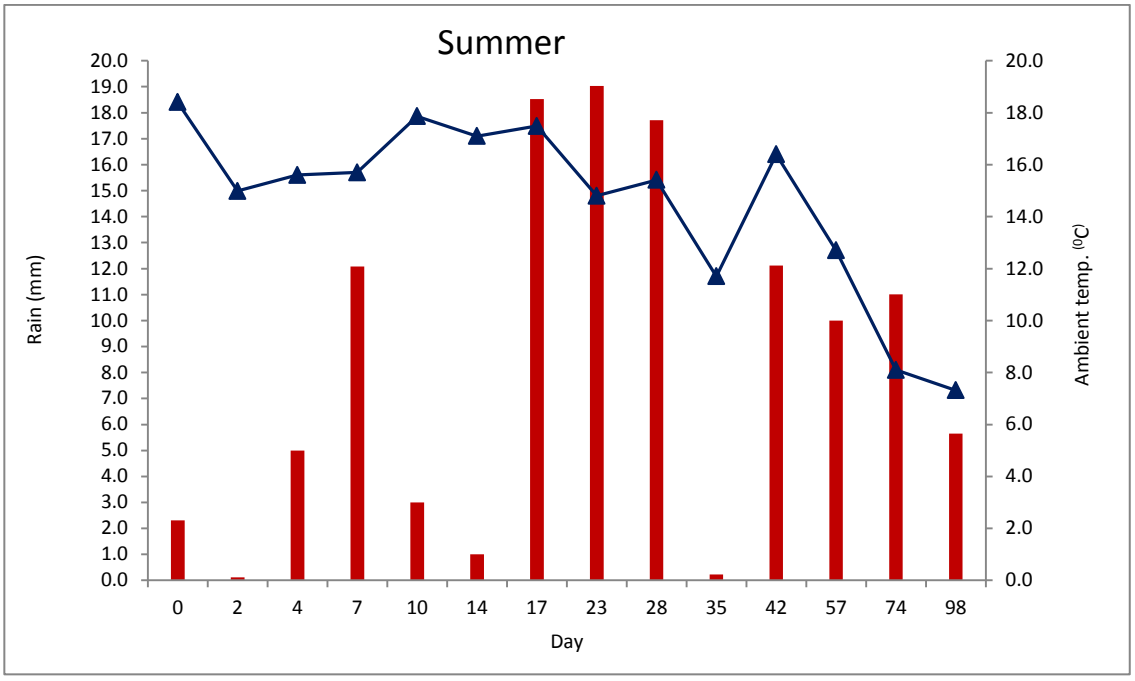

Figure 2.4: Mean temperature and cumulative rainfall (mm) per sampling throughout decay for pig placed in summer.

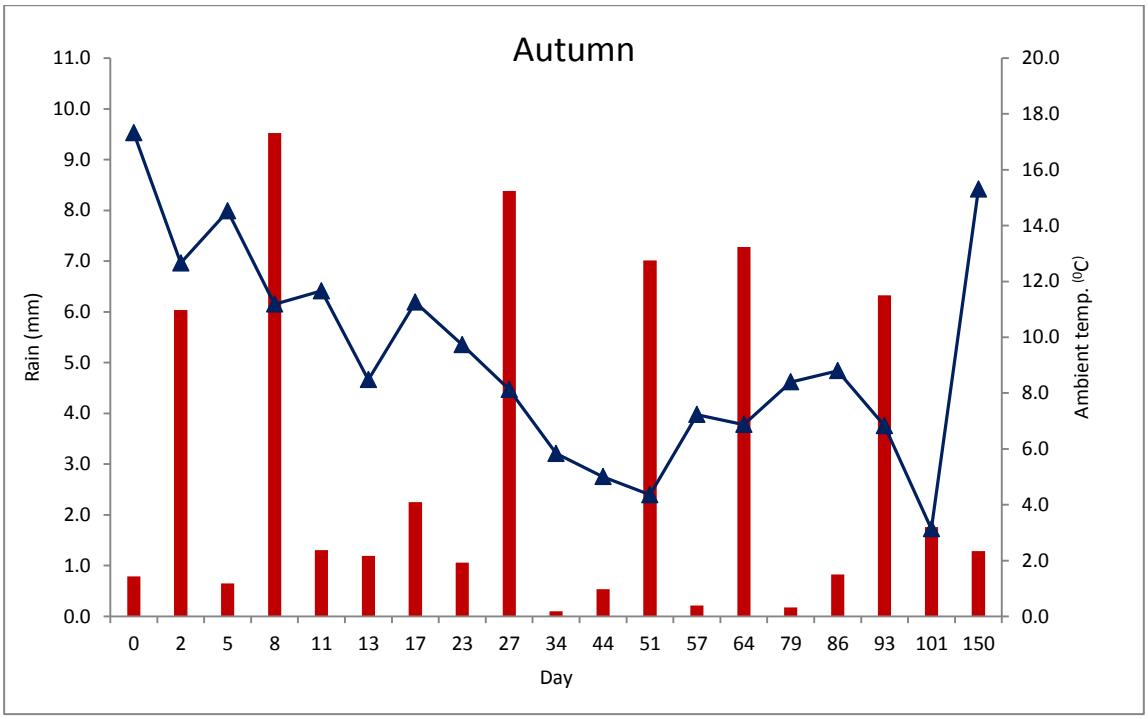

Figure 2.5: Mean temperature and cumulative rainfall (mm) per sampling throughout decay for pig placed in autumn.

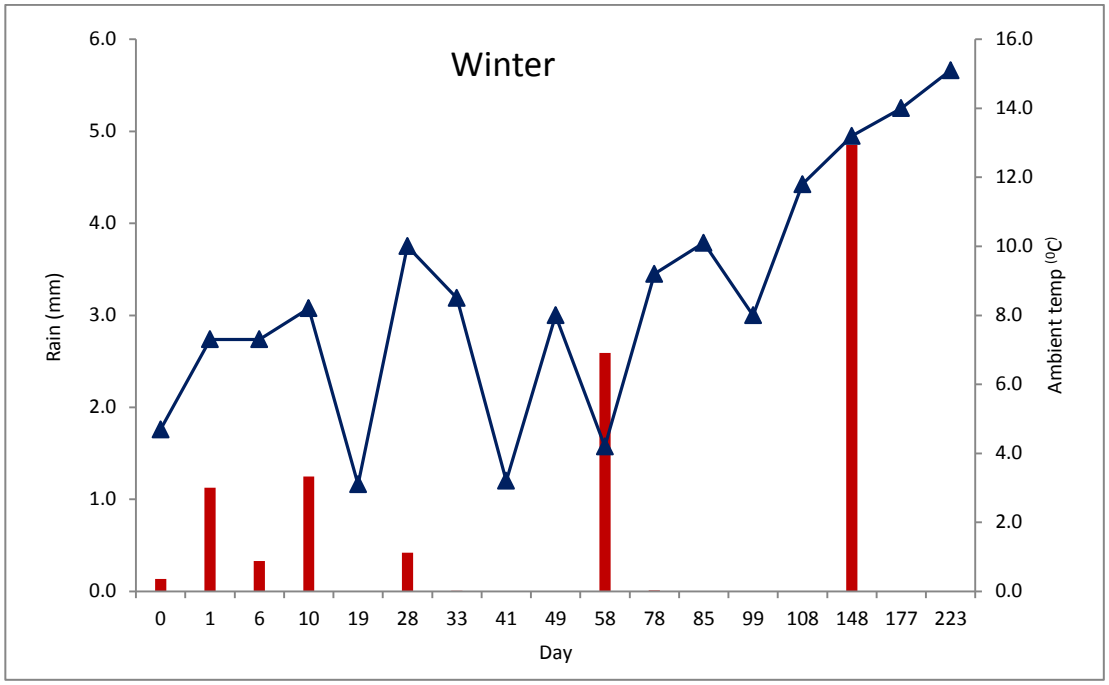

Figure 2.6: Mean temperature and cumulative rainfall (mm) per sampling throughout decay for pig placed in winter.

Table 2-3: The average of other factors (±SE) in the research study (2013-2014)

Season	Soil temp. (^{0}C)	U_2 (wind speed at 2m)(m/s)	RH (%)	Soil pH	Body temp. (surface) (^{0}C)	Noise (dB)	Light intensity (lux)
Spring	14.257 ± 4.678	2.843 ± 1.689	65.227 ± 11.658	7.9 ± 0.2	12.266 ± 5.186	86.983 ± 21.134	224.979 ± 214.125
Summer	14.379 ± 4.305	3.103 ± 2.709	57.171 ± 18.407	8.0 ± 0.23	13.654 ± 4.226	79.774 ± 19.713	227.030 ± 97.401
Autumn	9.505 ± 3.011	2.423 ± 1.432	88.302 ± 18.372	7.4 ± 0.42	8.185 ± 4.094	63.451 ± 4.188	242.350 ± 304.24
Winter	7.467 ± 4.193	4.363 ± 2.153	77.962 ± 9.569	7.3 ± 0.4	6.429 ± 7.025	71.208 ± 10.09	159.037 ± 152.51

2.3.2 Decomposition stages

The total duration of the decomposition was determined by the rate of individual carcass decay processes, starting from fresh stage, until the skeletal (dry/remain)

stage. Determination of stage change over time is subjective because there were no clearly defined boundaries between stages. The stages were recognised visually by characteristic morphological changes on the carcass body as described in Table 2.4. The carcass appearance and odour were the main criteria to divide decomposition into the stages. Five stages of decomposition; fresh, bloating, active decay, advanced decay and skeletal were observed on pig carcasses in four seasons; autumn, winter, spring and summer. It is literally impossible for any carcass to decompose fully in exactly 3 months or less as it completely depends on environmental factors. The temperatures year round in UK are quite low therefore it is unlikely the decomposition will be very fast in any season. The period for each stage of decomposition between seasons was different as shown by a bar chart (Fig. 2.4). Observation made on the rates of decomposition among seasons has produced the same conclusions as the statistical analyses. The decomposition stages were longer in autumn and winter and shortest in spring. In autumn and winter, the onset of decay was prolonged and carcasses remained relatively fresh for a month. From daily observations, the details of each stage were recorded and are discussed:

Fresh stage – Carcasses did not produce discernible odour. The skin had the original colour; pink or white, with purplish or greenish discoloration on the underside abdomen. The body orifices at the head (eyes, nose, mouth and ears), anus and genitals, and cut wounds had attracted the first insect invasion.

Bloating stage – The body had an inflated appearance. The leakage of body fluids with strong smell came from the natural orifices; nose, mouth and ears. The carcass developed a marbled-appearance on the skin. The entire body bloating occurred at a different time for carcasses in each season. Green mold grew on the skin of carcasses during autumn and winter.

Active stage – This stage was characterised by greater mass loss, resulting from the large feeding masses of maggots and fluid decomposition leaking from the body to the surrounding environment. The body started to deflate due to skin rupture. The fluid accumulated around the body and created a cadaver decomposition island (CDI). A strong odour of decomposition persisted.

Advanced decay stage – A very strong odour of decay was recorded. The exposed body parts had a black appearance. Only skin, cartilage and bone were left at this stage. Skin colour darkened; changed from brown to tan and hair loss increased. Decomposition fluid drained from the carcass, and seeped into the soil.

Skeletal (Remains/dry) stage– This stage was recognised when only bones and hair remained. The skin, cartilage and exposed bones became dry and bleached, with a cheesy smell. There were a few insects on the carcass.

Table 2-4: Description of key features characterising stage of decomposition of the 4 carcasses.

Stage	Skin colour change	Decomposition fluid leakage	Odour	Body condition	Arthropod succession
Fresh	No discoloration	Nil	Nil	Nothing changes	1st flies lay eggs on face
	White-pink				
Bloating	Brownish in certain areas; nose, ear	Small leakage from the orifices	Strong	Skin slippage	1st instar of maggots
Active decay	Green discoloration	Fluid leaked and pooled under bodies	Strong	Produce sagging of flesh	Extensive maggot activity
	Marbled appearance			Decayed in different body part	
Advanced decay	Black or darkened on arms and legs	Pools accumulated under bodies	Peak levels	Leathery appearance	Large maggot masses
Skeletal (Remains)	Black mummified	Black stained	Cheesy smell	Mummified tissues	Few maggots feeding tissue left
				Dry bones	

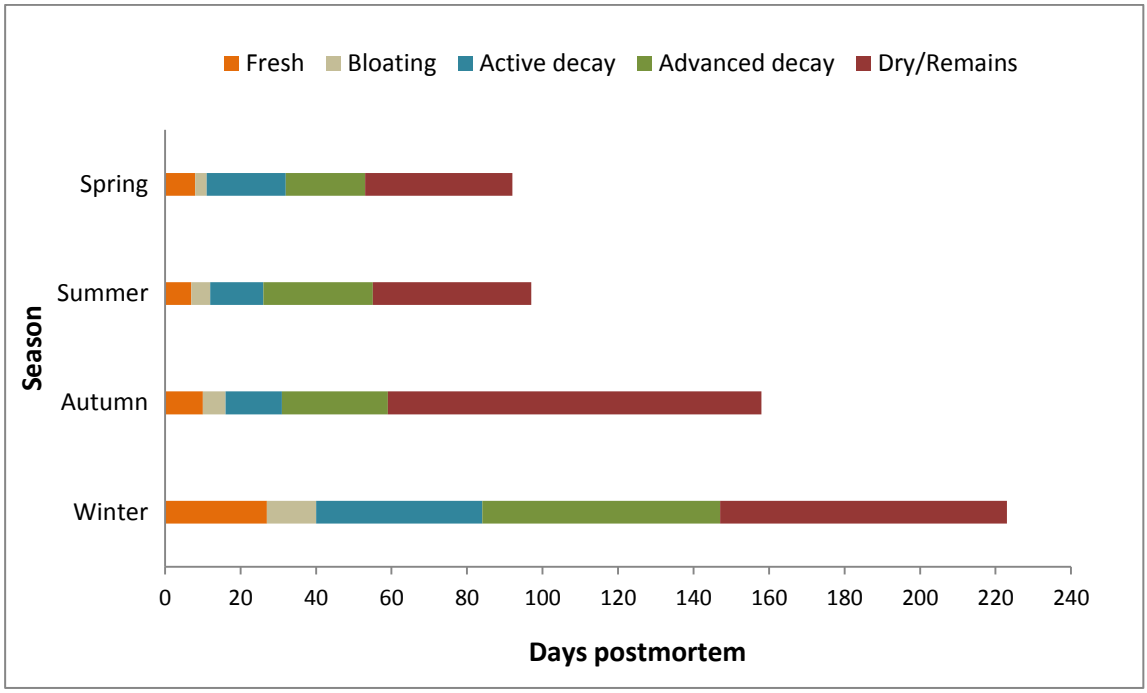

Figure 2.7: The duration of decomposition of each stage for the carcasses

2.3.3 Mite community

The succession of mites of each decomposition stage was compared among the seasons (Figure 2.5) with a grand total of 550 individuals of mites from 50 species belonging to the four main groups; Mesostigmata, Prostigmata, Astigmata and Oribatida were identified from the 4 experimental seasons (one carcass used for each season). Until present many prostigmatid, astigmatid and oribatid species were not confirmed; hence species identifications for these three groups were not included. Thirty six species were identified in Mesostigmata followed by six species of Oribatida, four of Astigmata and four of Prostigmata.

The highest number of mites was recorded in spring (46.8%) followed by summer (26%), autumn (18.3%) and the least in winter (8.9%) of total mites counted. The most abundant and consistent group present in all seasons was Mesostigmata that consisted 68.2% of total mites. Mesostigmata family composition was similar among seasons except that more species were counted in summer and spring. The ANOVA test (Table 2.5) showed the decomposition stages have significantly effect on the abundance of Mesostigmata, while changing of seasons has no significantly effect.

Table 2-5: Analysis of variance on the abundance of Mesostigmata on decomposition stages and seasons

Anova: Two-Factor Without Replication

SUMMARY	*Count*	*Sum*	*Average*	*Variance*
active decay	4	84	21	414
advanced decay	4	37	9.25	62.91666667
bloating	4	5	1.25	0.916666667
fresh	4	6	1.5	5.666666667
skeleton	4	81	20.25	15.58333333
Autumn	5	25	5	65.5
Spring	5	93	18.6	254.3
Summer	5	68	13.6	214.3
Winter	5	27	5.4	49.3

ANOVA

Source of Variation	SS	df	MS	F	P-value	F crit
Rows (stage)	1493.3	4	373.325	5.331310246	0.010549843	3.259166727
Columns (season)	656.95	3	218.9833	3.12721647	0.065879083	3.490294819
Error	840.3	12	70.025			
Total	2990.55	19				

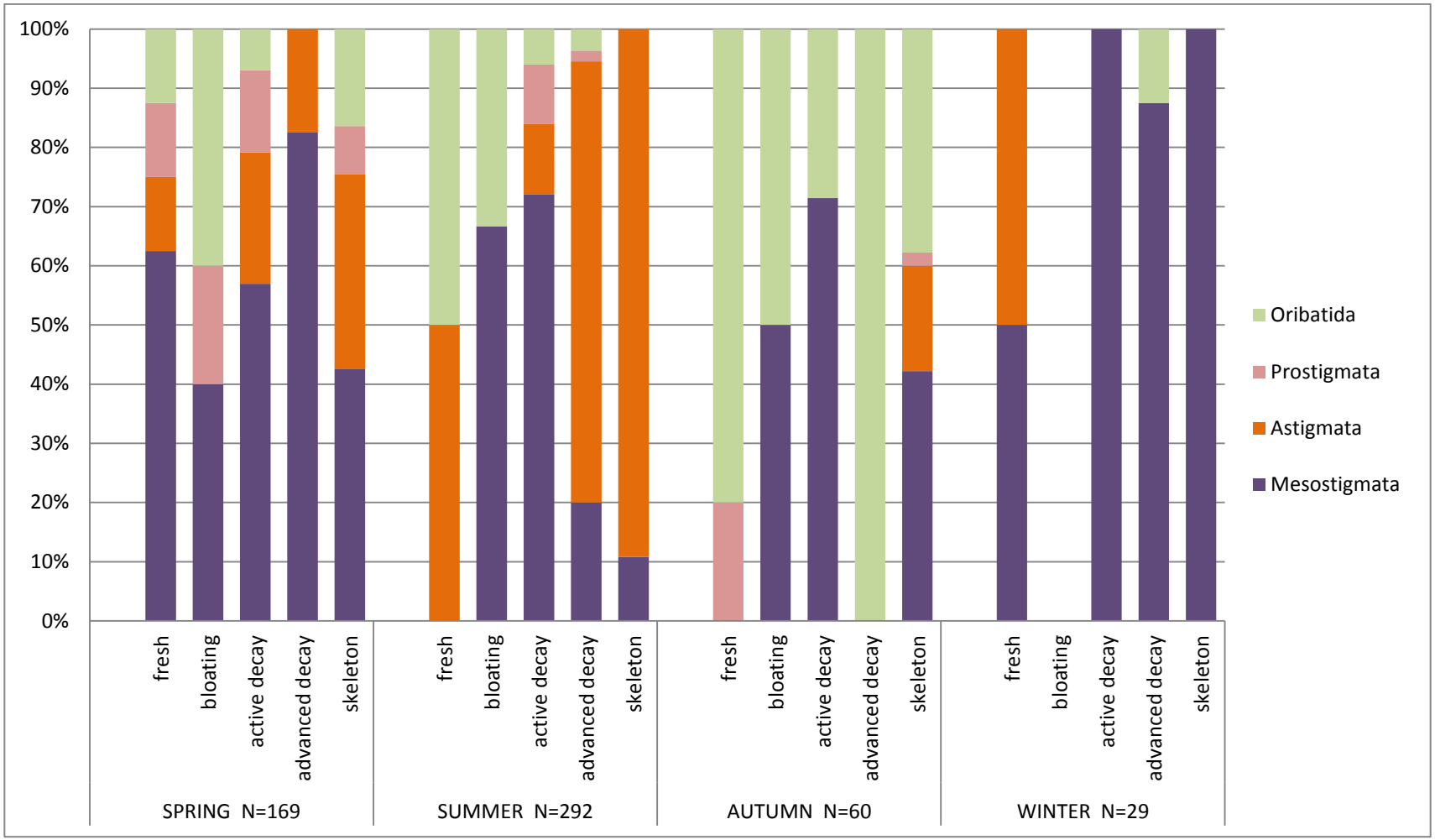

Figure 2.8: The proportion of mites' main groups according to decomposition stages in four seasons.

