A STUDY OF FORENSICALLY IMPORTANT NECROPHAGOUS DIPTERA IN KUWAIT

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Declaration

Hereby I declare that the thesis entitled (A STUDY OF FORENSICALLY IMPORTANT NECROPHAGOUS DIPTERA IN KUWAIT) submitted to the university of Central Lancashire, UK for the degree of master in forensic entomology is based on my own research and that, to the best of my knowledge, it contains no material previously published, or substantially overlapping with material submitted for the award of any other degree at any institution or university, except where due acknowledgment is made in the text.

HANADI A. AL-MESBAH **July 2010**

Dedication

To all my family particularly:

Mother & father,

Sisters & brothers,

Daughters

And my relatives

Whose love and support at different times in my life made this work possible

ABSTRACT

Medico-legal entomology is the use of insects in legal matters. Knowledge of the distribution, biology, ecology and behavior of insects found at a crime scene can provide information on when, where and how the crime was committed. Although insects are known to have been used in the detection of crimes for a long time, there has been no great increase in the popularity of the subject until the last 30 years. Forensic entomology is recognized in many countries as an important tool in legal investigations. Unfortunately, it has not received much attention in some parts of the world such as Kuwait and other Arab countries where the value of insect as indicators in criminal investigations has not been fully realised.

For forensic entomology to be effective in legal investigations, knowledge of local insect assemblages and their population dynamics is essential. This study was conducted to gather database information on necrophagous flies and their succession on carrion using rabbits as experimental models. It allowed quantitative and qualitative comparisons to be made in four habitats (desert, urban, agriculture and coastal). In addition, the insects sampled from the carcasses were compared with those collected from human corpses as part of recent investigations in Kuwait. Eleven families of necrophagous flies were collected at four habitats; the most important families to breed on carrions were Calliphoridae such as *Calliphora vicina* Robineau-Desvoidy 1830, *Chrysomya albiceps* (Wiedemann 1819), *Chrysomya megacephala* (Fabricius 1794) and *Lucilia sericata* (Meigen 1826), and Sarcophagidae such as *Parasarcophaga ruficornis* (Fabricius 1794), *Sarcophaga Africa* (Wiedemann), *Wohlfahrtia nuba* (Wiedemann 1830) and unidentified *Sarcophaga* sp. which can used as forensic indicators to estimate the PMI. The most important species in respect to abundance and frequency were *Chrysomya albiceps* and *P. ruficornis*.

There was a significant diversity of fly species between different habitats. Four stages of decomposition were observed (fresh, bloated, decay, and dry). The decomposition of carcasses in urban habitats was faster than that of other habitats. There was an interaction between decomposition of carcasses and colonization by insects and correlation between the stage of decay and the succession of insect families and species arriving at a carcass. The decomposition was slower when the arrival of the

insects was delayed. The abundance and diversity of flies increased with the onset of bloated and dry stages.

Of the 13 human case studies, 10 were male and 3 females; their ages ranged from few days (newborn) to 53 years. They were of different nationalities. The cause of death was either homicide, suicide, drug abuse, toxicity or abortion. The necrophagous fly composition and their succession patterns obtained from real forensic cases showed a number of basic similarities to those observed on rabbit carcasses as experimental animals. The involvement of the study in legal investigations helped determine the manner and cause of death in two cases and estimate the PMI in one case. Although this study was conducted in one season and for a short period, it adds to the known species richness in Kuwait as it recorded several species of flies for the first time in Kuwait. The results provide additional information to forensic entomologists by having identified species that are associated with carrion in this region of the world. The study made the officials and the criminal investigation team aware of and familiar with forensic entomology, a step which may initiate future studies and interest in the application of insect evidence in legal investigations.

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ABBREVIATIONS

°C	Degree Celsius
A	Adult
ANCOVA	Analysis of covariance
ANOSIM	Analysis of similarity
ANOVA	Analysis of variance
C. albiceps	*Chrysomya albiceps*
C. megacephala	*Chrysomya megacephala*
C. vicina	*Calliphora vicina*
DNA	Deoxyribo Nucleic Acid
E	Egg
e.g.	For example; *exempli gratia*
et al.	And others; *et allii*
F. canicularis	*Fannia canicularis*
I	Immature
i.e.	That is; *id est*
ID card	Identification card
L. sericata	*Lucilia sericata*
M. domestica	*Musca domestica*
M. sorbens	*Musca sorbens*
mid-	Middle
NHM	British Natural History Museum
no.	Number
p value	Statistical value; the probability that the observed difference could have happened by chance alone
P	Pupa
P. alceae	*Physiphora alceae*
P. ruficornis	*Parasarcophaga ruficornis*
PAST	A comprehensive statistics package
PMI	Post Mortem Interval
R value	The test statistic R is defined as $R = (rb\text{-}rw)/(N(N\text{-}1)/4)$. Large positive R (up to 1) signifies dissimilarity between groups.
S. africa	*Sarcophaga africa (haemorrhoidalis)*
Site A	Desert habitat
Site B	Urban habitat
Site C	Agriculture habitat
Site D	Coastal habitat
sp., spp.	Single species, several species (plural)
t value	Intercept; the equality of regression slopes (as assumed by the ANCOVA). Intercept
UK	United Kingdom
USA	United States of America
W. nuba	*Wohlfahrtia nuba*

Chapter 1: INTRODUCTION

Forensic entomology is the use of insects in legal matters. Lord and Stevenson (1986) divided it into three components: urban, stored products and medico-legal. The latter field is also termed medico-criminal entomology because of its focus on violent crime (Haskell *et al.* 2008). Knowledge of the distribution, biology and behavior of insects found at a crime scene can provide information on when, where and how the crime was committed (Hall 2008).

Many insects can be attracted to a decomposing body; however, flies (Diptera) and beetles (Coleoptera) represent the majority of total carrion fauna (Greenberg 1991). The insects invade the carrion in a typical sequence or succession; each group of insects is attracted to a certain stage of decomposition. So, the type and composition of fauna found on a corpse are indicative of its stage of decomposition (Anderson 2009). Necrophagous flies are the first arrival at the carrion; they have ecological importance in its decomposition and represent an important tool in criminal investigation as well (Catts and Goff 1992). Blowflies provide valuable clues in many aspects of legal investigations, particularly in estimating the time of death in cases where the postmortem interval (PMI) is prolonged and the value of other methods is limited (Campobasso and Introna 2001). Two ways are used for estimation of PMI of human remains based on information on the development and succession of carrion insect species (Tabor *et al.* 2004).

The rates of insect development and their pattern of succession on the carrion differ from country to country and even from area to area within the same country, mainly because of the variation in the topography and climate or weather. Thus, it is not possible to use the data available in one country and apply it to the crime entomology in another country. Collection of information about the composition and dynamics of the local communities of necrophagous arthropods in the area where the crime took place is essential to use entomology effectively in legal investigations.

In Kuwait (and more widely, the Middle East), there is a lack of essential entomological information, which inhibits the progress of forensic entomology as an investigative tool. Only four species of blowflies are known to exist, and these are *Calliphora vicina* (Meigen). *Chrysomya albiceps*, *Lucilia cuprina*

(Wiedemann), *Lucilia sericata* (Al-Houty 1989). It is not known what species of Calliphoridae will colonize a carcass, or whether there are differences in the species found in different locations. The speed of decomposition of the carcasses has not been quantified, nor has the successional sequence been described and quantified.

This study fills in many of these gaps using rabbit carcasses as experimental models. It allowed qualitative and quantitative comparisons to be made in four habitats (coastal, agricultural, urban and desert). Sticky traps were used for sampling necrophagous flies which were attracted to visit the carcasses. The necrophagous flies collected from rabbit carcasses were compared with those collected from human corpses as part of recent investigations in Kuwait.

1.1. Kuwait Geography

Kuwait is a small country of about 17.820km² total area, occupying the north eastern part of the Arabian Peninsula. It lies on the Arabian Gulf and is bordered to the north and west by Iraq and to the south by Saudi Arabia. Topographically, the country is considered predominantly desert, however, it also has a rich marine environment that includes the shoreline (170km) and open water. Desert in Kuwait includes smooth, rough sand, flat areas, hilly areas, lush green parts and very dry desolate parts, but no real mountains or rivers (Jaman and Meakins 1998). Because of the scarcity of water resources, high salinity and poorness of sandy soil, only 1% of Kuwaiti land is cultivated. The main agricultural areas are in Wafra, located in the south, adjacent to the border with Saudi Arabia and Abdali, located in the north adjacent to the border of Iraq. Sulaibeya and Kabd, located Northwest of Kuwait, are the main dairy and sheep farming areas. Permanent pastures amount to about 8% of the total land supporting transhumant flocks of small ruminants and camels.

1.2. Kuwait Climate

Kuwait's climate is hot and dry with little rainfall, 250mm a year. Rains normally begin in November and continue intermittently until April. Apart from a few days in spring and autumn, the relative humidity in Kuwait is normally low. The summer temperatures often reach 50ºC and the winter temperatures can drop to 10°C and even to -4°C in some nights. The prominent feature of the climate is fluctuation of temperatures not only seasonally but also daily; the cool mornings can be followed by scorching afternoons. Another unpleasant feature associated with the desert is sandstorms which occur throughout the year, but are most common between March and August (Jaman and Meakins 1998).

The harsh climate limits the number of animal species that can survive and thrive throughout the year (Jaman and Meakins 1998). For example, after dispersal of the Old World screwworm fly, *Chrysomya bezziana* Villeneuve, to Kuwait from Iraq in November 1997, it could not live long in this environment and disappeared in the next summer; screwworm flies need a wet and warm climate to thrive (El-Azazy 1992; 1993; 2004). In such climate only the hardiest of plants and animals are tough enough to survive the wide variations of temperatures, the unpredictable and meager rainfall and the discomfort of sandblasting by dust storms. Microclimates are of vital importance in helping the animals that live in Kuwait make the best of their situation and maximize their chance of survival (Clayton and Wells 1987). Dead animals act as microhabitats in the desert give support to detritivores and decomposers including blowflies (Calliphoridae) which are the first visitors to carrion and are considered most important in recycling this organic material in the ecosystem in the desert.

1.3. Blowflies in Kuwait

Blowfly species were enumerated in the screen surveys of Clayton and Wells (1987), Al-Houty (1989) and Jaman and Meakins (1998), and myiasis-causing flies were recorded in human and animal cases in the published reports of Hira *et al.* (1993; 1997; 2004) and in unpublished reports of veterinary and health sectors. These investigations are useful and informative with respect to animal biodiversity and fly species list in Kuwait.

1.4. History of research and development of forensic entomology

Forensic entomology as it is today is derived from a long sporadic history of research dating back to 13^{th} century China (Greenberg and Kunich 2002). Insects are known to have been used in the detection of crimes for a long time and number of researchers have written about the history of forensic entomology (Benecke 2001; Greenberg and Kunich 2002; Gennard 2007).

During medieval times, the correlation between maggots on a corpse and the oviposition of adult flies was not recognized. However, the realistic and detailed illustration of corpses containing maggots was not unusual (Amendt *et al.* 2004). Illustrations of corpses colonized by maggots were recorded since the 16th century (Klotzbach *et al.* 2004). The importance of insects in the decomposition of human bodies was documented, as early as the 19th century: the biologist Carl von Linné (Linnaeus) in 1767 said that three flies would destroy a horse as fast as a lion would (Benecke 2001).

Until the mid-17th century, it was believed that under the right conditions maggots spontaneously arose from rotten meat. In 1668, Francesco Redi refuted the hypothesis of the spontaneous generation of life after the analysis of the results of his experiments in which rotting meat was either exposed to or protected from flies (Hall and Huntington 2008). Redi proved by his experiments that maggots come from fly eggs deposited on rotten meat or putrefying carcasses (Cruz 2006). Even today, some people still think that maggots are worms with no connections to flies (Goff 2001).

The first use of entomology as a forensic tool occurred in the 13th century in China. The sickle of the murderer, which was used to kill a fellow farmer, attracted blowflies. The murderer confessed to the killing when he was confronted with this entomological evidence (Greenberg 1991; Hall and Huntington 2008). Half a millennium later, during mass exhumations in France and Germany in the 18th and the 19th centuries, medico-legal doctors, such as Reinhard and Hofmann, observed that buried bodies are inhabited by arthropods of many kinds (Benecke 2001).

The first application of forensic entomology in the west was in the mid-19th century in France where Dr. Marcel Bergeret, a hospital physician used insects in a legal case of a murdered newborn baby found mummified behind a chimney (Goff 2001). During autopsy, Dr. Bergeret found larvae of a flesh fly, *Sarcophaga carnaria* (Linnaeus 1758). In estimating the PMI, he assumed that metamorphosis occurred within one year and concluded that the current occupants of the building could not have been the murderers. The court accepted this proof (Gennard 2007). However, in that case, the forensic examiner was of the opinion that the development of the adult flies took about one year; clearly his results would be questionable today. At that time, forensic examiners had only a poor understanding of insect biology (Amendt *et al.* 2004). In spite of any putative errors, Bergeret was the first westerner to use insects in a forensic investigation (Hall and Huntington 2008) and paved the way for later studies (Hall and Huntington 2009).

From 1883 to 1898, J.P. Mégnin, the accredited founder of forensic entomology, published several articles dealing specifically with forensic entomology (Hall and Huntington 2008). However, Amendt *et al.* (2004) considered Yovanovich beside Mégnin the first contributors to forensic entomology as they attempted to evaluate insect succession on corpses.

In the 20th century, a lot of scientists worked on many aspects of entomology including the biology and behaviour of insects. These works have contributed to the advancement of entomology in general and increased understanding of insect biology and ecology; many publications appeared dealing with the identification and taxonomy of insects (e.g. Aldrich 1916; Knipling 1936 and 1939; Hall 1948; Hall and Townsend 1977), assemblage of insect fauna associated with carrions (e.g. Fuller 1934; Reed 1958; Payne 1965) and bionomics of insects (e.g. Norris 1965). Although the objectives and results of these entomological studies are not relating directly to legal matters, they constitute the foundation upon which forensic entomology is built.

In the early 20th century, entomological evidence has been used sporadically in several murder cases in Europe with increased success (Smith 1986). Nuorteva *et al.* (1967) and Nuorteva *et al.* (1974) were among the first to discuss the use of forensic entomology for the estimation of PMI in this continent.