Among the Mesostigmata, species from the family Macrochelidae were the most frequent throughout all seasons, followed by Parasitidae (Table 2.6). Two species of Macrochelidae; *Macrocheles glaber* and *M. matrius* and three species of Parasitidae; *Cornigamasus lunaris, Poecilochirus carabi* and *Gamasodes spiniger* were the most collected from all carcasses during the last three stages of decomposition. While most of these species were recorded in spring and summer, there were some seasonal differences in relative abundance noted and different species exhibited peaks in each season. The most abundant species collected in last three stages was *Macrocheles matrius* with peaks from the advanced decay (10 individuals), skeleton stage (7 individuals) and active decay (4 individuals). All were collected in spring. While *Cornigamasus lunaris* and *Poecilochirus carabi* were collected in spring, summer and autumn. *Macrocheles nataliae* was the only species collected in the bloating stage and only in the summer season. On the other hand, environmental conditions also play an important role, since there were species such as; *Cornigamasus sp., Parasitus coleoptratorum*, *P. loricatus, Gamasodes spiniger* and *Holostaspella sp.* which tolerate cold better than other species when they were found in winter. Two species of Macrochelidae and three species of Parasitidae have been selected as marker as they were present abundantly or with a single appearance throughout the decomposition stages in the four seasons. *M. glaber*, *M. matrius*, *Cornigamasus lunaris*, *P.carabi* and *Gamasodes spiniger* confirming their association with the decomposition of carrion.

Table 2-6: The presence of Mesostigmata families and species in four seasons throughout all decomposition stages. A:Autumn, WI:Winter, SP:Spring, SU:Summer

Specimen	Fresh				Bloating				Active decay				Advanced decay				Remains			
	SP	SU	AU	WI	SP	SU	AU	WI	SP	SU	AU	WI	SP	SU	AU	WI	SP	SU	AU	WI
Mesostigmata																				
Macrochelidae																				
Macrocheles carinatus									1								1			
M.muscaedomesticae									1				1				1			
M. punctatissimus																			1	
M.perglaber									1								1			
M.glaber									5	12							1			
M.matrius									4				10				7			
M.subbadius										7				2						
M.montanus																			1	
M.spiniger									1								1			
M.merdarius													1							
M.nataliae						1														
M.mammifer																		1		
M.punctoscutatus	1																			
Glyptholaspis confusa																		1		
Cornigamasus sp.												1								
Holostaspella sp.												1				1				
Parasitidae																				
Pergamasus sp																			1	
Cornigamasus lunaris									1				2	1				13	1	
Poecilochirus carabi									7	11	2			3			1	1		
P.austroasiaticus									1											
P.hyalinus										1			1	1			1			
P.fimetorum																		1		
P.coleoptratorum				1					1							2			4	3
P.loricatus																1				
Gamasodes spiniger									1							3			5	1
G. fimbriatus																				2
Parholaspididae																				
Parholaspis kewensis																				3
Laelapidae																				
Crytolaelaps sp							1				1									
Uropodellidae																				
Uropodellidae1	2																		1	
Uropodellidae2											1			1			1		1	
Heterozergonidae																				
Heterozergonidae1																			1	
Antennophoridae																				
Antennophoridae1																			1	

the most abundant species

Faunistic diversity indices (Table 2.7) were calculated by referring to the Mesostigmata species and families in all four seasons. According to Simpson's Index, summer scored the highest (D=4.89), followed by spring (D=3.24) while in terms of species evenness, winter showed the highest while autumn showed the lowest. High Simpson dominance index indicate high diversity. Evenness measure the equal abundances in the community. In winter the proportion all species are equal abundance even it has a low diversity.

Table 2-7: Diversity indexes associated to the four seasons.

Season	Richness (S) (n)	Simpson Index (D)	Evenness (J')
Autumn	22	1.3967	0.0457
Spring	60	3.24	0.0798
Summer	33	4.8935	0.1026
Winter	16	0.1183	0.1183

According to GLM, fitting the data to a Poisson distribution, there were strong positive interactions between the decomposition stages and Mesostigmata species, with higher significance in three stages, namely the fresh, bloating and active decay stages (Z=6.574, P<0.001; Z=-4.570, P<0.001, Z=-6.187, P<0.001,

respectively; see Table 2.8). Meanwhile, there was no interaction between seasons as there was no replicate of seasons.

Table 2-8: Regression analysis on the abundance of Mesostigmata with the decomposition stages

Box 1

```
Deviance Residuals:
    Min       1Q   Median       3Q      Max
-2.0242  -1.8371  -0.5732  -0.0342   5.5190

Coefficients:
                    Estimate Std. Error z value Pr(>|z|)
(Intercept)           0.7172     0.1091   6.574 4.91e-11 ***
Stagesadvanced decay -0.5721     0.1973  -2.899  0.00374 **
Stagesbloating       -2.1035     0.4603  -4.570 4.89e-06 ***
Stagesfresh          -2.6144     0.4226  -6.187 6.14e-10 ***
Stagesskeleton       -0.1940     0.1557  -1.246  0.21285
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

(Dispersion parameter for poisson family taken to be 1)
    Null deviance: 595.92  on 180  degrees of freedom
Residual deviance: 485.89  on 176  degrees of freedom
```

2.3.4 Mites as forensic marker

Based on the data collected throughout all the decomposition stages, mite species with the potential to be markers for season and/or decomposition were sorted and selected. Mites of several orders inhabit carcasses and the colonization taxa are in a predictable sequence is forensically significant. Here the list of the selected mites as markers with their characteristic and previous relevant literature (Table 2.9).

Table 2-9: Summary of the phoretic mites and their association with insect described in literature.

Mite	Peak abundance (Stage/Season)	Hosts described in literature	Reference
Macrocheles glaber	Active decay, skeleton/ spring, summer	*Onthophagus spp.*	Halliday, 1980
M.matrius	Active, advanced decay, skeleton/spring	Mammals	Krantz & Whitaker, 1988
Poecilochirus carabi	Active, advanced decay, skeleton/autumn, spring, summer	Silphidae (Coleoptera)	Brown & Wilson, 1192
Gamasodes spiniger	Active, advanced decay, skeleton/ autumn, spring, winter	Muscidae, Drosophilidae	Hyatt, 1980
Cornigamasus lunaris	Active, advanced decay, skeleton/ autumn, spring, summer	Small mammals	Hyatt, 1980

Family : Macrochelidae

Macrocheles glaber (J. Müller, 1860)

Twelve female individuals were collected from the summer carcass in the active decay stage while five individuals were recorded in spring also in the same stage. Only one male was collected in the final stage of decomposition in spring. *Macrocheles glaber* (Fig. 2.10), decomposes organic material especially manure, when there is enough humidity and nitrate (Masan, 2003). This species is

commonly related to decomposition of carcasses and with different Coleoptera families such as Silphidae, Carabidae, Scarabaeidae, Staphylinidae, Histeridae, as well as several Diptera families (Perotti & Braig, 2009). It feeds on newly hatched eggs and small larvae of *Musca domestica*. the house fly (Pereira & Castro, 1945). This mite species is cosmopolitan and found in Europe, Asia, North America and Australia (Kontschan, 2005). This species has shown the association with the decomposition stages that taking place in summer and spring.

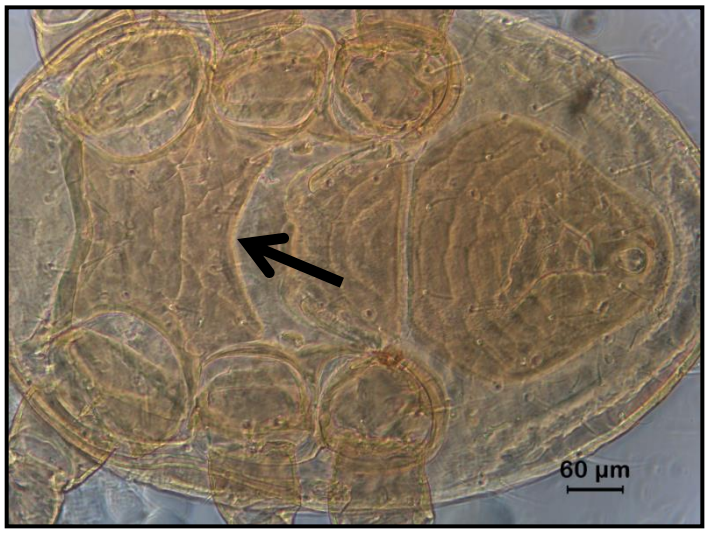

Figure 2.9: *Macrocheles glaber* female with sternal shield with one linea arcuata.

Macrocheles matrius (Hull, 1925)

Twenty-one individuals of females collected in spring were found during active and late decay stages (advanced and skeleton). This species feeds on house fly eggs and nematodes (Cicolani, 1977). It is a cosmopolitan mite that is abundant in Southern European countries (Greece, France and Italy). Most of the findings were from Silphidae, Lucanidae, Trogidae beetles, as well as on other dung beetles (Niogret, Lumaret & Bertrand, 2006). *M. matrius* (Fig. 2.11) could be used as seasonal and temporal markers; where it found abundantly in spring in stage of advanced decay of decomposition.

Figure 2.10: *Macrocheles matrius* female with sternal shield coarsely punctate.

Family : Parasitidae

Poecilochirus carabi (G. & R. Canestrini, 1882) (Fig. 2.12)

Twenty-five individuals were collected and identified throughout the seasons. Half of them were deutonymphs. They were collected in all seasons except winter, and at the end of the decay processes (active, advanced decay and skeleton). It is primarily associated with the silphid genus *Nicrophorus*, the burying or sexton beetles (Coleoptera: Silphidae) (Hyatt, 1980). The mites ride on carrion beetles as deutonymphs and feed on the carcass.

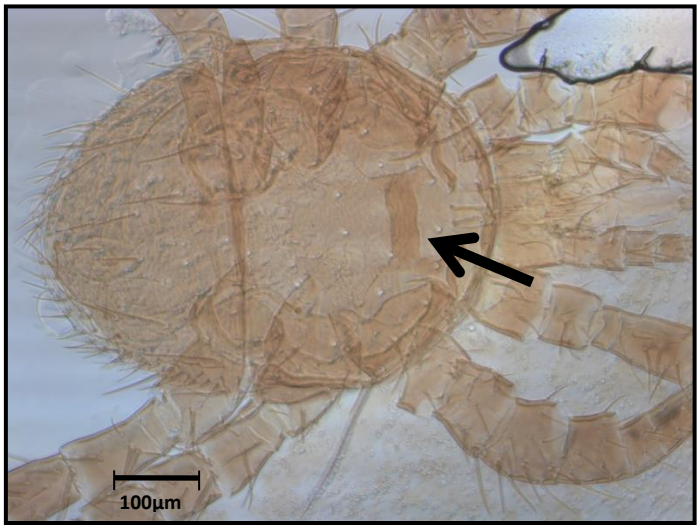

Figure 2.11: A *Poecilochirus carabi* deutonymph with lintercoxal shield with granular tranverse band

Gamasodes spiniger (Trägårdh 1910)

Five deutonymphs and one female adult were collected and identified throughout the experiments. This species is identified through its unique and special sternal plate shape (deutonymphs). The mites are predators and were observed to feed on small collembola. They are commonly found on birds, small mammals and small scavenger insects such as flies of Sphaeroceridae (Diptera). They are recorded to be found in many European countries such as Sweden (Trägårdh, 1910), France (Cooremann, 1954) and Italy (Valle, 1955). *G. spiniger* (Fig. 2.13) could be used as a temporal marker as they were collected at late stages of decomposition in all seasons (except in winter).

Figure 2.12: *Gamasodes spiniger* deutonymph with unique sternal plate shape.

2.4 DISCUSSION

2.4.1 The effects of seasons on the decomposition process

The decomposition changes shown in all seasons in this study are similar to those recorded by Payne (1965); however, there were stages that have a combination of decay stage characteristics. From the study, it is shown that the carcasses in summer and spring decayed at a faster rate compared to autumn and winter. However the decomposition periodfor each decay stage for both carcasses varied.. For example, even though total cadaver decomposition from fresh to skeletal for spring lasted 94 days and summer was 98 days, the fresh stage for spring lasted 6 days and for summer lasted 4 days. Meanwhile, the advanced decay stage for spring lasted 21 days and summer lasted 29 days. However, the initial decomposition stages; fresh and bloating of carcass in summer lasted for 2 weeks, whereas in spring it lasted approximately 3 weeks. The delay in spring is assumed to be due to the drop in average ambient temperature (10.85° C) in both stages, as compared to summer (16.33° C)., The onset of the skeletal/dry stage took place after 60 days from the start of the experiments. The temperature and rainfall in summer showed a fluctuating pattern. The increase of temperature sped the chemical reaction rate, triggering the increase of growth and feeding rate of necrophagous insect larvae (Anderson & Vanlaerhoven, 1996; Gill-King, 1997). This was coupled with higher relative humidity, influencing the decomposition rate. This contributed to rapid decay, whereas cold temperature prolonged the decaying process.

In winter, the decomposition period for each stage was prolonged. Temperature that ranged from 3 °C to 14 °C and rainfall that ranged from 0 mm to 1.7mm for the whole season; had affected decomposition period. The carcass appeared fresh for nearly a month and did not show the sign of bloating stage. However, the stages were both separated, in which the bloating stage was determined via the changes of skin colour after a long period of fresh stage. All the decomposition stages took longer in this season. On most days, soil sampling occurred in wet soil, due to moisture by more constant rainfall. However, soil moisture and increased relative soil humidity within the soil environment can also increase rate of decay and soil humidity is not only affected by rainfall but also other factors such as fluid leakage from the decomposing carcass. Continuous wet soil prevented the carcass from drying out and encouraged maggot and bacterial action in the fleshwhich prolonged the period of certain stages. According to Smith (1986), the rehydration of dried remains during rainfall sometimes allows carcass recolonization by blowflies. The carcass internal temperature was recorded as maggot masses developed from the inside of the body. The highest body temperature was recorded in spring (21.40 °C) while the lowest has been recorded in winter (-5.3 °C) which both were recorded during the advanced decay.

The designation of decomposition stages is relatively subjective and some invertebrates may vary in their stage associations (Archer, 2003). Carcass decomposition time and rate was profoundly influenced by the season during which exposure first occurred and this will affect the number and diversity of

insects visiting carcasses (Lopes de Carvalho & Linhares, 2001; Archer, 2002). The changes of seasonal temperatures affect decomposition rate, which differ greatly in insect succession (Archer & Elgar, 2003). As most of the mite species that colonise decomposition are associated with insects, it is important to use this information in this study. This study considered seasonal changes in mite succession on pig carcasses, following experiments that were held in the same habitat. In summary, mite assemblage significantly differed over time, with the progression of carcass decomposition within seasons. The decomposition stages used in the previous research used PMI estimations; such decomposition stages are determined by physical changes in the carcass (Segura et al., 2009). The stages often follow one after the other but there is no clear distinction when one stage ends and another begins. Decomposition stages could be characterised by distinctive mite communities. As expected, over a year, mite succession changed; largely due to shift of climatic conditions. Therefore in general, the surrounding environment; was an influential factor on outdoor decomposition due to variations in temperature, rainfall, humidity and other factors associated with each season (Jalil & Rodriguez, 1970).

2.4.2 Mite abundance throughout seasons

The influence of seasonal variation in meteorological conditions on mite species abundance has never been documented before. However, the studies of insect succession in seasonal variation are well documented (Souza & Linhares, 1997; Linhares & de Carvalho, 2001; Arnaldos et al., 2005), thus this information was

used to study mite behaviour. After temperature, access to the body by insects is the most important factor affecting the decay rate (Meadows, Mann, & Bass, 1990). In the cold seasons (autumn and winter), there are fewer insects infesting the carcass. As the two most abundant groups of carrion arthropods are insects and mites (Bornemissza, 1957; Braack, 1987; Perotti & Braig, 2009), in this condition the presence of mites will be used to gather information for the decomposition. Mite assemblage at carrion is dominated by phoretic mesostigmatid mites (Perotti et al., 2010; Barton, Weaver, & Manning, 2014). In general, the biodiversity of mites was very low. The species that were the most eminent throughout the decomposition process were mesostigmatid mites. According to the result, the most abundant families belong to the Mesostigmata order; that makes up 64% of the total mites collected from the carcasses. The dominant families Macrochelidae and Parasitidae played a fundamental role in the carcass decomposition. These families were present at carcasses in all decomposition stages throughout the four seasons, confirming their roles as major factors in carcass decomposition and significance to forensic acarology. Other species were identified up to family level and were then separated in to Laelapidae, Uropodellidae, Heterozergonidae and Antennophoridae families. There were several species across seasons and throughout decomposition stages which are the major contributors to the difference in the seasonal community of decomposition (Lopes de Carvalho & Linhares, 2001; Archer, 2002).

Based on the data collected throughout the year, mite species with the potential to be markers for season and/or decomposition stages were sorted and selected. The Mesostigmata species were numerically higher in the late stages, showing some distinctive seasonal peaks. *Macrocheles glaber*, *M. matrius*, *Cornigamasus lunaris*, *Poecilochirus carabi* and *Gamasodes spiniger* all are good taxa for forensic use. Each mite species depicts a unique set of seasonal adaptation, due to resource exploitation by their carrier in a variety of microhabitats. These mites have short life cycles and their timetables are predictable. Most of mites synchronise their life cycle and development with their hosts (Perotti & Braig, 2009; Perotti, Braig & Goff, 2012; Saloña-Bordas & Perotti, 2014). Perotti discovered that most mites collected from corpses were carried phoretically by flying insects (Perotti & Braig, 2009; Perotti et al., 2009; Perotti et al., 2010). Phoretic mites can be highly specific; they only arrive on a particular host or carrier, which has a unique complex of physical, chemical and behavioural characters (Athias-Binche, Schwarz & Meierhofer, 1993; Krantz, 1998). Deutonymphs of *Poecilochirus carabi* are phoretic on Silphidae burying beetles (Baker & Schwarz, 1997). This family from order Coleoptera has many species that occur on carrion (Cole, 1942; Payne, 1965; Andersen, 1982). *Poecilochirus carabi* was collected in spring, summer and autumn while *M. matrius* was only found in spring and this special presence could be selected as marker of season. Even though *Parasitus loricatus* and *Gamasodes fimbriatus* were only found from the carcass forwinter decomposition, their presence could not be considered as a marker of seasons since it was already spring. Therefore, eventhough their presence may not associated to a season, they

may be potential markers of late cadaver decomposition. The temperature increased according to the change of season. Thus, the possibility of these two species to be markers for seasons was rejected.

This specific characteristic of mites such as host specificity shows the potential value of phoretic mites as markers. Mites of several orders inhabit carcasses and the taxa that colonise carcasses in a predictable sequence are forensically significant. These species were highlighted as markers of specific seasons and stages.

2.4.3 Mesostigmata and environmental factors.

The colonization observed by Macrochelidae agreed with that reported in previous studies with species from this family being the most frequent to discover and colonize cadavers (Perotti & Braig, 2009). There was strong positive correlation between certain environmental factors and the abundance of Mesostigmata, specifically wind speed and the abundance of taxa. Mesostigmata associated with carcasses are phoretic and therefore, their occurrence is link to their scavenger insect hosts arriving on or leaving a carcass (Perotti et al., 2010). If they do not have carriers to leave the carcass towards a new habitat, they will likely increase in numbers until a 'transport' carrier is available. With strong winds, insects especially flies and beetles avoid flying because of movement

difficulty with most insects tending to follow the direction of air current although some are inclined to go against it (Hurd, 1920).

The carcass temperature and the light intensity too have a positive relationship with the abundance of Mesostigmata. Both environmental factors seem to affect mite occurrences, in which they increase in number under the warmer conditions and avoid direct sunlight by investing in reproduction and development. The carcass temperature affects the mites' surrounding. According to Voss (Voss, Forbes, & Dadour, 2008), they observed heat generation by larval aggregation that largely contributes to the decomposition process, regardless of ambient temperature changes. Larval aggregation produces heat, and thus increases the temperature by several degrees above the surroundings (Campobasso et al., 2001). This finding coincides with the previous studies that concluded that the carrion internal temperature is elevated during decomposition due to bacterial metabolic reaction (Payne, 1965; Rodriguez & Bass, 1983; Anderson & Vanlaerhoven, 1996). For the light intensity, mites are negatively phototropic, in which they avoid direct sunlight in their development. However, the results showed a higher abundance of mites when the light exposure was high. The mite phototropism behaviour does not apply to insects. Insects favour areas with light to produce progeny. Sun-exposed carrion attracts more species in terms of diversity and number as compared to the shaded area (Sharanowski, Walker & Anderson, 2008). This happens on the surface of a carcass since the lux meter was used to read the light intensity on the body. The mites collected in this experiment

were from the soil sampling, underneath the carcass and not where the light readings took place, therefore, this is an indirect effect. My interpretation of the positive correlation is that the more intense the light the more the mites avoid sunlight by seeking darker areas such as underneath the body or within the soil directly beneath the carcass (Walter & Proctor, 1999).

2.5 CONCLUSION

The results from this study are the first to demonstrate that there are possible variations in the abundance and diversity of mites associated with cadaver decomposition during different seasons. This was the first attempt to associate seasons and mites as forensic markers. During this study, five Mesostigmata species were chosen and considered as valuable forensic markers. The markers were selected according to their 'special' presence throughout decomposition stages in certain seasons. They became markers for certain seasons or stages. *Macrocheles glaber* for temporal and seasonal marker (active decay and warmer temperatures), *Macrocheles matrius* for both temporal and seasonal markers (later decomposition stages and spring), *Poecilochirus carabi* for a temporal marker (later decomposition stages), *Gamasodes spiniger* for both temporal and seasonal markers (later decomposition stages and colder temperatures) and *Cornigamasus lunaris* for a temporal marker (later decomposition stages). There were positive correlations between the abundance of Mesostigmata with certain microclimatic effect; wind speed and condition of the carcass; body's temperature and exposion

to sunlight. These correlations will effect the decomposition process and the presence of arthropods onto the carcass.

Chapter 3 : The contribution of Mesostigmata mites to the carcass position.

3.1 INTRODUCTION

This chapter discusses the process of decomposition in an outdoor setting that involves forensic analysis, considering direct contact with or hanging above the soil. The decomposition at outdoor environment consist two primary habitats, each with their own chemical profile; the cadaver itself and the soil into which the cadaveric fluids are released (Aitkenhead-Peterson et al., 2015). During decomposition, materials from the carcass physically enters the associated soil, providing a localized pulse of nutrients (Carter, Yellowlees & Tibbett, 2007) which results in the formation of a concentrated island of decomposition fluids that transform the landscape and it is known as a Cadaver Decomposition Island (CDI) (Carter, Yellowlees & Tibbett, 2010). CDI can alter steady-state edaphic and biological characteristics (Hopkins, Wiltshire & Turner, 2000). Each cadaver acts as a specialised habitat for several organisms and this is important in ecosystem processes. Numerous insect species and other arthropods will occur on or around a cadaver during decomposition (Amendt et al., 2011). Forensic entomology estimates an accurate PMI (Post-mortem interval) according to assumption made that insects, usually blowflies, will discover the dead bodies soon after death (Hall, 1990; Catts, 1992). The blowflies are attracted to body fluids like urine, saliva and

faecal material protruding from natural orifices or open wounds (Sumodan, 2002). Soil chemistry under decomposing bodies has been used to estimate PMI (Vass et al., 1992; Tumer at al., 2013) however the results depend on the environment factors that affect the soil properties. Not until recently, the use of soil in forensic investigations has focused on comparison of soil particles from the evidence and crime scenes (Pye, 2007; Ritz, Dawson & Miller, 2008).

Hanging is one of the most common methods of suicide around the world (Dedouit et al., 2007). This likely stems from the easy accessibility of victims to a myriad of possibilities to suspend themselves and the relatively rapid lethality of this method to commit suicide (Sharma, Singh, & Harish, 2005). Hanging resulting from suicide, accident and (more rarely) homicide, is not an uncommon form of death. A body suspended above the ground could present a unique environment for insect succession, particularly in the soil below. The process of decomposition of hanging bodies differs from bodies lying on soil and alters the colonization of insects by excluding some soil-dwelling taxa (Saloña-Bordas & Perotti, 2014). This can reduce the number of insects and influences the colonization of certain species on the remains (Goff & Lord, 1994). There are few cases reporting the insect fauna associated with decomposition of hanged corpses and carcasses (Arnaldos et al., 2005; Martins & Thyssen, 2005; Saloña-Bordas & Perotti, 2014). Different necrophilous insects are attracted to the corpse and changing over time thereby, the colonization of the corpse will occur in a predictable sequence (Amendt, 2004).

In this study, the information provided by Mesostigmata mites in the soil beneath several pig carcasses was examined with the aim to add information of forensic importance about the soil fauna and its role as indicator of a different decomposition process; that of hanging-remains. This study was part of a working group project that included scientists from Switzerland (Neuchatel University), Frankfurt (University of Frankfurt) and University of Reading. The collaboration focused on the effects of decomposing cadavers on the soil fauna and chemicals below (hanging-carcasses) and underneath (carcasses on the ground) by using several methods to establish new forensic indicators that may aid in solving criminal cases.

3.2 MATERIALS AND METHODS

3.2.1 Experimental site and design

The experiment took place in a forested area close to Neuchatel University, and six pig carcasses were used. The study site covered an area of 1200 m^2 in a small spruce (*Picea abies*) forest at the Bois-du-Clos, near Neuchatel, Switzerland. A total of 9 plots (ca. 4 metres from each other) with three treatments (and three replicates each) were set up. The treatments were the control (bare soil), surface pigs (carcasses placed directly on the ground) and hanging pigs (carcasses hanging 1 m above the ground) (Fig. 3.1). Six domestic pigs (*Sus scrofa domesticus*), were bought from a local farm and sedated with Stresnil® (Azaperone) and euthanized with T61® by a veterinarian. The carcasses were

immediately transported to the experimental site, weighed and placed on the plots. The average weight of carcasses was 27.8 kg ± 0.8 kg (SE). All carcasses were placed in cages (140 cm x 95 cm) surrounded by wire mesh fences to exclude any scavengers and larger animals, but allow free access of insects. The experimental area was surrounded by an electric fence for additional protection. Controls were marked with sticks and cords. One side each of the fences and cages was accessible for soil sampling and carcass-weighing. Carcasses were weighed just before the start of the experiment and on every sampling day, until Day 331 using a digital hanging scale (Fig. 3.2).

All procedures were approved by the Committee of Ethics in Animal Experimentation at the University de Neuchatel, Switzerland.

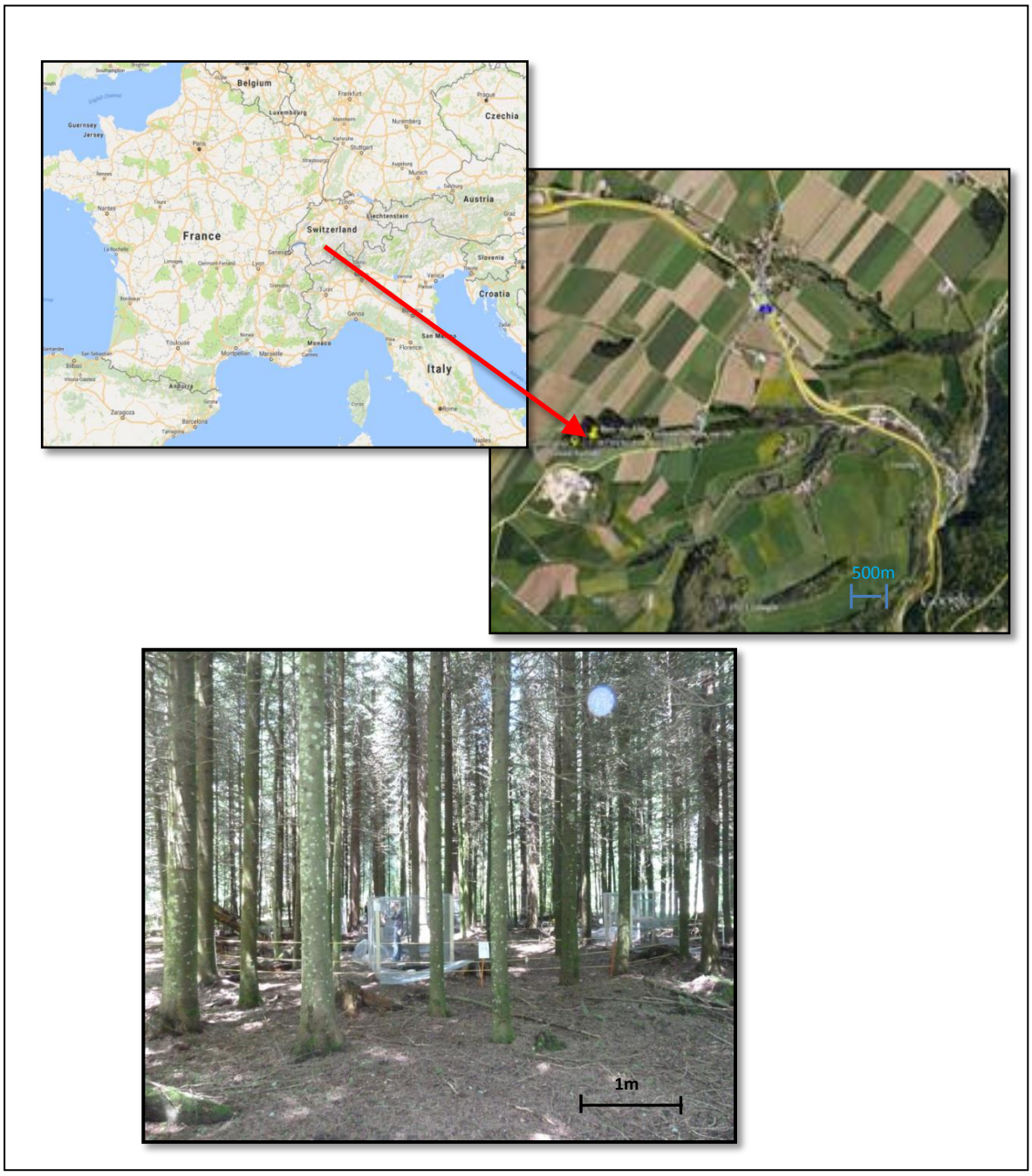

Figure 3.1: Study site, forest at the Bois-du-Clos, Neuchatel, Switzerland.

Figure 3.2: The experimental design and treatments (a) the plot for control soil (b) the carcass on the ground (c) the hanging carcass (d) the environmental probes.

3.2.2 Decomposition stages and sampling

Decomposition stages and patterns are described in detail in Payne, (1965)(Table 3.1). During the early stages (fresh until dry), each pig carcass was examined daily to record the state of decomposition based on the physical characteristics and arthropods present. When it started the dry stage, the carcasses were monitored at longer intervals (>9 days). Random sampling was done based on coordinates; a wooden frame identical in size with the experimental cages with x (letters) and y (numbers) was placed on the ground at each site. The coordinates corresponding to 10 subsamples per plot were selected by raffles for a random sampling and to avoid re-sampling on the same place.

Table 3-1: Description of decomposition stages in this experiment

Stage of decomposition	State of the carcass
Fresh stage	No visible external changes
Bloating	Accumulation of gases in the abdomen, bloating of the body
Active decay	Ruptures in the skin, release of cadaveric fluids, extensive loss of mass
Advanced decay	Body starts to dry
Dry	Dry skin, cartilage, and bones
Skeleton	Skin and flesh have been removed, leaving teeth, bones and hair

3.2.3 Environmental parameters

Mean temperature and total precipitation were measured on-site with a Decagon Em50 digital data logger that was centrally located and set as the experimental meteorological station.

3.2.3 Cleaning and identification of soil arthropods

The separation of arthropods from soil was performed in the Acarology Lab, School of Biological Sciences, University of Reading to proceed with the identification and studies on mites. The contents from collection jars were sorted based on taxa; mites, insects, myriapods, nematodes and other invertebrates under the stereomicroscope. Mites were collected in separate vials for each sample, labeled and preserved. Samples were preserved in 70% ethanol until further processed for identification.

The basic methods for cleaning, mounting and identification were identical with the previous experiment (Chapter 2).

3.2.4 Diversity Indexes and mite composition

Faunistic indexes for the major families of Mesostigmata were based on the indices of species richness, and diversity indices of Evenness, Simpson, and Shahnon Weiner (H') were applied.

3.2.5 Data and statistical analysis

Total individuals per plot for each species were counted and used for analyses. For the analysis of mite communities collected from soil, the whole dataset was used which is the total count of species, families and orders in three replicates. Then, further analysis compared the abundance of mites through the decomposition stages in different body positions. In order to compare the abundance of Mesostigmata between the hanging and the surface pigs, the non-parametric Kruskal-Wallis test was employed. Estimation of initials was conducted using the statistical software PAST (Hammer et al., 2001).

3.2.6 Multivariate analysis

Principal component analysis (PCA) was conducted on the abundance of Mesostigmata between carcass positions and decomposition stages using PAST (Hammer et. al., 2001) to reduce the number of variables. We therefore removed decomposition stages variable since it did not show a large different in the number of mesostigmata throughout the different stages. The relationship of mesostigmatid richness with different stages of decomposition was analysed using a Generalised Linear mixed-effects model; stages and plots are fixed effects and the number of Mesostigmata is a random effect. Model fitting and estimates were obtained with the linear mixed-effect package in R (R studio version 3.2.5, 2016) with a specified 'Poisson' error family. Wald's Z statistic and probability 'P' values of best fit models were quoted.

3.3 RESULTS

3.3.1 Decomposition stages between hanged and ground.

The decomposition process involves the study of hanging and on-the-ground carcasses (Fig. 3.3). The changes in stages were defined by body mass loss that was calculatedat each sampling. At the end of the experiment (Day 367), on-the-ground and hanging carcasses had already reached skeleton decay. The decomposition was faster in the hanging carcasses compared with the on-the-ground carcasses. The duration of each stage of decay in hanging carcasses was short which made it end faster. The decay process of hanging carcasses reached the dry (remains) stage on day 45, while the on-the-ground carcasses were still in the marble stages of dry and advanced decay. The carcasses on the ground have fully dried on day 50.

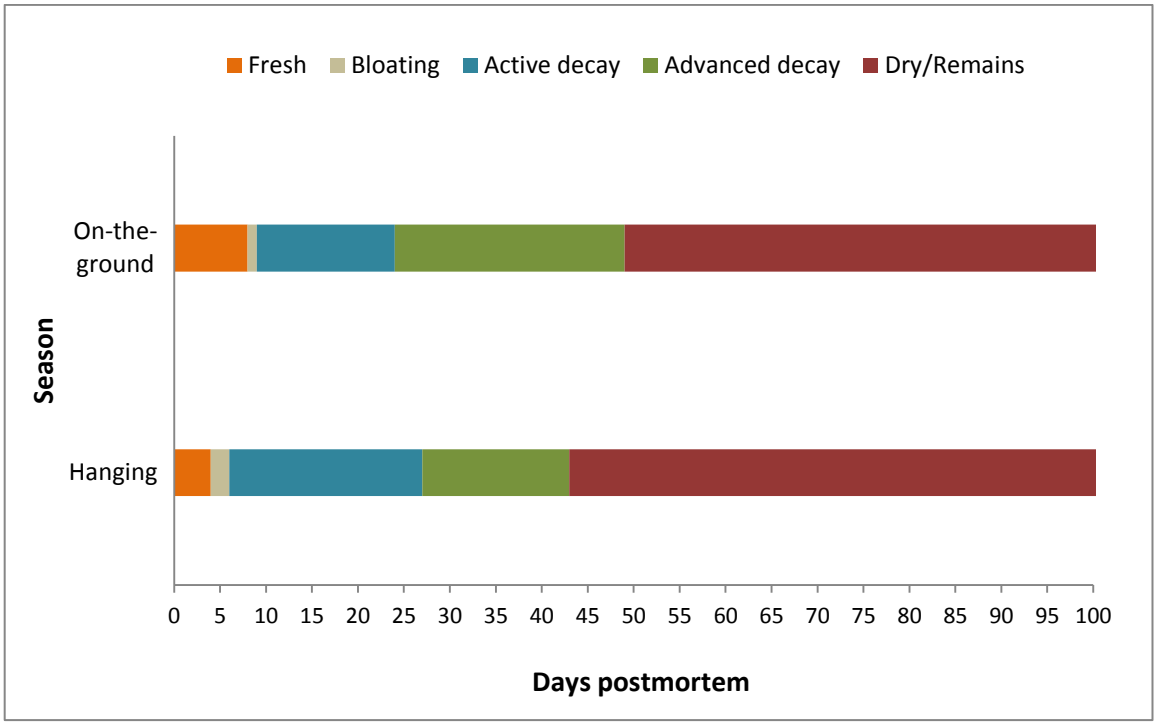

Figure 3.3: The duration (days) each of decomposition stages for hanging and on-the-ground carcasses.