In the second half of the 20th century there was been an increasing interest in forensic entomology and the topic was revived by many researchers such as Rodriguez and Bass (1983), Greenberg (1985), Lord *et al.* (1986), Goff *et al.* (1986), Introna *et al.* (2001). Although the amount of research increased in this field, there was no great increase in the popularity of the subject till the last thirty years (Gupta and Setia 2004). At the end of the 20th century and beginning of the 21th century in criminal investigations, forensic entomology is recognized in many countries as an important tool (Gennard 2007; Haskell *et al.* 2008; Byrd and Castner 2009; Amendt *et al.* 2010).

Forensic entomology continues to progress as a science, gaining credibility as research leads to a greater understanding of insects and refinement of methods. Employment of DNA analysis (Wells and Sperling 2001) and use of scanning electron microscopy (Liu and Greenberg 1989; Wells *et al.* 1999) has helped improve accuracy in the classification and identification of insect species. Recent research has brought the possibility of extraction human DNA tissue (Wells *et al.* 2001) and gunshot residue (Roeterdink *et al.* 2004) from the gut contents of feeding maggots. Entomotoxicology (detection of narcotics and toxins in the tissue of feeding insects on a decaying corpse) is an emerging field providing valuable tools in the investigation of homicides, suicides and other unattended deaths where chemicals are involved (Goff and Lord 2009).

Despite great strides having been made in fundamental and applied research, there are many questions yet to be answered (Amendt *et al.* 2010). Forensic entomology is still a young discipline and there is still much room for progress (Amendt et al. 2004). The scientific literature available on this topic, although constantly growing, remains small when compared to many other biological and legal subjects. Likewise, the number of qualified participating forensic entomologists capable of fully utilizing insect evidence is currently very small (Hall and Huntington 2008; Hall and Huntington 2009).

Forensic entomology as a science and profession faces many challenges as it attracts the public interest and also offers an opportunity for expanding research related to forensically important arthropods (Byrd and Castner 2009). This field of study has unfortunately not received much attention in some parts of the world such as in Kuwait and other Arab countries, where the value of insects as indicators in criminal investigation has not been fully realized. In addition, there is still a paucity of information on the composition of carrion insect assemblages and their succession patterns to be used in legal investigations, particularly the estimation of PMI. The challenge to apply forensic entomology is increased because of the fact that the pronounced geographic variability of the carrion insect community does not allow to use the available data from one biogeographic area to another (Anderson 2009). This supports the need for generating a database of sarcosaprophagous insects in a variety of geographic locations and habitats. The output of the combination of experimental studies using animal models and practical case work will familiarize officials with the forensic applications of entomology and will provide specialized individuals to analyze and interpret this type of data (Gill 2005). For maintaining advancement of the discipline, there is perhaps also a need to incorporate it in the academic curriculum at the Master's level under Applied Entomology/Zoology (Varatharajan and Sen 2000). Students provide a rich supply of enthusiasts who can contribute to forensic entomology as part of their studies or degree program (Gennard 2007). Without extensive research in the use of insects as indicators in death investigations under different scene circumstances and environmental conditions, this science will not be convincing in the rigid and factual countenance of law.

1.5. Decomposition and carrion insect succession

Decomposition is a continuous process, beginning at the point of death and ending when the body has become a skeleton (Goff 2010). Although this process is continuous (Schoenly and Reid 1987), many authors have divided it into three to six discrete stages (Fuller 1934; Reed 1958; Payne 1965; Coe 1978; Braack 1986; Early and Goff 1986). However, there does not appear to be a firm relationship between these and the total number of species of insect observed in each study (Goff 2010). These variations in the numbers of the stages of decomposition could be due to the type of carcass used, geographic region and author observation. Extremely small carcasses such as those of lizards and toads (Cornaby 1974), bird and mice (Blacklith and Blacklith 1990) and other small rodents (Payne 1965) are reported to decompose very quickly with no demarcation of any stages. However, Bornemissza (1957) and Moura *et al.* (1997) described five stages using guinea pig and rat carcass respectively.

Some studies like that of Parikh (1999), Vij (2001) and Pillay (2004) have described the changes after death under three headings of immediate, early and late stages. Reed (1958) using dog carcasses in Tennessee, Rodriguez and Bass (1983) using human corpses in Tennessee, Braack (1986) using Impala carcasses in South Africa and Tantawi *et al.* (1996) using rabbit carcasses in Egypt, proposed four stages of carrion decomposition, namely fresh, bloat, decay and dry. This categorization is perhaps the most convenient to be easily applied and marked out in experimental studies and case work (Morris 1988; Aggarwal 2005). However, in Hawaii, Goff and other authors have observed five stages of decomposition using pig or cat carcasses (Early and Goff 1986; Tullis and Goff 1987; Avila and Goff 1998; Davis and Goff 2000; Shalaby *et al.* 2000).

A corpse, whether human or animal, goes through a process of dramatic physical, biological and chemical changes during decomposition (Henssge *et al.* 1995). Thus, the carrion becomes attractive and presents a temporary and progressively changing habitat and food source for a wide variety of organisms ranging from bacteria and fungi to vertebrate scavengers (Goff *et al.* 1986). The arthropods comprise a major element of the carrion community (Payne 1965; Anderson 2009; Kreitlow 2009). Of the different groups of arthropod that have been categorized based on their attraction to the corpse in different stages of decomposition, four basic arthropods corpse relationships have generally been accepted: Necrophages e.g. blowflies, which feed and breed on the decomposing tissue of the carrion; Necrophiles e.g. beetles, which are predators and parasites of necrophagous fauna, feeding on other arthropods; Omnivores e.g. wasps and ants, which feed on the carrion and its colonizers and Opportunists e.g. spiders, which are found in and around the carrion but only use it as an extension of their own environment (Smith 1986; Goff 1993; Kreitlow 2009).

There are over 60 insect families which play an important role in carrion ecology (Smith 1986). However, only the families (Calliphoridae, Sarcophagidae and Muscidae) of Diptera (Flies) and the families (Silphidae, Staphylinidae, Cleridae and Dermestidae) of Coleoptera (beetles) are the most important to be used in forensic entomology (Aspoas 1994; Anderson and VanLaerhoven 1996).

These insects do not visit carrion at the same time, but in sequential 'waves'; each stage of decomposition is attractive to a different groups of sarcosaprophagous insects. Some are attracted directly by the carrion, which is used as a food source or an oviposition medium, whereas other species are attracted by the large aggregation of other insects, which provide them a food resource (Anderson 2009).

The postmortem changes during decomposition process and insect activity has been described by Aggarwal (2005), Kreitlow (2009) and Goff (2010). The fresh stage begins at the moment of death and continues until bloating is first evident.

In this stage, there are no visual or odor effects obvious to humans; however, some insects such as calliphorids and sarcophagids are able to detect the early stages of decomposition (Anderson 2009; LéBlanc and Logan 2010). Due to the autolysis of tissues and the activity of bacteria and fungi, from both the intestine and the external environment, gasses are produced (Knight 1991; Clark *et al.* 1997; Introna and Campobasso 2000). This results in abdomen inflation and the commencing of the bloated stage. In this stage more necrophagous flies are attracted to the carrion reaching a peak and early larval instars are observed in natural openings and injuries as well. The beginning of the decay stage is marked by release of gasses as the abdomen deflates. At this stage decomposition is advanced with a pronounced odor; most of the flesh has been removed with the departure of the calliphorids and sarcophagids and arrival of coleopterans. The dry stage is the final stage of decomposition where most soft tissue has disappeared with that remaining in the abdomen. The odor starts to fade. At this stage, the carcass consists of only dry skin, cartilage and bones and the beetles prevail on the remains.