3.3.2 Analysis the diversity of mite community.

A total of 1017 individual mites were sampled during the research, which were ascribed to 4 orders comprising 45 morphospecies; recovered from both treatments (experimental) and control. The majority of mites collected from the soil near the pig carcasses were from the order of Mesostigmata (N=461), closely followed by Oribatida (soil mites) (N=448). The proportions of mite diversities were higher in fresh stage, dominated by soil mites of Oribatida and being outnumbered by Mesostigmata in later stages (Fig. 3.4). Mesostigmata mites collected from on-the-ground carcasses were recorded in the highest number (N=281), which is three times the count mites recovered from the hanging carcasses,N=77.

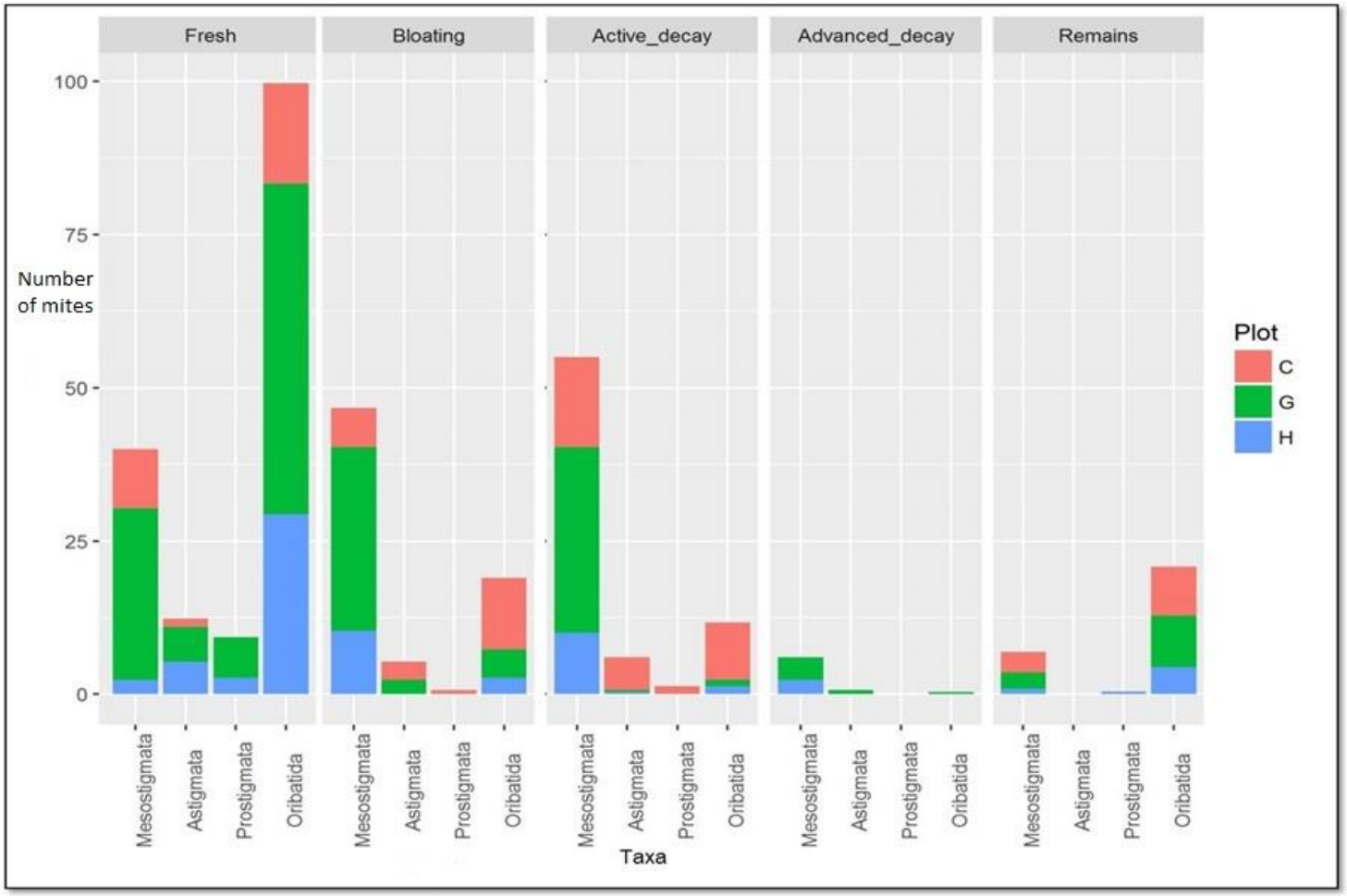

Figure 3.4: The composition of mites from on-the-ground (G), hanging (H) and control (C) treatments throughout the decomposition stages. The mite counts were the total number of three replications.

To analyse the diversity of mites in the control and experimental carcasses, several indices were used; Simpson's Diversity Index, Shannon Weiner Index, evenness and species richness (Fig. 3.5).

Meanwhile, the highest diversity family of Mesostigmata was recorded from on-the-ground carcasses (N=20; N is family), and the hanging plot recorded the least number (N=12). The composition of species in all plots throughout the decomposition stages is shown in Table 3.2. *Macrocheles glaber* dominated the species abundance throughout all decomposition stages. The highest count was recorded from on-the-ground carcasses during the active stage, followed by *Macrocheles muscaedomesticae* collected during bloating decay. There were three species found only associated with the hanging carcasses; *Parasitus copridis* and *Macrocheles punctoscutatus* (in bloating stages) and *Macrocheles scutatus* that was collected during the active decay stage.

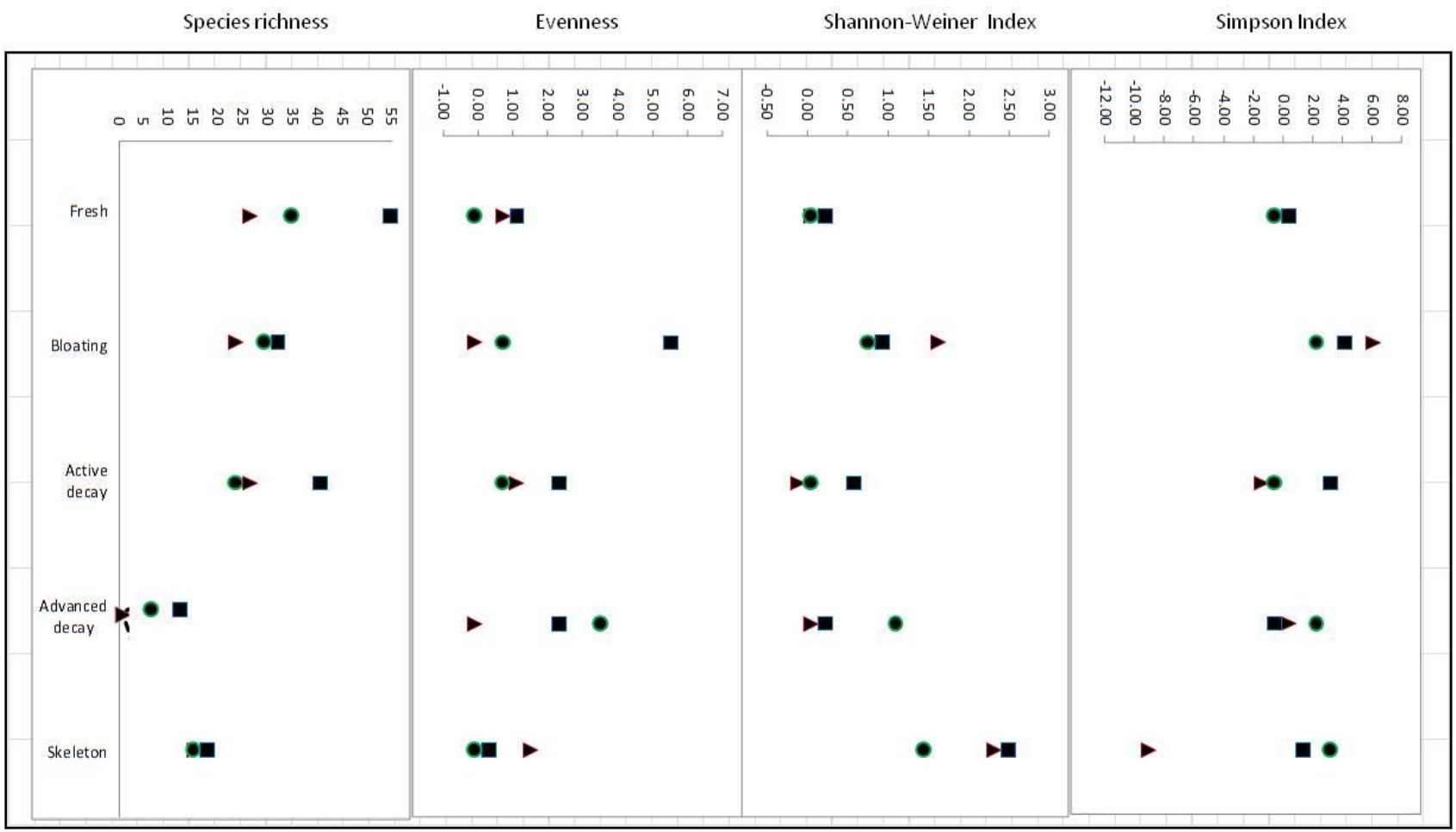

Figure 3.5: Temporal patterns of Mesostigmata (species occurrence) in the three treatments (control, on-the-ground and hanging) through the decomposition stages, using diversity indexes. From left; Species richness, Simpson Index, Evenness and Shannon-Weiner Index. Carcass conditions are indicated by point shape. Square: on-the-ground; Circle: hanging; Triangle: control.

Table 3-2: Comparison of Mesostigmata sampled from the experimental carcasses (on-the-ground and hanging) with the control plots (without carcass).

Key H= hanging G=on-the-ground C=control red numbers >0

Stages	Fresh			Bloating			Active decay			Advanced decay			Skeleton		
Plot	H	G	C	H	G	C	H	G	C	H	G	C	H	G	C
Macrochelidae															
Cornigamasus sp.	0	1	0	0	0	0	2	3	0	0	0	0	0	0	0
Macrocheles scutatus	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
M.muscaedomesticae	0	0	1	4	33	1	1	1	0	0	0	0	0	0	0
M.glaber	0	1	0	5	9	0	8	25	7	1	3	0	0	0	0
M.tardus	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0
M.subbadius	0	0	0	0	8	2	3	1	3	0	0	0	0	0	0
M.merdarius	0	0	0	4	21	0	0	1	0	0	0	0	0	0	0
M.nataliae	0	2	0	1	0	0	0	5	0	2	0	0	0	0	0
M.insignitus	0	0	0	4	2	0	0	1	0	0	0	0	0	0	0
M.punctatissimus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2
M.punctoscutatus	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
Parasitidae															
Vulgarogamasus remberti	0	1	0	0	0	5	0	0	0	0	3	0	0	0	0
V.kraepelini	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0
Cornigamasus lunaris	0	0	0	0	0	0	2	7	0	0	1	0	0	0	0
Parholaspis kewensis	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0
Parasitus hyalinus	0	0	0	0	0	2	1	2	0	0	1	0	0	0	0
P.evertsi	0	0	3	0	0	3	0	0	0	0	0	0	0	0	1
P.copridis	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0
P.loricatus	0	1	0	0	0	0	0	0	4	0	0	0	0	0	0
P.fimetorum	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0
P.mustelarum	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
P.coleoptratorum	0	0	0	0	0	0	6	1	1	1	0	0	0	0	0
P.consanguineus	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0
P.kempersi	1	11	0	0	1	0	0	0	10	0	0	0	0	0	2
Parasitellus crinitus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Eugamasus cavernicola	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
E. berlesei	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0
Trachygamasus ambulacralis	0	4	7	0	0	3	0	0	3	0	0	0	0	0	2
Parasitidae2	0	0	0	4	0	0	0	0	0	0	0	0	0	0	0
Uropodidae															
Uropodidae1	0	2	0	0	3	0	0	0	0	0	0	0	0	1	0
Uropodidae2	0	2	0	0	2	0	0	0	0	0	0	0	0	2	0
Uropodidae3	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0
Uropodidae4	2	0	3	0	0	0	0	0	0	0	0	0	0	3	0
Phytoseiidae															
Phytoseiidae1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Uropodellidae															
Uropodellidae3	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Eviphididae															
Eviphis ostrinus	0	5	1	0	0	1	1	0	4	0	0	0	0	0	2

Table 3.2: continued

Stages	Fresh			Bloating			Active decay			Advanced decay			Skeleton		
Plot	H	G	C	H	G	C	H	G	C	H	G	C	H	G	C
Heterozergonidae															
Heterozergonidae1	0	0	0	0	0	0	0	0	0	0	**1**	0	0	0	0
Ascidae															
Ascidae1	0	0	0	0	**1**	0	0	8	0	0	0	0	0	0	0
Zerconidae															
Zerconidae1	0	**2**	0	0	0	0	0	0	0	0	0	0	0	0	0
Zerconidae2	**2**	**12**	**9**	0	0	**1**	0	0	**1**	0	0	0	0	0	0
Zerconidae3	0	0	**1**	0	0	0	0	0	0	0	0	0	0	0	0
Zerconidae4	0	0	0	0	0	0	0	0	**11**	0	0	0	0	0	0
Parazercone sp.	0	**10**	0	0	0	0	0	0	0	0	0	0	0	0	0
Heatherellidae															
Heatherellidae1	0	0	0	**4**	**5**	0	0	0	0	0	0	0	0	0	0
Pachylaelapidae															
Pachylaelapidae1	0	**1**	0	0	0	0	0	0	0	0	0	0	0	0	0

3.3.4 Mites as forensic markers of carcass positions

Using the list of mites collected from 3 replications for each plot, species that visited the carcasses in specific positions were selected for the analysis. In total, six species from Mesostigmata were identified as valuable markers associated with the carcasses' positions of decompose. The abundance of the species were compiled in Table 3.3. *Macrocheles scutatus, Macrocheles punctoscutatus, Parasitus copridis* and *Parasitus coleoptratorum* were found almost uniquely from the hanging carcasses (lower abundances). *Macrocheles merdarius* and *Parasitus kempersi* were greatly abundant from on-the-ground carcasses. The high number of *Macrocheles glaber* collected from experimental pigs but none from the control plots show the species is a great marker for outdoor decomposition. In order to analyse it as a forensic marker, we eliminated the

factor of different decomposition stages since it did not show a large difference in the number of mites throughout the different stages.

Table 3-3: The abundance of forensic marker mites in different treatments. In yellow; hanging body markers, blue; on-the-ground markers, red; generalist forensic markers. H: hanging; G: on-the-ground; C: control plot

Species	Treatments (body positions)			
	H	G	C	TOTAL
Macrocheles merdarius	4	22	0	26
Macrocheles punctoscutatus	1	0	0	1
Parasitus copridis	2	0	0	2
Parasitus coleoptratorum	7	1	1	9
Macrocheles gl aber	14	38	0	52

3.3.5 Principal Component Analysis

PCA diagram (Fig. 3.5) shows the patterns of colonization of Mesostigmata in different plots treatment and decomposition stages. PCA on mite orders (decomposition stages and treatments as variables) have resulted in 4 components, whereas the first two components account for 82.552% and 13.216% of the percentages of variance. The first component (PC1) was strongly dominated on carcass on the ground. Analysis of variance on PC1 and PC2 for Mesostigmata, indicated significant effects Parasitidae, Ascidae and Zerconidae with fresh and active decay stages. Neither the effect of hanging carcasses nor the control plots with the abundance of major families of Mesostigmata.

3.3.6 Generalised Linear Model (GLM)

Analysis on the number of Mesostigmata throughout the decomposition stages in two different treatments (on-the-ground and hanging) has shown that the overall number of Mesostigmata collected were highest from on-the-ground carcasses, followed by one of the three plots of hanging carcass (H3) (Table 3.5), while there were almost no interactions or small numbers of Mesostigmata from the control and other plots (H1 and H2) of hanging carcasses. There was also a strong interaction of Mesostigmata in all stages of decomposition, except when the decomposition reaches to bloating phase in which the significance of interaction was low.