The main objective of insect succession studies is to study the colonization patterns of insects in a particular area, often with the goal of obtaining data that can be applied to forensic investigations involving insects in the event that a death occurs in a similar environment (Kreitlow 2009). Because of ethical, moral and logistical constraints, few researchers (Rodriguez and Bass 1983, 1985) have had the chance to work experimentally on human corpses (Hart *et al.* 2008). Most published information on insects involved in human decay comes from case studies (Smith 1986; Williams 2008). As useful as these may be, they are usually just a “snapshot” of the process; therefore, the majority of the studies on decomposition and insect succession have been done on non-human carcasses (Wells and LaMotte 2009). However, experimental animals are not always available for use, such as in Italy, where, bureaucracy makes it prohibitively difficult. Here, researchers resort to studying the carrion fauna on the human cadavers which turn up as part of real legal investigations (Magni *et al.* 2009).

A variety of different types and sizes of animal carcasses have been used to study decay processes and insect succession including rabbits (Denno and Cothran 1976; De Jong and Chadwick 1999), cats (Early and Goff 1986), dogs

(Reed 1958, Jirón and Cartín 1981), guinea pigs (Fuller 1934; Bornemissza 1957), mice (Putman 1978), rats (Baumgartner 1988; Moura *et al.* 1997; Tomberlin and Adler 1998), chickens (Hall and Doisy 1993; Arnaldos *et al.* 2005), deers, bears and alligators (Watson and Carlton 2005), lizards and toads (Cornaby 1974), turtles (Abell *et al.* 1982) and elephants (Coe 1978), foxes (Easton 1966; Smith 1975) and Monkeys (Omer *et al.* 1994).

Pig carcasses are now used in most carrion ecology and forensic entomology studies as they are the best models for human corpses. Pigs are relatively hairless, monogastric and have similar skin type and comparable sizes with humans (Catts and Goff 1992; Anderson 2001). However, these animals are not easy to obtain in Arab countries including Kuwait. Rabbits have been used satisfactorily as animal models to study carrion ecology and insect activity by many researchers in different habitats and geographic locations (Denno and Cothran 1976; Tantawi *et al.* 1996; Bourel *et al.* 1999; De Jong and Chadwick 1999; Shah and Sakhawat 2004). These animals are easily handled and can be obtained in nearly uniform size and weight to compare decomposition rates, carrion insect compositions and succession patterns in different habitats. However, caution in the use of successional sequences produced using non-human models is necessary, even if a good model such as a pig is employed (Lane 1975; Catts and Goff 1992).

The studies referenced above, that used rabbits to study forensic flies, were in different localities e.g. in temperate regions (France), in subtropical regions (Egypt) and at high altitudes in Colorado where temperatures are low (USA). The decomposition process of rabbit carcasses and the pattern of arthropod succession were the same as those were observed in other experimental animals and the forensically important insects collected were representative of the geographical areas where each study was conducted

Generally, the size of a carcass has no obvious effect on the composition of the insect fauna or succession pattern, but there is a positive correlation between the size of the carrion and the number of flies bred (Kuusela and Hanski 1982; Hewadikaram and Goff 1991). No differences were observed between patterns of succession based on the use of the cat or pig carcasses in the same habitat (Early and Goff 1986).

Carrion fauna associated with pig carcasses have been compared with those on human corpses in the same habitat (Carvalho *et al.* 2000). However, no data are available to compare the insect fauna on rabbit carcasses with those on human corpses. Arnaldos *et al.* (2005) used the entomological data obtained from experimental studies using chicken carcasses in order to determine PMI in real forensic cases. They concluded that the experimental data may be considered as representatives of the circumstances occurring in human corpses and may act as a valid database of sarcosaprophagous fauna in the same habitat.

Decomposition of the carrion and insect succession patterns are influenced by many factors e.g. geographic location, habitat, season, temperature, humidity, insect abundance and carrion accessibility. Furthermore, many of these factors are inter-related and even the decomposition rate and insect succession are inter-dependent. It has been found that decomposition of the carrion is accelerated when the insects, particularly necrophagous flies, are present (Payne 1965; Abell *et al.* 1982). Braack (1986) found that arthropods were capable of reducing a medium-sized Impala carcass to keratinous remains and bones within five days in summer and within 14 days in winter, without the aid of vertebrate scavengers. Simmons *et al.* (2010) found that body size is important factor in decomposition rate only when carcasses are accessed by insects; the small carcasses are decomposed faster than large ones. However, in the case of insect absence, the decomposition of bodies becomes at the same rate whatever they are indoors, submerged or buried.

In general climatic conditions, particularly temperatures, play an important role in the insect activity and carrion decomposition. Variations in climatic conditions lead to differences in the decomposition speed, insect development rate and succession pattern in different habitats, seasons and geographic locations (Anderson 2009).

In a dense urban setting in Chicago, Baumgartner (1988) reported increasing fly diversity and abundance with the advance of warm weather. In contrast, persistent cold temperatures combined with other properties of high elevations in Colorado have a negative influence on the rabbit carcass decomposition and insect succession as illustrated by the drastically reduced number of taxa, delayed loss of biomass, prolonged bloat and retarded larval development. Even in burial conditions, cold weather combined with type of soil effectively inhibited the decomposition process and accessibility of buried pig carcass to insects (Turner and Wiltshire 1999).

High humidity and rainfall could decrease the insect activity (Parman 1945) and larval development (Anderson and VanLaerhoven 1996) as well as affect the microbial activity within the carcass (Gill 2005). This in turn influences the rate of decomposition. Payne (1965) found that the reduction of carrion was slower on cool, cloudy days. Differences in decomposition were seen in the fall carcasses, probably due to greater rainfall (Tantawi *et al.* 1996). The length of bloated stage is more dependent on the abundance and larval development than climatic conditions, particularly temperatures, as this stage is ended by the penetration of insect larvae into the body, resulting in the release of gases (Anderson and VanLaerhoven 1996). Tantawi *et al.* (1996) found that in summer, bloated stage lasted longer than in spring. They attributed this observation to the differences in breeding biology of the primary flies colonizing the rabbit carcasses in these seasons.

Many studies have been conducted to study the carrion ecology and associated arthropod fauna in different geographic locations and habitats: In mainland British Columbia, which has a typical coastal rainfall habitat, Dillon and Anderson (1995) compared the insect succession and decomposition speed of exposed pig carcasses in sun and shade and found higher temperatures and faster decomposition in sun, but a greater diversity of successional insects in the shade. In Manitoba, Canada, Gill (2005) reported no differences in the arthropod community collected from sun- and shade-exposed pig carrion. In contrary, in the Boreal forest region of Canada, pig carcasses situated in sun were colonized by more abundance of dipteran species than those were placed in shade. Similarly, Isiche *et al.* (1992), in southern England, found a difference in the calliphorid species abundance and diversity when observing mouse carrion in sunny and shaded habitats. Four species were recorded in the sunny areas and only two of these species were observed in the shaded areas. On the island of Oahu, Hawaii, Goff (1991) recorded significant differences in the insect colonization of remains indoor and outdoor situations. The peak of insect diversity is reached faster indoors; however, at later stages, few indoor species contrast to a wide diversity of outdoor species. The indoor carcass fauna was richer in Diptera species while the outdoor fauna was characterized by a higher abundance of Coleoptera species. Goff also observed differences in insect succession in different areas of a rainforest habitat where distinct changes in species composition were observed (Early and Goff 1986; Tullis and Goff 1987). Tessmer and Meek (1996) noted several differences in species composition of calliphorids on carrion placed in woodland versus pastureland habitats. They found that a greater abundance of blowflies was associated with the pasture habitat.