Table 3-4: Interaction of Mesostigmata in all decomposition stages and different carcass positions

```
Deviance Residuals:
    Min       1Q   Median       3Q      Max
-4.2053  -1.5079  -0.7408   0.9674   4.3345

Coefficients:
                    Estimate Std. Error z value Pr(>|z|)
(Intercept)           2.8307     0.1603  17.656  < 2e-16 ***
StagesAdvanced decay -2.2970     0.2473  -9.290  < 2e-16 ***
StagesBloating       -0.2457     0.1128  -2.178 0.029399 *
StagesFresh          -0.3999     0.1180  -3.389 0.000700 ***
StagesSkeleton       -2.2970     0.2473  -9.290  < 2e-16 ***
PlotC2               -0.4055     0.2357  -1.720 0.085388 .
PlotC3               -0.5108     0.2434  -2.098 0.035867 *
PlotG1                0.4560     0.1905   2.393 0.016700 *
PlotG2                1.1133     0.1718   6.480 9.19e-11 ***
PlotG3                0.6707     0.1833   3.660 0.000253 ***
PlotH1               -0.3102     0.2292  -1.353 0.175959
PlotH2               -0.2513     0.2254  -1.115 0.264809
PlotH3               -1.6094     0.3651  -4.408 1.05e-05 ***
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

(Dispersion parameter for poisson family taken to be 1)

    Null deviance: 664.35  on 44  degrees of freedom
Residual deviance: 175.24  on 32  degrees of freedom
  (3 observations deleted due to missingness)
AIC: 331.11

Number of Fisher Scoring iterations: 6
```

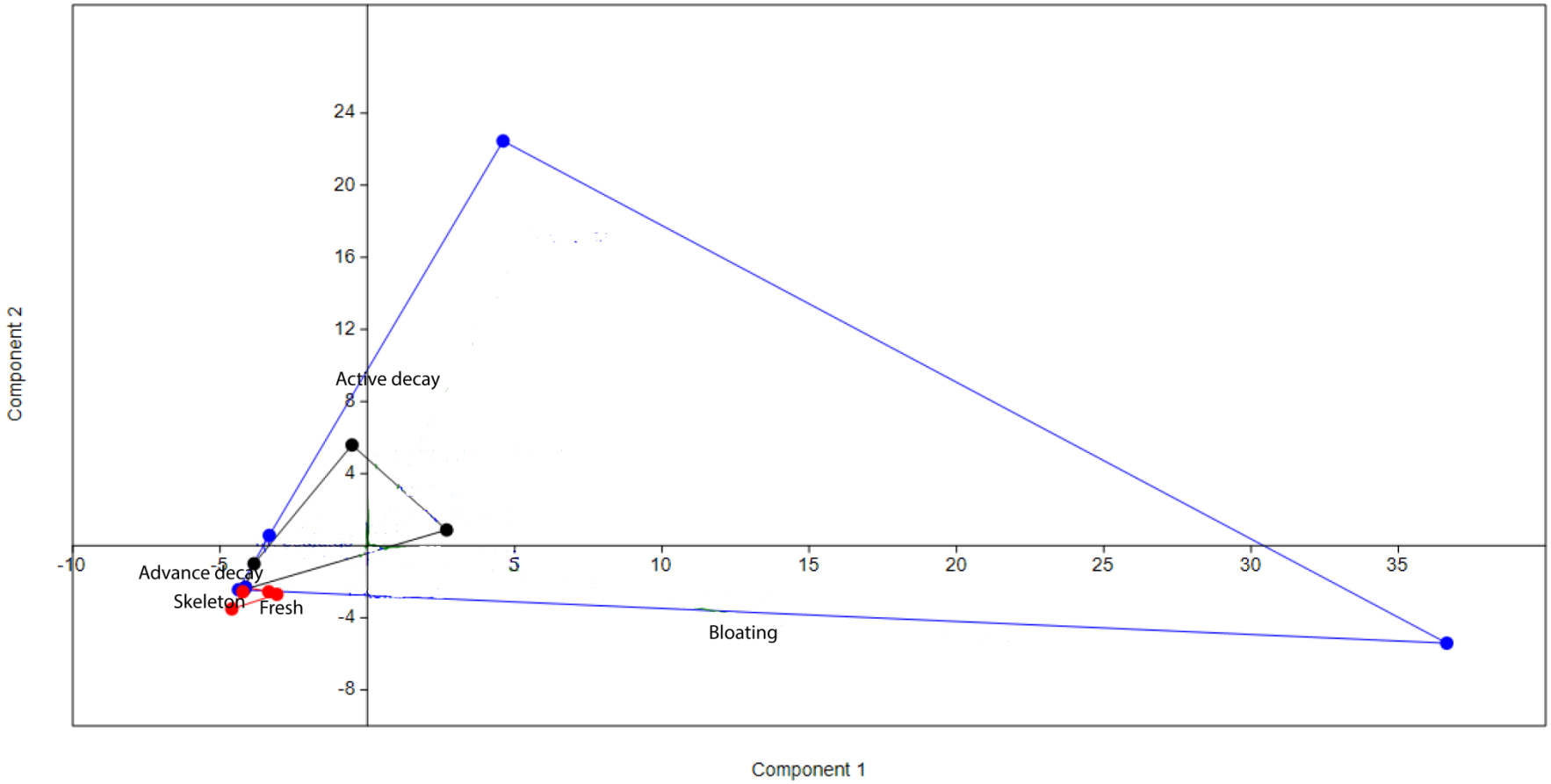

Figure 3.5: Patterns of colonization of pig carcasses by Mesostigmata in different body positions, according to the stages of decomposition, as produced by principle component analysis (PCA). Colours and dot reflect the position (blue = on-the-ground; black = hanging; red = control).

3.4 DISCUSSION

3.4.1 The patterns of decomposition stages between different body positions.

Decomposition is a sequential process which is depends on many factors, including the biotic (i.e. bacteria and insects) and abiotic (i.e. weather conditions) of habitat (Gunn, 2011) among which temperature and insect activity are the most influential (Meadows, Mann & Bass, 1990). These factors were minimised by replicating the experiment three times in the same habitat, soil properties, types of habitat (forest floor), the characteristic of forest appearance (tree cover) and environmental factors were in the same range. It has been demonstrated that the suspended position can affect the rate of biomass removal and of decomposition as well as the overall insect activity (Lynch-Aird, Moffatt & Simmons, 2015; Shalaby, Carvalho & Goff, 2000). The number of decomposition stages and their characteristics for the terrestrial carcasses revealed in this study were similar to the previous (Payne, 1965; Early & Goff, 1986; Archer, 2004). The different decay rates are clearly indicated in the hanging and on-the-ground carcasses. The hanging carcasses reached the earlier stages (fresh and bloating) faster but stay significantly longer in active and advanced decay stages due to the reason of lower insect activity in this position. This finding is similar to the study by Shalaby (Shalaby & Goff, 2000), that compared a single hanging and on the ground pig, found that the mass decreases more slowly in the hanging carcass with each stage of decomposition was prolonged. The continuous dripping and loss of maggots from the hanging carcass, resultedin reducedtissue consumption which delayed the process of decomposition (Saloña-Bordas & Perotti, 2014). The prolonged time for these stages

was mainly related to the inability of larvae that fell to the ground or the ground-dwelling insects to regain access to the carcass that prevented the formation of a well-established internal maggot mass (Shalaby, Carvalho & Goff, 2000). There is a different approach if a hanging body is partially in contact with the ground, crawling insects would likely return to the carcass if they fall into the drip zone (Shalaby & Goff, 2000). Only certain insects can reach a hanging body to continue the process of decay. Whilst the on-the-ground carcass was continuously colonised by successive waves of actively feeding insects throughout, which resulted in faster total body decomposition in comparison to the hanging carcass.

3.4.2 Mite distributions

The abundance of mites collected at the research site resulted from mites colonising and following the progress of the carcass decomposition. When the decomposition happened in the vertical or hanging position, gravitational effect related to body position causes the body fluids falling to soil beneath. However the body fluids were not directly absorbed into the soil however the soil beneath became a medium for the falling body fluids and a trap for insect larva from the decomposing body (Saloña-Bordas & Perotti, 2014). The species of insects attracted to a hanging body differ from carcasses on the ground because the carcasses were not touching the ground and this could give different composition of mite community (Hunter & Rosario, 1988). Goff and Lord (1994) already found that hanging could alter the insect colonization pattern excluding soil-dwelling taxa, thus changing the drying pattern of body, indirectly altered the presence of mites associated with it. Previous studies on arthropod succession on

animal and human remains have demonstrated a strong relationship between different insect communities and specific decomposition patterns (Mégnin, 1894; Payne, 1965; Schoenly, Goff & Early, 1992). The order of succession can be affected by various factors including burning, burial, habitat variation, sun exposure and hanging (Payne, 1965; King & Beinhart, 1968; Anderson & Vanlaerhoven, 1996; Avila & Goff, 1998; Shalaby & Goff, 2000). This experiment demonstrated that the processes of insect succession (a host for the mite) and decomposition were linked. It showed the reduction of mite numbers in the soil beneath the decomposing hanging bodies. The composition of mites at the family and genus level was much greater for on-the-ground carcasses compared to other plots, and most were found during the earlier stages of decay until it reached the advanced decay stage. Certain species of mites were present only during specific stages of decay. Previous studies demonstrated that mummification and the suspended position of the hanging carcass were two main factors to the lower diversity and the amount of insects (Lynch-Aird, Moffat & Simmons, 2015; Bugelli et al., 2018). The scarce diversity and scattered occurrence of insects associated with hanged bodies minimise the chances of finding the correct forensic marker of time (Saloña-Bordas & Perotti, 2014). There was not much difference in diversity of mites in all plots. Both the treatment and the control show the same pattern which was high values of Shannon-Weiner Index when carcasses reached the bloating stage and then went down during the active decay stage. The highest value of Simpson index was counted from on-the-ground carcass in the bloating stage. The species richness line graphs show decreasing number of species starting from fresh to the final stage of decomposition.

The members of the Mesostigmata were still the most frequently collected order from this study and highly dominated all the carcasses in all stages, except the fresh and remains stages. Macrochelidae is the most frequently collected family. *Macrocheles muscaedomesticae, Macrocheles glaber* and *Macrocheles merdarius* were the most abundant macrochelids collected. Macrochelid mites are commonly found phoretic on insects or in soil samples (Glida, Bertrand, & Peyrusse, 2003). The small number of Prostigmata and Astigmata did not provide any relevant information on the carcasses' conditions. Phoretic mites of forensic importance are highly specific and can provide clues, on a particular host or scavenger visiting a hanging-corpse, helping or aiding in the PMI estimations, and they are found in the soil below (Perotti & Braig, 2009). *Macrocheles muscaedomesticae* is a wide spread phoretic mite of forensic interest that attaches to the Diptera group such as Muscidae, Fanniidae, Calliphoridae and Drosophilidae. During carcass decay, blowflies (Diptera; Calliphoridae) are the main agents of flesh removal. They also remove uneaten soft tissue through enzymatic and mechanical action (Putman, 1978; Archer, 2004). They usually visit carcasses during the early stages (Anderson et al., 2002; Perotti et al., 2010). Their presence can be associated with the collected of phoretic mites, *Macrocheles glaber* and *Macrocheles muscaedomesticae.* Both mites prey on fly eggs and young larvae (Wade & Rodriguez, 1961; Glida, Bertrand & Peyrusse, 2003; Perotti & Braig, 2009). This explains the significance of the abundance of *M. glaber* and *M. muscaedomesticae* in the bloating and active decay from on-the-ground carcasses during which they are large numbers of fly eggs and larvae feeding on the soft tissue of the cadaver. With the characteristic of host-specificity of the phoretic mite, which means specific mite species have been brought to

the dead body by different insect species (Perotti et. al., 2001), the infestation of blowflies could be predicted even the taxa were not sampled. In most environments, an exposed body will be colonised within hours under suitable weather conditions (Greenberg, 1991). The reliable appearance of blowflies and their phoront mites is important forensically in establishing a time of death.

Although two species, *Macrocheles scutatus* and *Macrocheles punctoscutatus* were only found on hanging carcasses, they were collected in low frequencies. *Macrocheles scutatus* is a commonly found phoretic mite on the most abundant family of dung beetles, Scarabaeidae. (Niogret, Lumaret & Bertrand, 2006).However it also has been found living in the fur of *Rattus exulans* (Emberson, 1973), while *Macrocheles punctoscutatus* as described before, has been found from a mole nest, rodents and small mammals (Lundqvist, 1974; Plumari, 2010). They choose to live on the fur of their hosts . The small mammals that act as the host for these two species of mites, may have been attracted to the soil directly underneath the hanging carcasses rich in decay fluids during the bloating and active decay stages. These mites species were found on the drier parts of the carcass, which fits in with their preference for habitats. The decomposition fluids from the hanging dead bodies seep and drop down into the soil below; resulting in some parts of the carcass to dry out. This allowed these two species to inhabit the dry regions of the carcass where they were collected from. The conditions were different with the on-the-ground carcasses. The decay fluids from cadavers that are in direct contact with soil accumulate directly around and beneath the carcass resulting in a very wet environment; such wet conditions would be unsuitable for the existence of

these species of mites due to their preference of dry habitats. Hence their presence only on the hanging carcasses.

Parasitus copridis is another phoretic mite on the dung beetle (*Copris hispanus*). It spends a long period as deutonymphs on the host beetle (Masan & Halliday, 2009) and will moult into an adult when the phoriont beetle is present. It feeds on nematodes and fungi, which were produced in both positions of carcass. The hanging body allowed faeces and gut contents to reach the soil faster than the carcass on the ground, thus access to faeces by coprophilous beetles was fast and easy.

3.5 CONCLUSION

The abundance of mites on the carcass depends on many factors, and one is the carcass position. The present study details the predictable succession of mite taxa under specific conditions of decay in habitats in Neuchatel, Switzerland. Additionally, the intermittent presence of order Mesostigmata mites is highlighted in the abundance tables. In the experiment that was set up to understand this factor, carcasses on the ground attracted the most mites compared with the hanging position. The results demonstrated variations in mite abundance patterns in different plots within a year of research study. The stage of decomposition was the most significant factor for the observed variation. The mite samples were collected from the soil underneath the bodies (from the ground) and the soil below the hanging bodies. Mites were not collected directly from the bodies since this study aimed for mites in the soil. The

presence of certain mite species could provide a marker for the carcass positions and could be used forensically to estimate the place of death.

Chapter 4 : *Macrocheles* species (Acari: Macrochelidae) associated with human corpses in Europe.

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Naila A. Che Kamaruzaman: identification of mites, analysis of data and writing of the 1st draft of manuscript

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Yelitza Velásquez, Alejandro González Medina, Anders Lindström: provided mite samples from cases and identified the insects, revision of final draft

Henk R. Braig: revision of final draft

M. Alejandra Perotti: identification of mites, analysis and interpretation of data and writing the manuscript.

Abstract

The biology of macrochelid mites might offer new venues for the interpretation of the environmental conditions surrounding human death and decomposition. Three human corpses, one from Sweden and two from Spain, have been analysed for the occurrence of Macrochelidae species. *Macrocheles muscaedomesticae* (Scopoli) females were associated with a corpse that was found in a popular beach area of southeast Spain. Their arrival coincides with the occurrence of one of their major carrier species, the filth fly *Fannia scalaris*, the activity of which peaks during mid-summer. *Macrocheles glaber* (Müller) specimens were collected from a corpse in a shallow grave in a forest in Sweden at the end of summer, concurrent with the arrival of beetles attracted by odours from the corpse. *Macrocheles perglaber* Filipponi and Pegazzano adults were sampled from a corpse found indoors in the rural surroundings of Granada city, south Spain. The phoretic behaviour of this species is similar to that of *M. glaber*, but it is more specific to Scarabaeidae and Geotrupidae dung beetles, most of which favour human faeces. *Macrocheles muscaedomesticae* is known from urban and rural areas and poultry farms, *M. glaber* from outdoors, particularly the countryside, whereas *M. perglaber* is known from outdoor, rural, and remote, potentially mountainous locations. *Macrocheles muscaedomesticae* and *M. perglaber* are reported for the first time from the Iberian Peninsula. This is the first record of *M. perglaber* from human remains.

4.1 INTRODUCTION

Mites (Acari) are ubiquitous in the human environment and interact with animals and humans both during life and after death (Braig & Perotti, 2009; Goff, 1991; Leclercq & Verstraeten, 1988a; Perotti, 2009; Perotti & Braig, 2009a). They inhabit the surrounding environment of any dead body, for instance by living within garments, by nesting in clothing or fabrics, by walking into a corpse or in the form of phoretic mites by arriving on a dead body by taking advantage of flying insects and other scavengers for transport (Goff, 1991; Perotti & Braig, 2009b; Perotti et al., 2010). Despite having been overlooked due to their minute dimensions, mites represent the most diverse eukaryotic organisms of the scavenger community; for each insect species landing on a corpse or a carcass, it is expected that between 1 and 11 + mite species will be carried into the remains (Perotti & Braig, 2009b).

That mites occur in human and animal decay is not new. Over 160 years ago, Jean-Pierre Mégnin studied the entomological and acarological fauna of corpses (especially in the morgue of Paris) and already established a sequential colonization of arthropods following stages of decomposition (Braig & Perotti, 2009; Leclercq, 1978; Mégnin, 1894). Within 3 years of the publication of Megnin's book, Johnston and Villeneueve confirmed the 'eight waves' for Canada (Johnston & Villeneuve, 1897). The eight waves entered the Manual of Forensic Entomology and became a fundamental part for the understanding of decomposition (Lefebvre & Gaudry, 2009; Smith, 1986; Wyss & Cherix, 2013). The recognition of these waves is very important because it

acknowledges that mites in wave 1 are among the first colonizers of a corpse. Mégnin's wave six, for example, which is associated with a specific stage of decomposition, is exclusively composed of mite species. After Mégnin, it took nearly 100 years until the Belgian pathologist Marcel Leclercq started using mites again in forensic case work to estimate the time of death (Leclercq & Verstraeten, 1988a, b, 1993; Leclercq & Watrin, 1973). Unfortunately, most of his work was published in French or Dutch and did not reach the English-speaking forensics. Forensic acarology can assist, complement and, at times, even replace forensic entomology (Perotti & Braig, 2009a; Perotti et al., 2009). Insects are less likely to colonise corpses during winter months, particularly at high latitudes and altitudes. A corpse that has been covered with lead arsenate as an insecticide and a repellent for police dogs, with the aim of compromising entomological evidence, will still carry forensically important mites (Leclercq & Vaillant, 1992). Corpses decomposing indoors or concealed in any other way often carry an abundance of exclusive mites (Frost et al., 2010; Russell et al., 2004; Szelecz et al., 2018).

By adding mites to case work evidence, corrections on the insect activity on remains can be made. These include information on insect arrival times, oviposition times, insect life span and departure times even the end of insect waves can be predicted (Mégnin, 1894; Perotti, 2009; Perotti et al., 2009, 2010). Used in a similar manner to insects, mites alone can provide timelines too. In this respect, they are of great help in time estimations of later stages of decomposition, when most flesh has disappeared (Mégnin. 1894; Perotti, 2009; Russell et al., 2004; Saloña-Bordas & Perotti, 2014). In addition, mites can become reliable indicators of geographical location or origin (Hani et al.,

2018). At genus or species level, mites have micro-habitat specific requirements, offering themselves as potentially one of the most informative pieces of biological trace evidence gathered from a crime scene (Perotti, 2009; Pimsler et al., 2016; Prichard et al., 1986; Russell et al., 2004; Szelecz et al., 2018; Webb et al., 1983).

The family Macrochelidae includes over 470 species in 20 genera, and *Macrocheles*, with around 325 described species, is the most diverse genus of the family (Beaulieu et al., 2011; Emberson, 2010; Krantz, 1962, 1998, 2018; Krantz & Moser, 2012; Lindquist et al,. 2009; Makarova, 2012). New species of macrochelid mites and new phoretic associations are constantly described (Acs et al., 2017; Alatawi et al., 2018; Azevedo et al., 2017; Haloti et al., 2005; Hartini & Dwibadra, 2017; Knee 2018; Kontschan, 2018; Ozbek, 2017). Most *Macrocheles* species are predators feeding on small invertebrates, with the exception of only a handful of non-phoretic, detritivorous species (Manning & Halliday, 1994). As predators, they influence population growth of other micro-invertebrates (Geden et al., 1988; Perotti, 1999, 2001) and, thereby, may have effects on the advancement and composition of ephemeral micro-ecosystems.