1.6. Forensically important flies

There are about 86000 fly species described worldwide (Castner 2009). A fly community in a given geographic comprises mainly synanthropic, myiasis-causing and forensically important flies. The latter ones are those associated with remains and provide evidence in the death investigations (Greenberg 1985; Smith 1986). Some fly species can be placed in more than one of these mentioned categories, such as *Lucilia (Phaenicia) cuprina*, *Lucilia sericata*, *Chrysomya albiceps* and *Calliphora vicina* which can infest living tissue and breed on carrions, feces or decaying matter.

In Arab countries including Kuwait, data on synanthropic and myiasis-causing flies are available in publications of some authors (e.g. Büttiker *et al.* 1979; Al-Houty 1989; Hira *et al.* 2004) who are interested in studying biodiversity and ecosystems or who are interested in recording myiasis cases. However, there is a paucity of basic information on forensic flies as this field of study has unfortunately not received much attention.

There is great variability in the fly species involved in the sequential colonization of animal remains and their times of arrival with respect to geographic location (Early and Goff 1986; Anderson and VanLaerhoven 1996; Archer and Elgar 2003; Tabor *et al.* 2005). Each biogeographic zone has specific climatic, seasonal and developmental requirements of certain fly species.

So, the occurrence and abundance of forensic fly species differs among geographic regions, and even among habitats in the same geographic area. For example, in the Old World, species of genera *Calliphora*, *Lucilia* and *Chrysomya* are considered the most important carrion colonizers (Smith 1986), whereas in the New World, *Phormia regina* (Meigen 1826), *Cochliomyia macellaria* (Fabricius 1775) and species of *Lucilia* and *Calliphora* are important carrion breeders (Hall 1948). Also, the habitat can influence the carrion fauna composition as blowflies exhibit preferences within their regional distribution (Erzinçlioğlu 1996). *Phormia regina* is prevalent in the urban environment in the Netherlands as they prefers shade rather than brightly sunlight; in urban areas shade is available in and around buildings (Huijbregts 2004; Haskell *et al.* 2008). In another geographical location, in southwestern British Columbia, pig carrion in open fields attracted only *Phormia regina* and *L. illustris* (Meigen 1826) (Anderson and VanLaerhoven 1996). The latter species frequents corpses located in more rural wooded areas (Williams 2008). *Calliphora vicina* and *L. sericata* are commonly considered urban species (Reiter 1984 cited in Anderson 2009), but have collected in rural regions (Dillon and Anderson 1995; Haskell *et al.* 1997). Similarly, *L. sericata*, *Bercaea Africa* (Wiedemann 1824) *(Sarcophaga haemorrhoidalis* Fallén*)* and *Chrysomya albiceps* have been trapped indoors and outdoors in Saudi Arabia (Büttiker *et al.* 1979). It is obvious that a fly species could prefer and subsequently is abundant in a certain habitat but in the same time it may extend its range to another habitat. Therefore, caution must be used in determining location of death or whether remains have been moved based on insect evidence.

Wherever the geographic location, it has been accepted that the most significant and the first insect colonizers on carrion are typically species of necrophagous flies in the families Calliphoridae (blowflies) and Sarcophagidae (flesh flies) (Greenberg 1991; Hall and Doisy 1993; Anderson and VanLaerhoven 1996; Tabor *et al.* 2004). But the species involved will vary according to the biogeographic region. In most European countries including Britain, France and Germany, *Calliphora vicina*, *Calliphora vomitoria* (Linnaeus 1758) and *Lucilia sericata* are common primary colonizers of corpses (Bourel *et al.* 1999; Schroeder *et al.* 2003; Gennard 2007). In contrast, *Chrysomya albiceps* is the common first colonizer in Afro-tropical regions, oriental regions, central South America and Southern Europe (Baumgartner and Greenberg 1984; Hall and Smith 1993). Whilst in another tropical region such as Hawaii, it has been found that the initial colonizers are *Lucilia cuprina*, *Chrysomya megacephala* and *Chrysomya rufifacies* (Macquart 1843) in the family

Calliphoridae and *Bercaea africa (Sarcophaga haemorrhoidalis)*, *Parasarcophaga ruficornis*, *Sarcophagula* (*Sarcophaga) occidua* (Fabricius) and *Helicoba morionella* (Aldrich) in family Sarcophagidae (Early and Goff 1986).

Blowflies are attracted to the body immediately after death to feed and oviposit (Smith 1986; Dillon 1997; Anderson and VanLaerhoven 1996). Sometimes, oviposition is delayed when the death occurs at night. It is commonly thought that flies are nocturnally inactive (Haskell *et al.* 1997). Generally, some fly species such as *Musca* and *Muscina* spp. may visit the carrion to feed but not to breed (Dear 1978; De Souza and Linhares 1997). Mostly, these 'secondary' flies are attracted by exudates rather than the carrion itself (Gennard 2007).

Knowing the developmental data of the primary colonizers of the corpse and the meteorological data at the crime scene, it is possible to estimate the PMI (Wells and LaMotte 2009). This is like a biological clock; it starts at the time of oviposition (Greenberg 1991). However, if insect access is limited it complicates analysis of specimens by the forensic entomologist (Catts 1992). Whether access is denied by climatic conditions (Smith 1986), by burying the remains (Payne *et al.* 1968; Rodriguez and Bass 1985) or by any other factors (Anderson 2009). Flies that are attracted to a corpse are also influenced by their environment (Gennard 2007).

Low temperatures (De Jong and Chadwick 1999; Shah and Sakhawat 2004) and heavy rain and humidity (Smith 1986) influence blowfly activity and delay their arrival at carrion. Greenberg (1990) has stated that calliphorids do not fly in the rain. Similarly, Digby (1958) found that strong wind inhibited the ability of *Calliphora vicina* to fly. In contrast, sarcophagid flies are considered to be unimpeded by rain (Erzinçlioğlu 2000); as a result, flesh flies may be the initial colonizers of the body if there is a long period of rainy weather. Temperatures above 30°C and below 12°C are also known to inhibit blowfly activity (Gennard 2007). Kuwait has a harsh environment; the climate is intensively hot and dry with sandstorms, particularly in hot season when temperatures reach 50ºC. The influence of this environment on the fly activity and ecology needs to be investigated as it is important in the determination of the PMI.

Many blowfly species are cosmopolitan and those which occur in Kuwait could be found in other geographic regions. It is likely that, the same fly species, in different geographic locations, has different developmental requirements and ecological features which enable it to be adapted and thrive in such markedly different environments. Anderson (2009) has stated that when a species is found to be present in many different regions, it is possible that there may be within-species differences. However, if we suppose there is geographical differences between blowfly species, it is unknown if this is due to genetic variations or simply a reflection of the behavioral response to different environmental conditions (Wells and LaMotte 2009). Using gas chromatography/mass spectrometry, Byrne et al. (1995) identified the extracted cuticular hydrocarbons of 3 geographical populations of *Phormia regina* and after discriminant analysis of the cuticular hydrocarbons profiles, they could separate the flies according to both location and gender. A morphological and molecular analysis of *Chrysomya bezziana* from some countries in Africa and Asia revealed that there are two geographical races of this species, one from sub-Saharan Africa and the other from the Gulf region and Asia. The latter race could be further divided into two lineages, i.e. one from mainland Asia (from Iraq to the Malay Peninsula) and the other from two islands of Papua New Guinea (Hall *et al.* 2001).

Distribution range of Calliphoridae changes according to the season and climatic conditions. It has been found that *Phormia regina* is the dominant species in the summer months in the northern USA, whilst being the dominant species in winter months in southern USA (Byrd and Allen 2001). This may also be the case in Kuwait where flies may migrate from the desert to urban habitat or to agricultural areas in hot weather and sandstorms to seek for suitable microhabitats in other places to maximize their chances of survival.

There are many questions which need to be answered and a lot of data required to be gathered in order to apply insect evidence in legal investigations in Kuwait. This study provides baseline information on the forensically important flies and is the first step for more investigations in the ecology, biology and behavior of these flies in this country.

1.7. Use of insects in criminal investigations

Although forensic entomology includes three categories (urban; stored-products; medico-legal), the third category, medico-legal forensic entomology, is the most popularized aspect of the science and more frequently used in legal matters (Catts and Goff 1992).

Medico-legal entomology deals with the use of insect or other arthropods associated with a corpse at a murder scene in legal investigation to provide data not available by using the normal methods of classic pathology (Wolff *et al.* 2001). These data helps to solve litigation in criminal cases (Haskell *et al.* 2008).

Forensic entomologists can provide an objective estimate of time since death as well as other valuable information concerning the circumstances surrounding the victim's demise including season of death, location of death, movement or storage of remains after death, specific sites of injury on the body, postmortem artifacts on the body, use of drugs, and even more in linking a suspect to the scene of a crime, in child neglect and sexual molestation as well as in the identification of suspects (Campobasso and Introna 2001).

The crime scene is usually the focal point of any criminal investigation. A death scene properly processed can yield information useful to reconstruct events and circumstances, link a suspect to the victim or scene and establish the credibility of the statements made to investigators by witnesses (Meek and Andrew 2005).

Therefore, the task of the entomologist starts at the crime scene to gather insect samples and evidence with regards to the habitat type, corpse condition and meteorological data. He/she should contact other authorized personnel responsible for the legal investigation such as police officers, technicians and medical examiners for additional information about the identity of the victim, circumstances of the crime and verification of essential evidence (Haskell *et al.* 2008).

The next step is in the laboratory where entomological, molecular and toxicological analysis are performed on the insect samples to determine their species, life stage, age and if there is a drug or chemical in their tissues. The analysis of the data collected from observations and notations at the crime scene and the results of the laboratory work combined with utilization of the basic concept of insect systematics, ecology and biology, the entomologist may help to answer the question when, who and where the death occurred. It should be kept in mind that each case has its own set of circumstances and peculiarities that defy exact matching of events to some predetermined scenario (Haskell *et al.* 2008).

Necrophagous flies are attracted to the carrion by the odour emanating from it. In the case of the concealment or hiding of a corpse after homicide, its place may be betrayed by numerous blowflies in the immediate vicinity of the site in which it is hidden. The case history described by Williams (2008) is an example of the help of entomology in this respect. The investigators could determine the site of the remains of a murder victim which were reported thrown down an open well on a farm in a rural area in south-central Indiana. The exact location of the well was unknown. During searching investigators could determine the site of the well after seeing thousand flies were hovering above it. The remains were at the bottom of the well covered by debris; they were in advanced stage of decay, but no insects had colonized them. Access to the body by the blowflies was prevented by the intervening material, but odors were still capable of attracting multitudes of insects.

Usually the female flies prefer to oviposit or larviposit in moist sites of the body such as natural orifices. Therefore, any maggot concentrations at other sites on the body reflect the presence of antemortem injuries. This is particularly so with bodies in the fresh or bloated stages or decay (Haskell *et al.* 1997; Williams 2008). Antemortem injuries are bloody areas, more attractive to the female flies to oviposit. Rodriguez and Bass (1983) have stated that a corpse may be more decomposed in the area of the body where there are injuries which result in exposure of blood and underlying tissue, thus providing access for carrion bacteria and insects. Careful examination of these sites could help to know the cause and manner of death. The wound may be the result of a knife stab or bullet. Extensive maggot activity in the chest and palms of hands, and less in the face, was used to indicate stab and defense wounds in the death of a young woman (Rodriguez and Bass 1983; Williams 2008). Also the presence of other traumatic antemortem injuries such as bruises, contusions, abrasions and lacerations, which are target for flies, could indicate that a victim was subjected to physical abuse such as dragging, hitting or falling before death (Gunn 2006; Haskell *et al.* 2008). The cause and manner of death can be also known in cases of drug abuse or toxicity by determination of narcotics or toxins in the tissues of feeding Instars on the severely decomposing human remains (Goff and Lord 1994; Goff and Lord 2009).

Insects can also be used as indicators in the cases of neglect and sexual abuse of elderly and children (Benecke 2009). Untreated wounds and traumatized and soiled urogenital areas are predisposing factors for fly infestation (Haskell *et al.* 2008). Also fecal material and urine attached to diapers and garments, due to neglect, are often as attractive to a fly species as a decomposing corpse. Neglect can not be automatically assumed if evidence of insect activity is found because eggs may be laid immediately after cleaning and in the case of flesh flies, because 1st instars are laid, maggots are instantly present (Gunn 2006). The extent of neglect i.e. colonization interval (not the postmortem interval) must be determined based on the temperature of the place and the species and age of insects found. Gherardi and Costantini (2004) described a case in which the maggot-infested body of an elderly woman was discovered in the filthy flat shared with her daughter. This resulted in her daughter being charged initially with concealment of a corpse although she claimed that she had given her mother a meal the previous night. An autopsy later in the day of discovery demonstrated that body's temperature was 34.7°C, indicating that the women had not been dead for long, and this was corroborated by finding only the early stages of postmortem decomposition. However, the presence of 3rd instar blowfly larvae and second instar flesh fly larvae on the skin and diapers indicated that the woman had not been kept clean for several days and the daughter was subsequently charged with elderly neglect.

The presence of certain fly species associated with the victim may indicate neglect/or abuse. This can be corroborated by estimation of the age of recovered maggots to reveal the length of neglect (Gunn 2006). For example, the presence of specific wound myiasis-causing flies such as *Chrysomya bezziana*, *Cochliomyia hominivorax* (Coquerel 1858) and *Wohlfahrtia magnifica* (Schiner) leads to the suggestion that the wounds were not clean while the helpless victim was alive. These flies themselves could be the cause of death, particularly when sensitive organs are infested. Also the presence of *Fannia canicularis* (Linnaeus) and *Muscina stabulans* (Fallen) associated with the crime scene or the corpse, particularly in the early postmortem stages call the attention that during life the sanitation and health care of the victim were poor.