Forensically important *Macrocheles* species arrive on carcasses through phoresy on flies and beetles. Those associated with corpses and carcasses can inform about circumstances of death, environment and habitat, making a link to a site or a location. In a recent crime case study, the inclusion of *Macrocheles matrius* as trace evidence a species highly prevalent in poultry manure allowed the reconstruction of the crime scene (Szelecz et al., 2018). Carriers for macrochelids linked to decomposing mammals

are necrophagous and necrophilous insects and micro-mammal hosts (Andreev, 1988; Halliday, 2000; Korn, 1983; Krantz & Whitaker, 1988; Leclercq & Watrin, 1973; Mašán, 1999, 2003).

One of the best known macrochelid species, *Macrocheles muscaedomesticae*, is highly prevalent on muscoid flies of the families Muscidae and Fanniidae (Axtell, 1964; Filipponi, 1960; Perotti & Brasesco, 1996, 1997; Rodriguez & Wade, 1961; Sacchi Carmona Rodrigueiro & Pires do Prado, 2004). These flies colonise and reproduce on a particular variety of organic material (e.g., poultry manure) as well as on sources of food decay abundant in urban areas. They are considered highly synanthropic insects (Legner & Bowen, 1973; Perotti, 1998). *Macrocheles muscaedomesticae* is much less common on other arthropods and mammals, to a point where it has rarely been reported on Calliphoridae, the dominating fly family of animal decomposition. It can occur on adult blowflies under special circumstances like in indoor decomposition, due to loss of phoretic specificity (Perotti & Braig, 2009b). *Macrocheles glaber* and *Macrocheles perglaber* are well known associates of dung beetles (Scarabaeidae, Geotrupidae) (Ciccolani et al., 1981; Filipponi & Pegazzano, 1962; Halliday & Holm, 1985; Halliday, 2000; Mašán, 2003; Niogret et al., 2006; Shereef et al,. 1990). In Europe, macrochelids on carrion or burying beetles (Silphinae and Nicrophorinae) are outnumbered by species of Parasitidae (Hyatt, 1980, 1990; Mašán, 1999, 2003). Therefore, any assumptions on phoretic specificity based on unusual or rare reports should be taken cautiously as they might represent a case of loss of phoretic specificity and can compromise the interpretation of the acarological evidence from a crime scene.

Most phoretic macrochelids have a haplodiploid sex determination system termed arrhenotoky, where males are parthenogenetically produced from unfertilized eggs (Manning and Halliday, 1994; Norton et al., 1993; Oliver, 1977). A few species can be thelytokous and phoretic, like *Macrocheles similis*, a species similar to M. muscaedomesticae (Manning & Halliday, 1994), and one species, *Macrocheles mycotrupetes*, phoretic on dung beetles, behaves like a diplodiploid (Krantz & Royce, 1994). Experiments on arrhenotokous and phoretic *M. glaber* indicated that virgin females can easily be fertilised by their sons, allowing the start of a population (Manning & Halliday, 1994). Fertilisation by sons—oedipal reproduction—has experimentally been studied for *M. muscaedomesticae* (Farahi et al., 2018). This is particularly important if the female is a virgin founder. Phoretic *Macrocheles* spp. can travel either as virgin or 'mated' females; still, mating does not guarantee fertilisation. The detailed experiments of Costa (1967) on the reproduction of the *Macrocheles pisentii* species complex proposed that wild phoretic females will produce a majority of males in their first progeny, independent of being mated, ruling out a 100% fertilisation. In a new population, as time goes and the number of mites increases, females dominate, to a point where a few males are left in an older dung pad (Kinn & Witcosky, 1977; Richards & Richards, 1977). This also explains many phoretic females leaving old dung pads unmated or unfertilised.

Recently, confusion has arisen on the matter of the virgin/mated status of phoretic females, with some reports overlooking the fact that founding females will be either virgin or mated—and if mated, they will not necessarily autofertilise their first oocytes

(Glida et al., 2003; Kinn & Witcosky, 1977; Niogret et al., 2010). Costa (1966) found that slightly old virgin females have difficulties mating, due to the hardening of the genital slits in coxae III, impeding males from the introduction of spermatophora; these females will stay virgin and produce only males, a finding later confirmed by Yasui (1995). Fertilised and unmated *M. muscaedomesticae* will attach to either gender of house flies to move to a new habitat (Jalil & Rodriguez, 1970), and the majority of fertilised females will have been exposed in their teneral stage to multiple matings, as males fiercely guard moulting females (Yasui, 1995). On the other hand, species such as *M. glaber,* living off the limited habitat offered by an ephemeral (isolated) dung pad, will have difficulties finding mates. Dung-breeding species might resolve sperm competition, sperm precedence and female control on oocyte fertilisation in different ways (Yasui, 1995), and might mate just once before departure, if sufficient males occur, otherwise will travel unmated. More research, especially on reproduction of *Macrocheles* species associated with corpses and carcasses, is critical to clarify this phenomenon. Interpreting gender bias of macrochelids would support estimations of time. If the *M. glaber* specimens found in/on a corpse exhibit a male bias, this is suggestive of a recent arrival, of both the carrying beetle and its mites. Under optimal environmental conditions, *Macrocheles* embryos will reach adulthood in just a few (3–4) days (Ciccolani et al., 1977; Singh et al., 1967; Wade & Rodriguez, 1961). The sex ratio of the first progeny from a majority of virgin phoretic females (F1 generation) will be mainly male-biased, having more males than females, or an even sex ratio within the adult *Macrocheles* population. A few days forward and the sex ratio will transition towards a higher number of females, leading much later towards almost female-only offspring,

ready to be transported to a new corpse or carcass, as it happens in nature within dung pads too (Ciccolani, 1992).

The biology of three Macrochelidae mite species collected from three corpses decomposing under different environmental conditions is discussed in the light of the potential value these species might offer as indicators of any special circumstances surrounding the death of these individuals.

4.2 MATERIALS AND METHODS

Macrochelidae mites from three case studies occurring in two European countries, Spain and Sweden were received at Reading University, studied and discussed

Case 1

On April 23rd, 2010 (early spring), the corpse of a homeless man was found outdoors, in a lot close to the beach, called 'Solar Vistahermosa', Alicante, southeast Spain. The body was found under an umbrella (used for shadow), lying on the ground and face up. It was fully dressed and covered with a blanket up to the neck, exposing only the head. The corpse was reported as in advanced decay, and slightly mummified (Fig. 4.1). According to the pathologist, there were no signs of violence and death was stated as natural. The deceased was last seen alive 30 days before the finding. A weather station of the Spanish Meteorological Agency (AEMET) closest to the scene reported an average temperature of 15.3 °C, for the 30 days prior to the discovery of the body.

Entomological evidence was collected from the corpse during autopsy at the Institute of Legal Medicine of Alicante (IMLA), Spain, and consisted of empty puparia and blowfly adults (Calliphoridae: Calliphora vicina, *Chysomya albiceps*, *Lucilia sericata*); larvae, pupae and empty puparia of *Hydrotaea capensis*; larvae, pupae, empty puparia and adults of *Synthesiomyia nudiseta* (both Muscidae); larvae of the filth fly *Fannia scalaris* (Fanniidae); and pupae of the scuttle flies *Conicera tibialis* and *Puliciphora rufipes* (Phoridae) (Velázquez et al., 2010). The postmortem interval (PMI) was estimated using more than one species of Diptera and gave a maximum of 31 and a minimum of 27 days, which coincided with the time when the person was last seen alive (Velásquez, 2011).

Mite samples were prepared for identification at the Acarology Lab (University of Reading) following standards for clearing and mounting of Acari, using Hoyer medium for permanent mounting (Faraji & Bakker, 2008). Mites were identified using appropriate taxonomical literature (Emberson, 1972; Evans & Browning, 1956; Evans & Hyatt, 1963; Hyatt & Emberson, 1988). Voucher specimens are deposited in the Forensic Acarology Reference Collection, University of Reading.

Figure 4.1: CASE 1. The corpse of homeless man found in a lot close to the beach, Alicante (Spain), in advanced decay and slightly mummified. Photo taken in the autopsy room (YW).

Case 2

On September 18th, 2009 (end of summer), the dead body of a woman was found by her boyfriend in a remote forested area in central Sweden. She was reported missing on August 2nd, almost 7 weeks earlier. The area where the remains were found is a boreal forest typical for Sweden with spruce (*Picea abies*), aspen (*Populus tremula*) and birch (*Betula pendula*). The corpse was lying in a very shallow grave, purposely covered with cut aspen branches and birch saplings, grass and moss, revealing only a minor portion of the left hip and right foot. The cover may have delayed the colonization by sarcosaprophagous Diptera for a while but was loose enough to allow colonization by the flies.

The forensic entomologist (AL) visited the crime scene the day after the body's discovery and sampled insects from the body and nearby surroundings. Another forensic entomology investigation of the scene was held on September 24th when the soil in a radius of approximately 2 m from the center of the grave was dug up to a depth of 15 cm, and collected in search for Calliphoridae pupae. No hatched puparia were found. Adults of *Calliphora vomitoria* started to hatch on September 26th from pupae collected the first time. A time of death was estimated for the first half of August. Due to heavy decomposition of the body, the cause of death could not be established.

Among the entomological specimens collected from the remains, there were many mites. All mite specimens were collected using a brush and transferred to 70% alcohol. Mites were then prepared for identification following the same protocol as for Case 1, and the voucher specimens were deposited in the Reading collection. The identification of the *Macrocheles* species of this case used a variety of keys and descriptions (Halliday, 2000; Hyatt & Emberson, 1988; Mašán, 2003). Mites of the family Parasitidae were also identified (Hyatt, 1980).

Case 3

In September 2011, the dead body of a mature woman, in her mid-fifties, was found in her house in the mountainous country side of Granada, south Spain, in El Sacromonte at an elevation of 820–840 m a.s.l. (González Medina et al., 2012). The discovery was prompted by the odours coming from the house, detected by neighbours. The pathologist determined the cause of death as an overdose of acetaminophen

(paracetamol). At the time of the finding, the body was in active decay (Fig. 4.2) (indoor decomposition; Galloway et al., 1989; Goff, 2009). The deceased suffered from Diogenes syndrome, characterised by self-neglect, isolation, hoarding and accumulation of garbage (González Medina et al., 2012). Insect data were recorded and identified by the forensic entomologist (AGM) and consisted of empty puparia of *Calliphora vicina* (Calliphoridae); adults, larvae and pupae of *Sarcophaga africa*, *Sarcophaga* sp. (Sarcophagidae); adults of *Musca domestica* and *Hydrotaea aenescens* (Muscidae); adults of *Megaselia* sp. (Phoridae); adults of the clown beetles *Saprinus subnitescens* and *Margarinotus brunneus* (Histeridae); and adults and larvae of the skin beetle *Dermestes frischii* (Dermestidae). According to the original analysis of the case, a PMI of 13 days was estimated based on insect succession and activity of Silphidae and *Poecilochirus austroasiaticus* (Acari: Parasitidae) (González Medina et al., 2012).

A list of mite species associated with the corpse was previously reported, together with an interpretation of the role of the Parasitidae, *P. austroasiaticus* (González Medina et al., 2012). For this study, an unpublished species of Macrochelidae was later rescued from entomological samples of the case, and is discussed here. Mites were kept in 70% alcohol and prepared for identification following the same protocol as for Case 1. Voucher specimens were deposited in the Reading collection. The identification of the *Macrocheles* species of Case 3 followed the description and key of Mašán (2003).

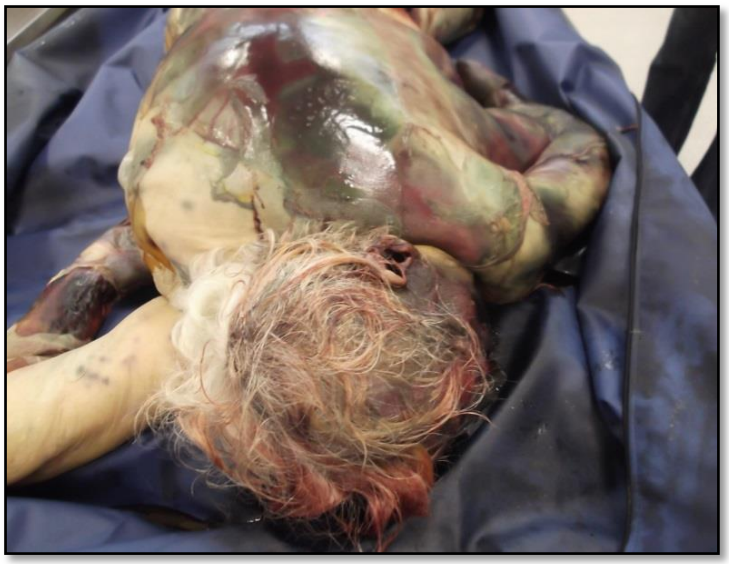

Figure 4.2: CASE 3. The body of a woman found in her house in Granada (Spain), in active stage of decomposition. Photo taken in the autopsy room (AGM).

4.3 RESULTS AND DISCUSSION

Three species of *Macrocheles* were identified, each corresponding to each case study, and the biology of these species was analysed in relation to the corpse and its environmental conditions. Table 4.1 compiles and expands literature records on habitat and geographic distribution of each species, the table presents a list of phoretic carriers that include common and specific-less-common-species of insects, birds and mammals.

Case 1: A dead man found close to a popular beach area, southeast Spain

Two mites were recovered and both were females of *M. muscaedomesticae* (Fig. 4.3). *Macrocheles muscaedomesticae* is highly synanthropic, its habitat is domestic, urban and semirural, being common in poultry farms (Farish & Axtell, 1971; Ho, 1990; Perotti, 1996, 1998; Perotti & Brasesco, 1996; Rodriguez & Wade, 1961; Wade & Rodriguez,

1961; Williams & Rogers, 1976). It disperses as phoretic on synanthropic animals, preferentially flies (Axtell, 1964; Nuorteva, 1963) of Muscidae and Fanniidae (filth flies), and much less frequently on other insects or small mammals that live with or in association with humans (Filipponi, 1960; Jalil & Rodriguez, 1970). Inaccurate identification of mite species riding on insects can lead to confusing reports on phoretic carriers. For example, the latest publication on phoretic mites associated with necrophagous flies in Brazil, reports *M. muscaedomesticae* on the abdomen of *Chrysomya albiceps* (Sato et al., 2018). From the photos included in the publication, disparities emerge from the morphology of the sternal shield of the mites that question the identification of the *Macrocheles* specimens. In fact, none of the mite specimens were identified using keys to species level; instead, the consulted literatures were two major keys of Mesostigmata families (methodology section in Sato et al., 2018).

Specific food items of *M. muscaedomesticae* adults are Musca and Fannia eggs, plus acarid mites. Larvae of *M. muscaedomesticae* feed on conspecifics (cannibalism), and proto- and deutonymphs feed on nematodes (Axtell, 1964; Farish & Axtell, 1971; Perotti & Brasesco, 1996, 1997; Rodrigueiro & do Prado, 2004; Rodriguez & Wade, 1961; Wade & Rodriguez, 1961). Coincidentally, in this case study fly larvae belonging to Muscidae and Fanniidae were collected. *Fannia scalaris* was found at larval stages. This is a highly synanthropic European species associated with food, decay, myiasis, faeces, and with sheltered corpses (Easton & Smith, 1970; Leclercq & Verstraeten, 1988b; Mégnin, 1894; Mihályi, 1965; Perotti, 1998; Velázquez et al., 2010).

The corpse was sheltered under a beach umbrella and covered by a blanket. Flies and mites independently link the scene of decomposition to a domestic/urban environment. In Europe, there are only two previous reports of *M. muscaedomesticae* on human corpses: (1) mites recovered from the brain, after a failed operation in a military hospital during mid-August in France (of the son of an acarologist), and (2) one female mite, recovered from a human corpse (Easton & Smith, 1970; Hermann, 1804; Oudemans, 1929). The latter was collected together with the Parasitidae species *Poecilochius necrophori*, from a corpse of a poison suicide. The body was found lying on a well-drained chalk hillside, in a small wood on the North Downs in southeast England. This occurred at the beginning of the autumn (October), when Fanniidae and Muscidae flies slow down their activity. Interestingly, according to Easton and Smith (1970), maggots of *Fannia* sp. were collected, although the mite occurred on adult *Musca domestica* (both flies are specific carriers). The body was found in a similar condition to the corpse in the present case. Exposed parts of the body were in advance stage of decomposition, while covered parts (inside a sleeping bag) still had soft tissue and were heavily colonised by the arthropods.

Table 4-1: Literature review and records of the Macrochelidae species with respect to habitat phoretic carriers and geographic distributionLiterature review and records of the three Macrocheles species, with respect to habitat, phoretic carriers and geographic distribution.

Macrocheles muscaedomesticae

Habitat

Animal carcasses			
Fresh	cat	xero + mesophytic	(Early and Goff 1986) (Goff 1989)
	kangaroo	grassy woodland	(Barton et al. 2014)
Bloating	cat	xero + mesophytic	(Early and Goff 1986) (Goff 1989)
Advanced decay	kangaroo	grassy woodland	(Barton et al. 2014)
Skeletal stage	cat	xero + mesophytic	(Early and Goff 1986) (Goff 1989)
	impala	woods	(Braack 1986; Braack 1987)
	bird	?	(Emberson 1980)
Human corpses			
Fresh	hospital		(Hermann 1804) (Oudemans 1929a)
Advanced decay	small wood		(Easton and Smith 1970)
	near beach		this report

Dung/Faeces: poultry, cattle (outermost layer), pig, wombats (USA: poultry: summer; cattle: winter and spring)

Bird nests: Ciconia ciconia, Fulica atra, Larus ridibundus, Merops apiaster, Perdix perdix, Remiz pendulinus, Tachycineta bicolor, Turdus merula, Zapornia tabuensis plumbea

Birds: Dryobates pubescens, Sayornis sp.