These fly species do not feed on the corpse itself but they are attracted to excreta (Benecke and Lessig 2001; Frost *et al.* 2010). Benecke (2009) in a case history found the larvae of *F. canicularis* and *Muscina stabulans* with the adult of *Dermestes lardarius* (Linnaeus) on the corpse of an elderly woman. The presence of only these fly species and intact skin and eyes give an indication that the colonization occurred antemortem. Similarly, *Lucilia sericata* are attracted to odors, such as ammonia, resulting from urine or faecal contamination (Gennard 2007). The oriental latrine fly, *Chrysomya megacephala*, is equally attracted to feces and can lead to errors in estimates of the postmortem interval in similar cases of neglect (Goff *et al.* 1991).

Entomological tools may also be used to determine if the body has been moved from one locality to another, and may provide information about the site of death itself because of relatively defined diversity of insects that exist in specific geographical area/habitat (Anderson 2009). In a landmark case in Belgium, forensic entomologist observed five species of Staphylinids on the bones of a corpse but the absence of other insect species in spite of favorable conditions suggested that the murder was committed elsewhere (Leclercq *et al.* 1991 cited in Varatharajan and Sen 2000).

As mentioned above, forensic entomology is used in many aspects of death investigations to solve or to produce additional information on crimes and their circumstances. However, the major contribution of the science is the estimation of the postmortem interval in human cases (Haskell *et al.* 2008; Wells and LaMotte 2009), and even in wildlife cases (Anderson 1999).

In very early stage of death (1-3 days), the PMI can be estimated by the pathologist based on the biological and physical changes of a corpse. The entomological findings can corroborate the results of the pathologist. In the early stages of decomposition blowfly eggs, and sometimes small larvae can be seen in the natural orifices or wounds of the body; it can be assumed that it has only been on the site for a very short time (1-2 days) (Smith 1986). After 72 hours or more after death, the medical procedures are no longer of value (Campobasso and Introna 2001) and then the value of entomological tools become increasingly important in the PMI estimate.

Information on the development and succession of carrion insect species is used in two primary ways to estimate the PMI of human remains (Tabor *et al.* 2004). In cases where death was recent (from one day to more than one month) the PMI can be estimated by analyzing the degree of development of early-arrival species which have colonized the corpse (Smith 1986; Goff 1993; Wells and LaMotte 2009). The technique is heavily dependent on correct identification, the accuracy of the estimated environmental conditions the insects are thought to have experienced and the reliability of the reference development data (Gunn 2006). Data on the development of forensically important species are consulted from controlled studies (e.g. Kamal 1958; Introna *et al.* 1989; Goodbrod and Goff 1990; Wells and Kurahashi 1994, Greenberg and Wells 1998; Byrd and Allen 2001). Based on the results of these controlled studies, the entomologists can estimate the time taken for the development of the stage of similar species collected on a corpse.

Another PMI-dependent process is the succession of arthropod species found on and within a body (Schoenly and Reid 1987). As insects arrive at a corpse in a predictable manner specific to the location and environmental conditions under which the remains are found (Payne 1965; Anderson and VanLaerhoven 1996; Anderson 2009), the time of death can be estimated using succession data when insect development data are unavailable (Tabor *et al.* 2004). In such cases, the composition of taxa found on a corpse at the time of discovery (corpse fauna) are usually compared with the composition of the arthropod assemblage at a given period of time derived from an animal model (base line fauna) to estimate the PMI (Schoenly *et al.* 1996). Succession data have been used to very accurately calculate a PMI as long as 52 days (Schoenly *et al.* 1996) and could be applied to a much greater interval (Wells and LaMotte 2009).

The two approaches for estimating the PMI are complementary (Tabor *et al.* 2004). However, the investigator must decide which model is suitable to be used. The use of development data is most applicable in the early phase of decomposition when the immature stage of the first colonizers of family Calliphoridae, Sarcophagidae and Muscidae are present. Whereas succession data are used the earliest colonizers are no longer present and the remains are occupied by predators of fly maggots such as beetles (Smith 1986; Schoenly and Reid 1987).

In a case study, Goff and Flynn (1991) estimated the PMI for the remains of a man using the succession pattern. The remains were removed from a sandy area near the ocean in Mokuleia, Oahu, Hawaii. At examination, at the city and morgue, the body was in dry stage of decomposition and the body cavities were markedly desiccated and decomposed. Specimens collected from the remains were three families of Coleoptera (beetles) and four families of Diptera (true flies). In addition one family (Staphylinidae) isolated from the soil underneath the remains at the crime scene. The collections were compared with computerized records of decomposition studies conducted in similar habitats on the island of Oahu. These comparisons for taxa developmental stages resulted in a PMI estimate of 34-36 days, based on interpretations of pattern of arthropod succession on the remains. This interval was primarily based on the presence of adult *Philonthus longicornis* (Stephens 1832) (Family Staphylinidae), mature larvae of *Piophila casei* (Linnaeus) (Family Piophilidae) and empty pupal cases of *Chrysomya rufifaces* (Family Calliphoridae). The period of postmortem interval (34-36 days) in this case fitted well with the last sighting of the decedent, 37 days period to discovery of the remains.

1.8. Aim and objectives

The study was conducted to address six objectives:

(1) To discover qualitative and quantitative differences in the Diptera assemblages associated with rabbit carcasses at four locations in Kuwait.

(2) To compare the successional changes and rate of decomposition of rabbit carcasses at the same four locations.

(3) To compare the necrophagous Diptera of rabbit carcasses with those from human corpses from criminal investigations.

(4) To provide baseline information on the forensically important flies and pave the way for more research on their ecology, biology and behavior.

(5) To familiarize officials with the forensic applications of entomology, and stimulate the involvement of entomologists in the criminal investigation team and entomological tools in the protocols which are used.

(6) The important objective of the study is eventually to advance forensic entomology and maximize the opportunity of entomologists to take part in the court in Kuwait and other Arab countries.

Chapter 2: MATERIAL AND METHODS

2.1. Study sites

The study was conducted in November and December of 2009 at four sites designated A through D, in Kuwait. This period is the beginning of rainy season. The study sites represented four different habitats (desert; urban; agriculture; coastal).

Site A (desert habitat) was an animal hutch within the campus of the Central Veterinary Laboratories in Amghara area. The campus is surrounded by expansive sandy areas; the main road is about half km away and the nearest buildings are about 4km from the site of the experiment.

Site B (urban habitat) was the roof of the building of the General Department of the Criminal Evidence in Farwaneya, which is a dense urban town.

Site C (agriculture habitat) is an animal hutch within a cattle farm in Sulaibeya area which is the collection of dairy farms in Kuwait. Some parts of the farm are cultivated with fodder for animal feeding.

Site D (coastal habitat) is an isolated place within the club of police officers, in Abo Al-Hasaneya area, which is situated on the Gulf coast.

Each site was selected on the basis of specific criteria. It was necessary also that the sites of the experiment were sufficiently isolated to minimize human interferences and scavenger disturbances.