Mammals: Apodemus agrarius, Cricetulus barabensis, Eothenomys melanogaster, Homo sapiens, Mus musculus, Myodes glareolus, Notomys alexis, Peromyscus leucopus, Rattus pyctoris, Sigmodon hispidus, Sciurus carolinensis, Spermophilus citellus

Reptiles: Crocodylus johnstoni (inside mouth), Terrapene carolina

Insect nests: Reticulitermes flavipes, bumble bees

Decomposing plants: litter

Other: facultative parasitism on adult drosophilid and muscoid Diptera

Phoretic carriers and parasite hosts

Diptera

Fanniidae: Fannia armata, F. canicularis

Muscidae: Australophyra rostrata, Hydrotaea dentipes, Musca domestica, M. sorbens, M. vetustissima, Muscina stabulans, Ophyra chalcogaster, O. ignava, Stomoxys calitrans (common)

Calliphoridae: Calliphora vicina, C. vomitoria, Chrysomya megacephala, Cochliomyia hominivorax, Lucilia cuprina

Sphaeroceridae: Copromyza equina
Syrphidae: Eristalis tenax, Syritta pipiens
Coleoptera:
Geotrupidae: Geotrupes stercorarius
Scarabaeidae: Bubas bubalus, Eupleurus (Aphodius) subterraneus, Catharsius dayacus, Microcopris hidakai, Onthophagus schwaneri, O. waterstradti, Osmoder

Macrocheles glaber sensu lato

Habitat

Animal carcasses

Advanced decay	fox	garden	(Smith 1975)
	kangaroo	grassy woodland	(Barton et al. 2014)
Human corpses			
Active decay	forest		this report
Active decay	?		(Leclercq and Verstraeten 1988a)

Dung/Faeces: chicken, boar, cattle, horse, sheep
Bird nests: Accipiter gentilis, Acrocephalus arundinaceus, Anser anser, Ciconia ciconia, Cygnus olor, Larus ridibundus, Merops apiaster, Nycticorax nycticorax, Parus major, P. montanus, Passer montanus, Remiz pendulinus, Vanellus vanellus
Mammal nest: voles
Decomposing plants: compost, silage, hay, straw, moss, lichen, bark, rotten wood, seaweed
Other: garbage, discarded food

Phoretic carriers
Diptera
Calliphoridae
Muscidae: Australophyra rostrata, Hydrotaea dentipes, Musca domestica, Stomoxys calitrans
Coleoptera:
Aphodiidae: Aphodius aestivalis, A. constans, A. erraticus, A. haemorrhoidalis, A. luridus, A. merdarius

Carabidae: *Carabus violaceus*
Geotrupidae:*Geotrupes mutator*, *G. spiniger*, *G.* (*Anoplotrupes) stercorosus, G. stercorarius, Sericotrupes niger, Trypocopris pyrenaeus, T. vernalis*
Histeridae: *Pachylister lutarius*
Scarabaeidae: *Aphodius fimetarius*, *Bubas bison*, *B. bubalus*, *Caccobius schreberi*, *Catharsius molossus*, *Copris lunaris*, *Euoniticellus fulvus, Euonthophagus crocatus*, *Onthophagus coenobita*, *O. lemur, O. ovatus, O. similis, O. taurus, O. vacca, O. verticicornis*, *Scarabaeus laticollis, S. sacer*
Silphidae: *Nicrophorus humator, N. marginatus, N. obscurus*
Staphylinidae

Distribution

Europe: Belgium, England, France, Hungary, Italy, Latvia, Poland, Slovakia, Sweden, Turkey, former USSR (from the Kola peninsula, Karelia and Yakutin in the north to the Caucasus and Central Asia in the south) Americas: USA; South America (reported here: considered absent) Asia: China, Indonesia, Iran, Iraq, former USSR (Central Asia), Taiwan. Africa: North Africa, Morocco; Réunion, Saudi Arabia. Oceania: Australia, New Zealand

Macrocheles perglaber

Habitat

Human corpse
Bloating to Advanced decay indoors new record, this report
Dung/Faeces: chicken, cattle, horse, sheep
Decomposing plants: compost, straw, weeds
Phoretic carriers
Diptera
Muscidae*: Musca domestica*, *Stomoxys calitrans*
Coleoptera:
Aphodiidae: *Aphodius constans*, *A. haemorrhoidalis, A. luridus, A. merdarius*
Geotrupidae: *Geotrupes mutator*, *G. spiniger*, *G. stercorarius, Sericotrupes niger*
Scarabaeidae: *Bubas bison*, *B. bubalus*, *Copris lunaris*, *Euoniticellus fulvus*, *Onthophagus taurus*, *O. vacca, Scarabaeus cicatricosus, S. laticollis, S. sacer, Sisyphus schaefferi*

Distribution

Europe: France, Italy, Spain (reported here: new record), Slovakia, Turkey, former USSR (Khabarovsk Territory)
America: USA; South America (reported here: considered absent)
Africa: Morocco; South Africa (reported here: considered absent)

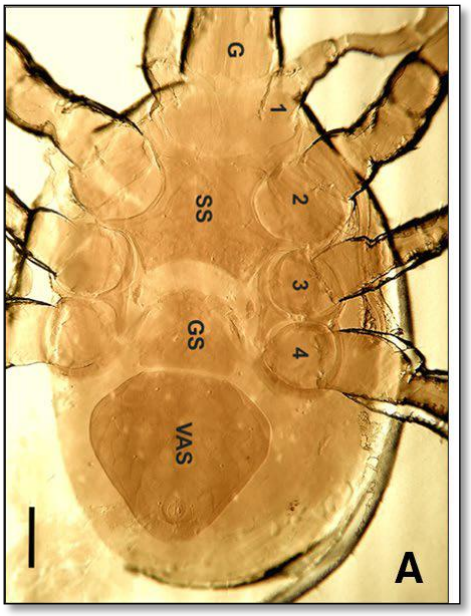

Figure 4.3: *Macrocheles muscaedomesticae* female, ventral view, identified from the corpse of Case 1 (Spain). Legs are numbered from front to rear; G gnatosoma, SS sternal shield, GS genital shield, VAS ventro-anal shield. Scale bars: 100µm

For this case study, *M. muscaedomesticae* adult males were absent and females were rare, possibly due to a late arrival of their carrier flies (likely *F. scalaris*). Indeed, *Macrocheles* first-generation offspring, which is almost exclusively male (Geden et al., 1990; Jalil & Rodriguez, 1970) did not complete development. The presence of only two females is in concordance with a minimum number of *F. scalaris* reaching the corpse, as a first generation of flies of the year, early spring, correlate with the moment the corpse was found. Fannia's activity peaks in the summer (Hewitt, 1912). Early colonisers, such as Calliphoridae and Muscidae specimens were used for PMI estimations, giving a time since death of approximately 1 month (Velázquez et al., 2010).

Despite being a cosmopolitan species, with most records from European countries, this is the first time *M. muscaedomesticae* is documented within the Iberian Peninsula possibly due to the lack of work on this taxon in Spain.

Case 2: A dead woman found in a forest in Sweden

A female (Fig. 4.4) and a deutonymph of *M. glaber* sensu lato were collected from the corpse, together with other Mesostigmata mites, mostly Parasitidae deutonymphs known to colonize corpses or carcasses (González Medina et al., 2012; Perotti & Braig, 2009a, b; Perotti et al., 2009; Saloña-Bordas & Perotti, 2014). *Macrocheles glaber* is the type species of the glaber group (Filipponi & Pegazzano, 1962), which comprises coprophilous mites associated with large herbivore's manure, and is less frequently found on carrion (Ciccolani, 1992; Fain & Miessen, 1997; Filipponi & Pegazzano, 1962; Mašán, 2003; Perotti & Braig, 2009b). It is a cosmopolitan species originally found and studied from the Mediterranean area (Europe and North Africa) (Halliday & Holm, 1985; Mašán, 2003); however, it has been reported in Sweden from 1998 (Lundqvist 1998; Lundqvist et al. 2000). In a recent survey in Hungary, 224 mites were found in rural forest patches, but only 26 in urban areas, in parks (Mizser et al., 2016). *Macrocheles glaber* is highly prevalent on dung beetles (e.g., Scarabaeidae), occasional on necrophagous and/or necrophilous beetles (e.g., Silphidae) and rare on Diptera (Fain & Miessen, 1997; Halliday, 2000; Hartini & Takaku, 2006; Hyatt & Emberson, 1988; Mašán 2003; Mašán & Krištofík, 1992; Perotti & Braig, 2009b; Perotti et al., 2010). Its phoresy on non-dung-related arthropods (e.g., carrion beetles or filth flies) is assumed as an opportunistic strategy used when its main hosts (dung beetles) are absent (Perotti

& Braig, 2009b). The corpse was partially covered with local vegetation, that restricted access of beetles and flies. This is not the first report of the species from human remains. Leclercq and Verstraeten (1988a) found *M. glaber* in a body that decomposed during the end of summer and begin of autumn, 3 months after the time of death, with the corpse found in October. Unfortunately, no details of the environment or potential fly or beetle hosts have been provided for this case in Belgium. No common carriers used by *M. glaber* in Sweden are known either (Lundqvist, 1998).

Macrocheles glaber's life cycle is slightly longer than that of *M. muscaedomesticae*, completing development after an average of 5 days at 30 °C (females) (Shereef et al., 1990). Like many other macrochelids, the species is haplodiploid, and the F1 of female colonisers is mainly male. With sufficient food resources, the female lays eggs that she held under her gnathosoma (oviparity), with poor resources she will lay eggs that hatch immediately (ovoviviparity), and with very poor resources, she will eat her eggs (cannibalism) (Marquardt et al., 2015). It is impossible to sex the deutonymph found but, considering that the accompanying fauna of mites was dominated by Parasitidae (Mesostigmata) and Histiostomatidae (Astigmata), a time of arrival can be drawn (Perotti & Braig, 2009b; Perotti et al., 2010; Saloña-Bordas & Perotti. 2014). The deutonymph might represent an immature male, offspring of the first females arriving. Seven deutonymphs of Poecilochirus carabi, two of *P. mrciaki* (Parasitidae) and three deutonymphs (hypopi) of *Spinanoetus pelznerae* (Histiostomatidae) were recovered from *Necrodes litoralis* sub-elytral cavity, justifying the very recent arrival of the carrion beetle (Silphidae), as much as 2 days before the finding of the body (González Medina et

al., 2012). Otherwise, the Parasitidae individuals would have moulted into adulthood. In this sense, the *Macrocheles* specimens have spent long enough on the corpse to produce offspring. If *M. glaber* females arrived earlier, they very likely did on dung beetles. Niogret et al. (2006) carried out a numerical survey of phoront-mite/host species proportions in France and *M. glaber* were highly prevalent on Geotrupidae and Scarabaeidae; proposing that Aphodius and Onthophagus are the major hosts for the glaber group species. Linking this to the geographical location of the case, the most northerly members of the Scarabaeinae are Onthophagus beetles with a record of nine species reported for Sweden alone (Ljungberg, 2002). The preference of Onthophagus for faeces of omnivorous animals, especially human stool, has long been known; some species are also attracted to carrion (Fincher et al., 1970; Howard, 1900; Whipple & Hoback, 2012; Woodruff, 1967). Post-mortem discharge of faeces can occur during fresh decomposition due to relaxing of muscles (algor mortis), as well as at the end of the bloating stage, when fluids and excrement exit the body (Shkrum & Ramsay, 2007).

In shallow graves, decomposition is delayed and there is no initial scavenger activity (Gaudry, 2010; Rodriguez & Bass, 1985). In the case study, insect and mite colonization took 6–7 weeks, despite carriers being highly active over the summer. *Macrocheles glaber* has even been recorded in high numbers in Australia at week 6 of decomposition during the summer months (Barton et al., 2014). The Australian study, which recorded a total of 1,003 *M. glaber* from 18 grey Kangaroo carcasses, also recorded very high numbers of beetles in the same week (Barton et al., 2014). Such abundance of *M. glaber* is expected when the mites have arrived earlier, because in this controlled experiment

there were no barriers that impeded colonization, the kangaroos were not covered. A small number of mites is expected if there is concealment of the body (as, e.g., in a shallow grave). Analysis of mite numbers should be exercised with caution in a crime scene.

Calliphoridae flies were used in the Swedish case, giving a PMI of 6 weeks, which is supported by the mite evidence.

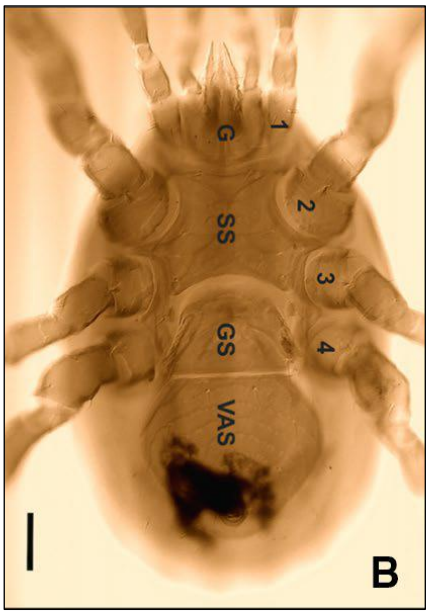

Figure 4.4: *Macrocheles glaber* female, ventral view, identified from corpse of Case 2. Legs are numbered from front to rear; G gnatosoma, SS sternal shield, GS genital shield, VAS ventro-anal shield. Scale bars: 100µm

Case 3: A dead woman found inside a house in Granada, south Spain

Three Macrochelidae specimens were recovered from the corpse together with other Acari reported elsewhere (González Medina et al., 2012). They were one female (Fig. 4.5) and two males of *M. perglaber*. The identification to species level was based on the males, as the morphological differences to females of the sister species *M. glaber* were not conclusive. This has also been the situation for populations from Slovakia (Mašán, 2003) and it is expected that many misidentifications of *M. glaber* and *M. perglaber* mites exist in the current literature (Halliday & Holm, 1985).

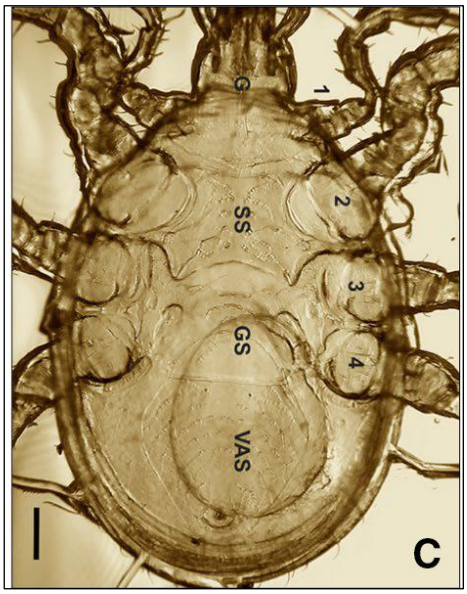

Figure 4.5:Macrocheles perglaber female, ventral view, identified from corpse of Case 3 (Spain). Legs are numberedfrom front to rear; G gnatosoma, SS sternal shield, GS genital shield, VAS ventro-anal shield. Scale bars: 100µm.

Macrocheles perglaber is a member of the glaber group, which comprise coprophilous species associated with manure of large herbivores that occasionally are also necrophilous (Ciccolani, 1992; Mašán, 2003; Perotti & Braig, 2009b). *Macrocheles*

perglaber presents traits different from *M. glaber*. *Macrocheles perglaber* has not been found simultaneously with *M. glaber* on the same carriers.

The original population described by Filipponi and Pegazzano (1962) was isolated from horse dung in central Italy. *Macrocheles perglaber*'s phoretic behaviour is similar to that of *M. glaber*, specialising in Scarabaeidae and Geotrupidae (Filipponi & Pegazzano, 1962; Glida et al., 2003; Niogret et al., 2010). Most of its dung beetle hosts favour human faeces (Fincher et al., 1970; Howard, 1900; Whipple & Hoback, 2012; Woodruff, 1967). Although *M. perglaber* can be found at any altitude between sea level and 1200–1400 m a.s.l., it seems more restricted to higher elevations compared to its sister species *M. glaber* (Filipponi & Pegazzano, 1962; Mašán, 2003). The country-side house where the dead body was found is located on a hill (pre-Sierra Nevada Mountains) at an elevation of just over 800 m a.s.l. Livestock is common in this semi-rural area, and considering the waste and abundance of faeces in the house (González Medina et al., 2012), *M. perglaber* was very likely brought inside the house, which had open windows, by scarabs of the surrounding area. According to an inventory of scarabs performed 5 km from the house, at similar altitude, in Pinos de Genil, 12 candidates can be considered: one Bubas, five Onthophagus and six *Aphodius* species for the pre-Sierra Nevada area (Avila & Pascual, 1987). All three genera are attracted to human, horse, cow and other wildlife animal faeces (Woodruff, 1967). Death occurred during the summer; therefore, no shortage of potential carriers is presumed.

Macrocheles perglaber is haplodiploid, like other macrochelids (Cases 1 and 2). The collection of adult males confirms the presence of the species in the house for at least 5–

7 days, as founder females will produce male offspring approximately 5–7 days after arrival to a suitable environment (Kinn & Witcosky, 1977; Richards & Richards, 1977). The new habitat, the corpse, and the exposition to faeces offered optimal conditions for starting a colony; otherwise, females would have kept attached to the carrier due to their specific requirements regarding the moisture level of the substratum (Niogret et al., 2010). *Macrocheles perglaber* would have arrived with one of the aforementioned dung beetles at an early stage of decomposition, like bloating, which has been confirmed by the presence of *Poecilochirus* mites. Taking into account all these factors, the period of activity of *M. perglaber* in the house would propose a PMI estimation of no less than 8–11 days. This estimation considers (1) the arrival of females at bloating stage, happening 3–4 days after death, and (2) males reaching adulthood in 5–7 days. This minimum period of mite activity agrees with the PMI estimation of 13 days given by the entomological analysis (González Medina et al., 2012).

This is the first report of *M. perglaber* from a human corpse and it is the first report from the Iberian Peninsula.

4.4 CONCLUSION REMARKS

The presence of *M. muscaedomesticae* on a corpse, even collected during autopsy, might provide information on the circumstances surrounding death; for example, it may provide links to synanthropic habitats. *Macrocheles glaber* is a mite species transported by beetles, widely prevalent in decomposition. Its specific association with rural

environments helps confirming exposure of remains or outdoor decomposition, especially in remote areas. This species cannot access sealed/closed buildings because it rides on large beetle carriers. If found indoors, it is either due to open doors or windows, or due to relocation of the body, from outdoors to indoors. *Macrocheles glaber* is a good indicator of rurality and outdoor habitats including shallow graves. *Macrocheles perglaber* occurrence in outdoor, rural or remote, potentially mountainous, locations is highlighted, due to its specific association with dung beetles. If a corpse is re-located to a new urban location, the presence of this species is indicative of a previous exposure to rural, likely mountainous environment.

Chapter 5 : Summary and concluding remarks

5.1 BACKGROUND

The concept of using arthropods in forensic investigations is not new. It dates to the 13th century in China with a documented case involving forensic entomology. The suspect in the case was identified by the attraction of insects (flies) to the blood on the murder weapon (translated by McKnight, 1981). It was easy to put the crime weapon and suspect together in the case when invisible traces of blood drew blowflies to single sickles that belong to the killer. Since then, there have been major contributions to the field of forensic entomology (Benecke, 2001; Gomes & Von Zuben, 2006). Bergeret (1855) was the first to give modern forensic entomology case reports that included an estimation of a post-mortem interval (PMI). A few decades later, Megnin (1894) documented his observations on the mummified body of a new-born girl in Paris. He estimated the PMI using information based on insect succession (Gomes & Von Zuben, 2006). Insect fauna is specific to the stage of decomposition and this was used to estimate when death occurred by identifying the species present on the cadaver. This was also the first case where mites provided substantial information on PMI. Most mites arrive at a carcass phoretically, that is, carried by other insects. Specific phoretic mites are found on a corpse at specific stages of decay. Because patterns of insect succession on a corpse are known based on the stage of decomposition, the arrival of phoretic mite on a carcass can be predicted.

The results presented from this research were covered up with the final findings for the experimental forensic settings for outdoor cases that were designed for a variety of scenes that involved the use of soil as a medium for collecting mites. The focus was on carrion-mites for use as forensic markers. Well-established forensic entomology methods from previous studies were used as additional templates to develop forensic acarology. The following sections will summarize the main findings from the different forensic settings used in these studies. In addition, the validity of using mites as forensic markers was demonstrated in a real-crime case study.

5.2 THE SEASONAL ABUNDANCE OF MITES IN A SOIL COMMUNITY

Seasonal experiments were conducted in the University of Reading for four seasons. Environmental variables were recorded and the patterns of carcass decay were monitored to explore their correlation with the abundance and diversity of mites. Determining the mite fauna associated with carcasses may significantly increase the amount of location- and time-specific information available for forensic evaluation. This study focused on the potential use of mites as forensic indicators of seasons and stages of cadaver decay in outdoor settings, a field of forensic science that has not before been studied in depth. Variability of the decomposition patterns throughout the seasons was determined. The succession of insects at different stages of decomposition is relatively significance and the abundance of mites has shown to follow a similar patterns to insects. It was determined that the abundance of mites collected in summer and spring season at its peak in the last stage. The collected mites represented four orders, but

only mites of the order Mesostigmata were identified to the species level, as they were dominant in all seasons and from each decomposition stage. This is consistent with the characteristics of Mesostigmata being free-living predators in soil and litter. The spring season and active decay stage of decomposition had the highest number of Mesostigmata.

5.3 SOIL MITES UNDERNEATH A CARCASS/CORPSE AS FORENSIC MARKERS

This study involved interaction with members of the Animal, Plant and Soil Traces (APST) working group, of the European Network of Forensic Science Institute (ENFSI) as well with European Association of Forensic Entomologist (EAFE) members. Field work was conducted outside England, in a spruce forest near Neuchatel, Switzerland, to compare the presence and diversity of mites from carcasses either on the ground or hanging.

This study demonstrated that a decomposing cadaver influences the abundance and composition of mites in the soil. Carcasses on the ground decomposed faster and attracted more insects and mites than did the hanging carcasses. The properties of the soil beneath a hanging carcass also changed, along with the presence of soil arthropods. It was concluded that the effects of liquefaction from the cadaver decomposition island (CDI) affect the original soil properties and the succession of mites in that soil. These findings are important, as they could be of forensic use in cases where bodies have been removed from the scene.

5.4 REAL-CRIME CASE INVESTIGATION

Three actual cases from European countries were investigated. The crime scene investigators and police provided soil samples from the crime scenes for us to assess if our approaches are reliable. *Macrocheles* species were identified in all cases. These species may be used as an indicator of time or location in forensic analyses, depending on the species. These results may provide missing information for the cases. The real-case investigation demonstrated the feasibility of combining well-established methods such as use of insects with methods that use mite biology.

5.5 EXTENDED RESEARCH

Although we have presented relevant results of mites in forensic settings in outdoor cases, more research is needed to complement our findings. These studies have provided a list of mites useful as markers related to seasonal weather and body/corpse condition or position, outdoors. However, this data serves as a baseline for only the region where the studies were performed. For other regions, the data may be used as a guideline, with local environmental conditions factored in. With the environmental data, decomposition patterns and the succession of mites were predictable. Further refinement may be achieved by collecting a greater number of samples, as well as collecting seasonal data for more years. In conclusion, the findings from this research provide relevant information for the use of mites as forensic markers, which can be added to the database of forensic literature. A baseline for the presence and abundance of mites in temperate climates was described and can be used for the future reference.

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APPENDICES

Environmental data for all seasons (Chapter 2)

Autumn

Stages	Time / day	Ambient temp (0 C)	soil temp	rain (mm)	U2 (winds speed at 2m)	RH (%)	Soil pH	Carcass temp. (0C)	Noise (dB)	light intensity
fresh	0	17.324	14.132	0.7895	2.64425	84.829	7.00	11.75	53.47	176.67
fresh	2	12.66042	14.321	6.0355	2.88958	79.173	7.00	12.25	61.57	35.333
bloating	5	14.52083	14.286	0.6484	3.50069	80.064	7.00	13.25	61.73	14
active decay	8	11.18354	13.276	12.525	3.7899	78.855	7.00	10.2	60.47	68.667
active decay	11	11.65854	11.97	1.3059	2.2554	87.324	7.00	12.25	60.67	54
active decay	13	8.47625	10.226	1.1909	2.95401	73.695	7.00	10.95	62.23	75.333
active decay	17	11.25313	10.794	2.2512	2.50767	89.467	7.00	13.7	64.33	57
advanced decay	23	9.727708	10.346	1.0589	1.14286	86.797	7.50	10.9	64.37	65.333
advanced decay	27	8.125417	8.0857	8.384	0.51463	90.117	8.00	7.6	65.67	36
advanced decay	34	5.834375	6.4505	0.1	1.58014	76.855	7.80	4.5	58.67	110.67
advanced decay	44	5.003542	6.7875	0.5334	1.18328	81.513	7.80	5.85	57.9	137.33
skeleton	51	4.353333	7.5499	7.0149	7.04645	165.2	8.00	0.6	71.17	318.33
skeleton	57	7.230208	7.6425	0.2125	1.25938	91.076	7.80	8	68.3	416
skeleton	64	6.874792	8.1028	7.2815	3.5128	79.182	8.00	5.85	68.97	141.33
skeleton	72	7.550208	6.5799	4.6084	3.3309	86.475	8.00	3.95	62.97	460.67
skeleton	79	8.395833	7.5958	0.1794	2.35347	86.609	6.80	6.85	60.97	429.33
skeleton	86	8.801875	6.576	0.8258	2.55764	90.114	7.50	8.25	69.27	448
skeleton	93	6.825625	6.224	6.3259	1.47118	80.649	7.50	5.75	64.9	202.33
skeleton	101	3.1325	5.141	1.7571	1.18958	92.627	7.20	-1.2	66.36	197.67
skeleton	150	15.3	14.005	1.289	0.784	85.412	7.20	12.45	65.55	1403

Spring

Stages	Time / day	Ambient temp (0 C)	soil temp	rain (mm)	U2 (winds speed at 2m)	RH (%)	Soil pH	Carcass temp. (0C)	Noise (dB)	light intensity
fresh	0	18.311	19.14	0.311	1.217	74.622	8.00	15.7	93.87	298.33
fresh	2	6.367	6.112	4.319	4.812	88.786	8.00	4	65.77	624.5
fresh	4	7.855	6.59	6.012	0.876	76.4	8.00	5.15	72.2	693
fresh	6	10.901	11.546	0.593	1.006	60.19	8.00	9.15	93.2	610.67
bloating	8	11	12.063	0.39	3.766	61.421	7.80	9.5	87	169.5
bloating	11	8.7	7.659	2.322	0.666	50.005	8.00	7.9	99.93	149.5
active decay	14	11.11	11.814	5.495	1.441	75.21	8.00	9.65	103.8	278.33
active decay	18	8.466897	12.79	0	44.5081	73.081	8.00	6.9	75.83	130.83
active decay	23	10.71318	14.214	0.6946	92.2487	56.5	8.00	10.6	106.2	138.5
active decay	28	9.7625	14.19	0	112.3	59.5	8.00	13.25	89.7	156.33
advanced decay	35	14.55618	17.966	0	64.8878	52.71	8.00	21.4	113.5	70.167
advanced decay	43	11.15382	14.14	1.3021	77.8612	55.8	8.00	11.9	104	63.5
skeleton	56	17.85417	20.051	0.3731	53.4797	63.7	8.00	19.6	112.6	74.833
skeleton	66	20.17206	19.057	0	34.2959	60.18	7.20	15.4	68.23	68.167
skeleton	84	14.75243	18.767	2.475	68.5275	50.76	7.80	15.95	29.27	43.167
skeleton	94	21.4375	22.017	0.1	40.6025	84.766	7.80	20.2	76.6	30.333

Summer

Stages	Time / day	Ambient temp (0 C)	soil temp	rain (mm)	U2 (winds speed at 2m)	RH (%)	Soil pH	Carcass temp. (0C)	Noise (dB)	light intensity
fresh	0	18.405	17.507	2.31	7.129	46.188	7.47	15.7	104.6	275.75
fresh	2	14.987	12.954	0.117	3.006	51.007	8.00	12.2	105.1	148
fresh	4	15.599	17.84	5.001	13.611	40.001	8.13	14.05	72.2	397.5
bloating	7	15.7	13.109	32.078	30.007	87.112	7.60	14.6	103.5	391.17
bloating	10	17.861	19.056	3	12.023	42.399	7.83	17.9	77.27	152.5
active decay	14	17.1	19.8	1.003	10.387	40	8.03	17.1	103.3	111.17
active decay	17	17.486	20.005	18.521	15.61	61.002	8.07	19.75	57.4	188.67
advanced decay	35	11.7	12.865	0.229	7.803	52.222	8.27	11.75	105.9	178.5
advanced decay	42	16.396	17.549	12.119	15.778	41.234	7.70	15.9	70.57	116.67
skeleton	57	12.702	10.871	30.004	48.054	88.004	8.17	12.85	69.17	125.17
skeleton	74	8.1	6.032	21.005	70.598	83.723	7.93	6.75	59.77	319
skeleton	98	7.317	7.001	5.6442	40.005	40.283	7.90	2.95	66.2	324.67

Winter

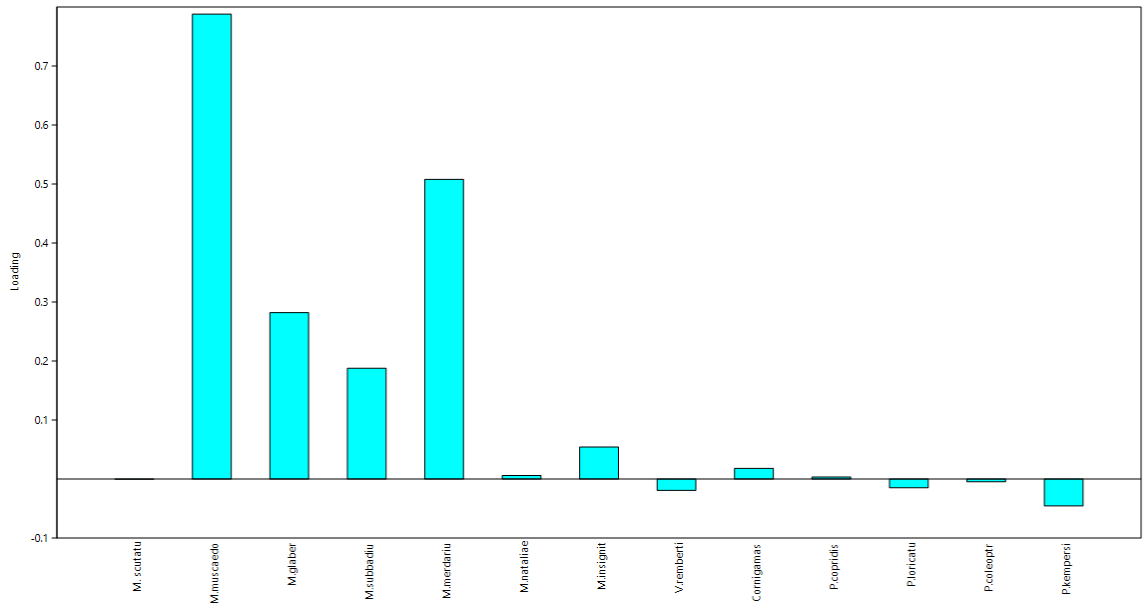
Stages	Time / day	Ambient temp (0 C)	soil temp	rain (mm)	U2 (winds speed at 2m)	RH (%)	Soil pH	Carcass temp. (0C)	Noise (dB)	light intensity
fresh	0	4.68941	4.6924	0.1333	4.96597	88.67	7.20	21	64.1	1.86
fresh	1	7.3	6.2934	1.1278	168.861	74.569	7.20	5.8	65.3	0.4
fresh	6	7.3	5.7708	0.3306	2.67813	90.16	7.15	3.25	74.77	14
fresh	10	8.2	8.9712	1.2493	6.86806	78.705	7.15	4.25	61.23	68.667
fresh	19	3.1	5.6185	0	2.59375	78.578	7.25	-1.95	62.63	54
bloating	28	10	5.4844	0.4208	3.93611	86.806	7.80	6.85	66.21	107
active decay	41	3.2	3.7601	0	1.88264	84.691	7.40	-3.2	70.3	103.2
active decay	49	8	3.2392	0	1.0309	88.865	7.35	3.15	66.93	33
active decay	58	4.2	3.3205	2.5917	5.74097	82.115	6.95	-5.3	65.8	115.5
active decay	78	9.2	5.0951	0.009	6.66875	72.212	6.65	6.05	65.5	137.5
advanced decay	85	10.1	3.9451	0	8.02708	57.917	7.45	3.7	97.2	332.67
advanced decay	99	8	7.9136	0	3.68715	68.982	7.20	6	83.47	192.33
advanced decay	108	11.8	10.194	0	1.37917	78.038	7.10	11.75	82.1	426.17
skeleton	148	13.2	13.031	4.8542	4.60868	90.197	8.20	10.2	86.9	150.67
skeleton	177	14	14.849	0	5.84965	60.139	8.00	17.7	70.6	470.67
skeleton	223	15.1	18.503	0	5.2059	70.643	6.75	17.55	57.2	439.5

Row Labels	Sum of Mesostigmata	Sum of Astigmata	Sum of Prostigmata	Sum of Oribatida	TOTAL
A	**25**	**8**	**2**	**25**	60
active decay	5	0	0	2	7
advanced decay	0	0	0	1	1
bloating	1	0	0	1	2
fresh	0	0	1	4	5
skeleton	19	8	1	17	45
SP	**93**	**25**	**17**	**18**	153
active decay	41	4	10	5	60
advanced decay	19	1	0	0	20
bloating	2	0	1	2	5
fresh	5	0	1	1	7
skeleton	26	20	5	10	61
SU	**68**	**1**	**6**	**10**	85
active decay	36	0	5	3	44
advanced decay	11	0	1	2	14
bloating	2	0	0	1	3
fresh	0	0	0	4	4
skeleton	19	1	0	0	20
WI	**27**	**1**	**0**	**1**	29
active decay	2	0	0	0	2
advanced decay	7	0	0	1	8
bloating	0	0	0	0	0
fresh	1	1	0	0	2
skeleton	17	0	0	0	17
Grand Total	**213**	**35**	**25**	**54**	327

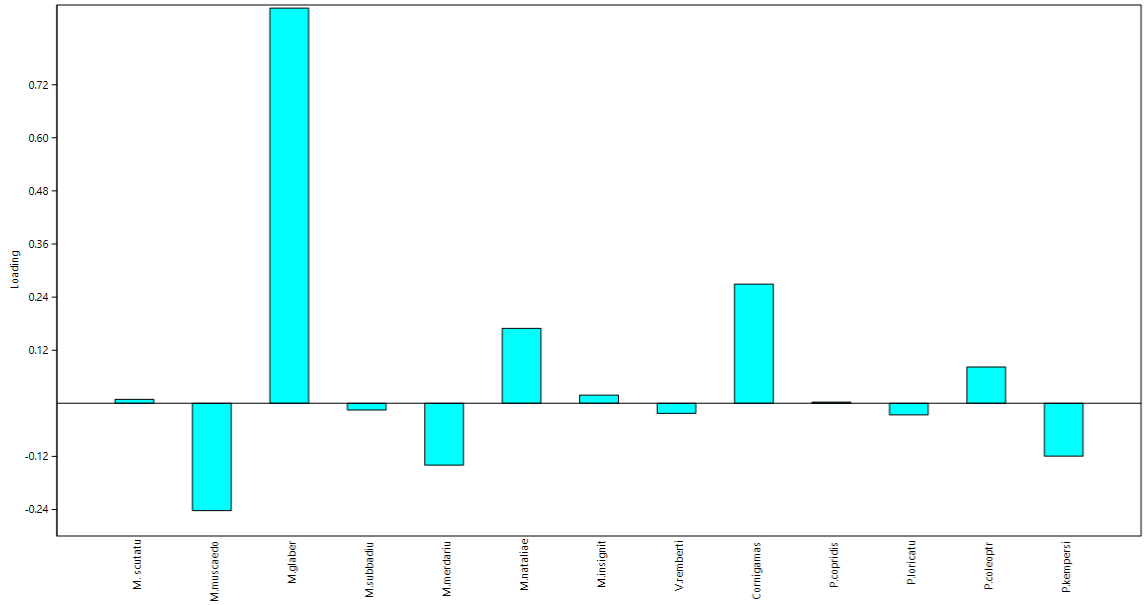
Calculation for diversity indexes.

Season	**Mesostigmata (N)**	Richness (S) (n)	pi (n/N)	ln pi	pi ln pi	Shannon's diversity (H')	pi2	Simpson Index (D)	H'max	Evenness (J')
A	**26**	22	0.8462	-0.1670541	-0.14135	0.141353	0.715976	1.396694	3.091042	0.0457
SP	**108**	60	0.5556	-0.5877867	-0.32655	0.326548	0.308642	3.24	4.094345	0.0798
SU	**73**	33	0.4521	-0.7939519	-0.35891	0.35891	0.204354	4.89348	3.496508	0.1026
WI	**29**	16	0.5517	-0.5947071	-0.32811	0.328114	0.3044	3.285156	2.772589	0.1183

First loading PC1 for PCA (Chapter 3)



Second loadings PC2



PC	Eigenvalue	% variance
1	110.353	62.765
2	44.7969	25.479
3	13.4034	7.6234
4	2.99963	1.7061
5	2.31988	1.3195
6	1.15737	0.65827
7	0.508268	0.28909
8	0.205172	0.11669
9	0.05782	0.032886
10	0.0170372	0.0096902
11	0.00023173	0.0001318
12	7.53E-32	4.28E-32
13	1.41E-33	8.03E-34