2.2. Experimental animals and cages

In total 40 mature rabbits weighing from 1.72 to 3.46Kg (2.44 ± 0.44) were purchased locally and used in this study. The rabbits were of different colors but comparable in size. They were euthanized with chloroform at the study sites. To exclude scavengers, the rabbits were placed in man-made fabric cages (55cm x 40cm x 24cm), which were designed specifically to allow insect access, but prevent them escaping, instead trapping them in a chamber at the top of each cage. For these purposes, each cage was made of a frame composed of two layers of wire screening (Fig. 1), the outer layer was a rigid steel 2cm mesh to keep out scavengers and allow insects access. The inner layer was a lean mesh cloth with slits small enough to prevent insects escaping. The top of the cage was covered with thin plywood which was opened in its middle for the fixation of a water bottle to act as a collection chamber. One side of the cage had a hinged opening (20cm x 15cm) to allow placing and taking out of the rabbit carcass. Two holes (2cm) were made on two sides of the inner cloth to allow access for the insects to oviposit on the carcass, but on attempts to escape, they made their way to the bottle fixed on the top of the cage to be collected (Fig. 2).

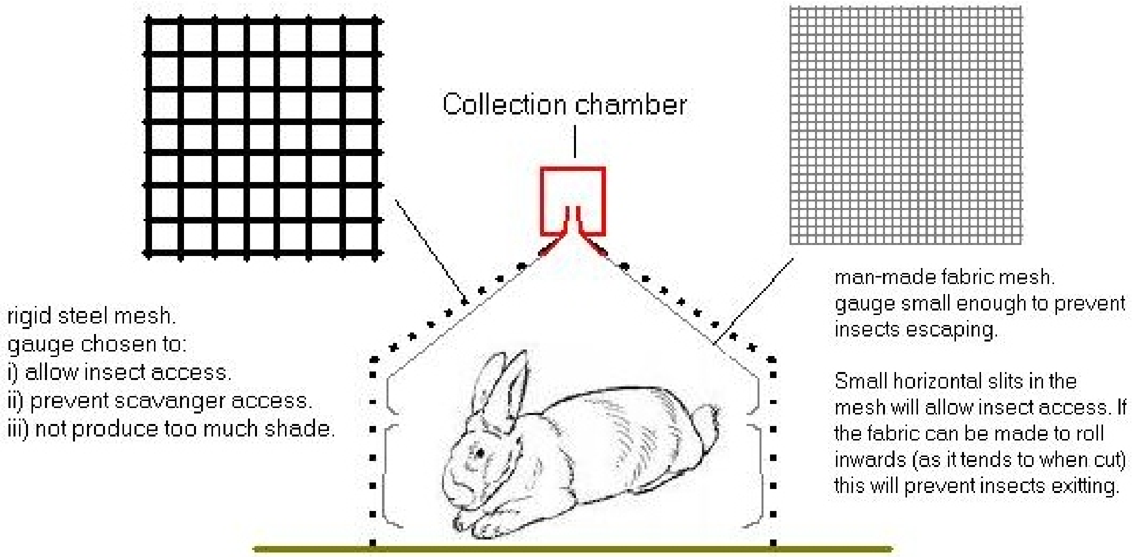


Fig. 1: Diagram shows the construction of a cage designed to protect the rabbit carcass from scavengers and trap the visiting insects.

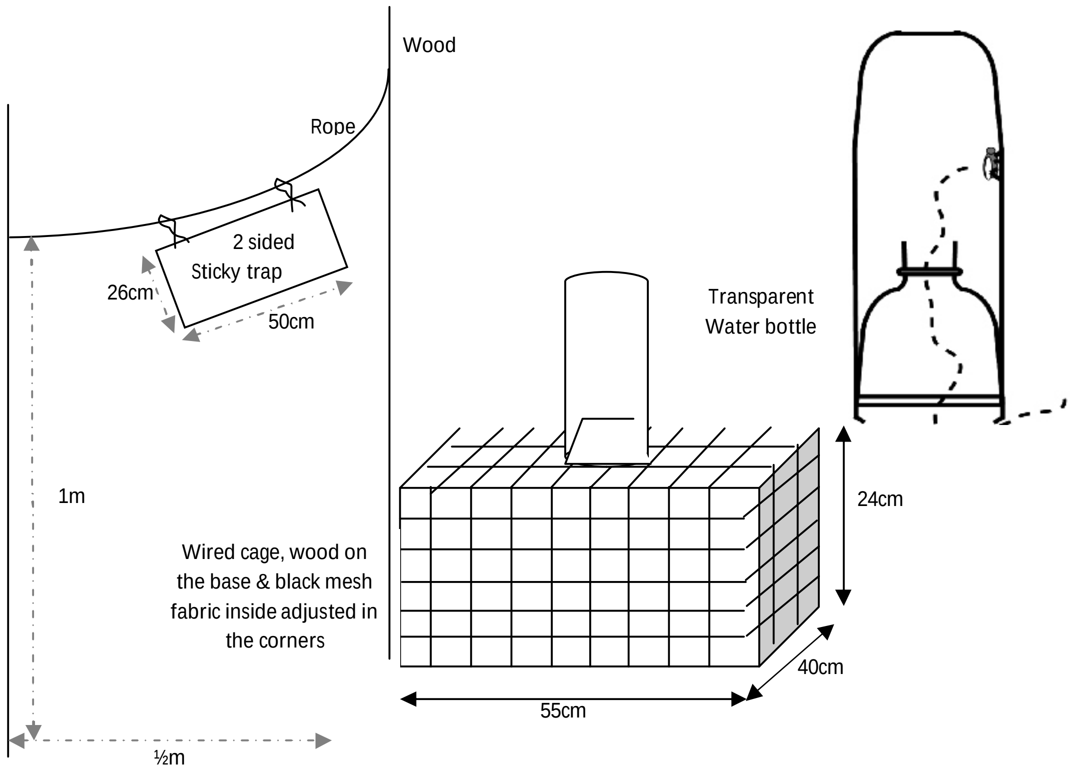


Fig. 2: Diagram shows the placement of a cage and sticky trap in a site.

2.3. Experimental design and sampling

The experiment lasted from 16-30 November 2009 at site A, and from 10-26 December at the other sites. It was not applicable to bring and examine in the lab more than four rabbits per day. Therefore, sampling at site A was done separately. Ten rabbits were used in each site (habitat) where they were killed, weighed and placed in the cages. Then, the caged carcasses were divided into two groups (a; b) which were distributed 10m apart in two lines. The distance between any two carcasses in the same group was 2m. A sticky trap was positioned half a meter from each caged carcass (Fig. 2 and 3). The cages were labeled with plastic coated ID cards. These cards stated specific letters for sites and groups, and numbers for carcasses; for example, the ID (such as Aa1) represented the site A, the group a, and the carcass number 1. The ID cards were with different colors, each color represented a site of the experiment. The sticky traps were labeled with the same ID as the corresponding cage. The date of death was designated as day 0. Each site was visited after 2, 4, 7, 10, 14 days for sampling the carcasses (Fig. 4). A pilot study had been carried out to arrive at this sampling strategy. At each visit, two caged carcasses, one from each group, with the corresponding sticky traps were removed and placed in large plastic bags which were closed and transferred to the laboratory for entomological examinations.

The protocol of placement and sampling of carcasses is shown in (Fig. 3 and Table 1). For example the rabbit carcasses sampled after two days from placement at site A was designated A2. Such a protocol allowed sampling of two carcasses at specific date without any disturbance of other carcasses which were removed later according to the schedule. In addition, this sampling design allowed collection of information on necrophagous fly succession and their composition on the rabbit carcasses at different stages of decomposition and at different habitats.

Site A: Desert habitat

The Central Veterinary Laboratories - Amghara area

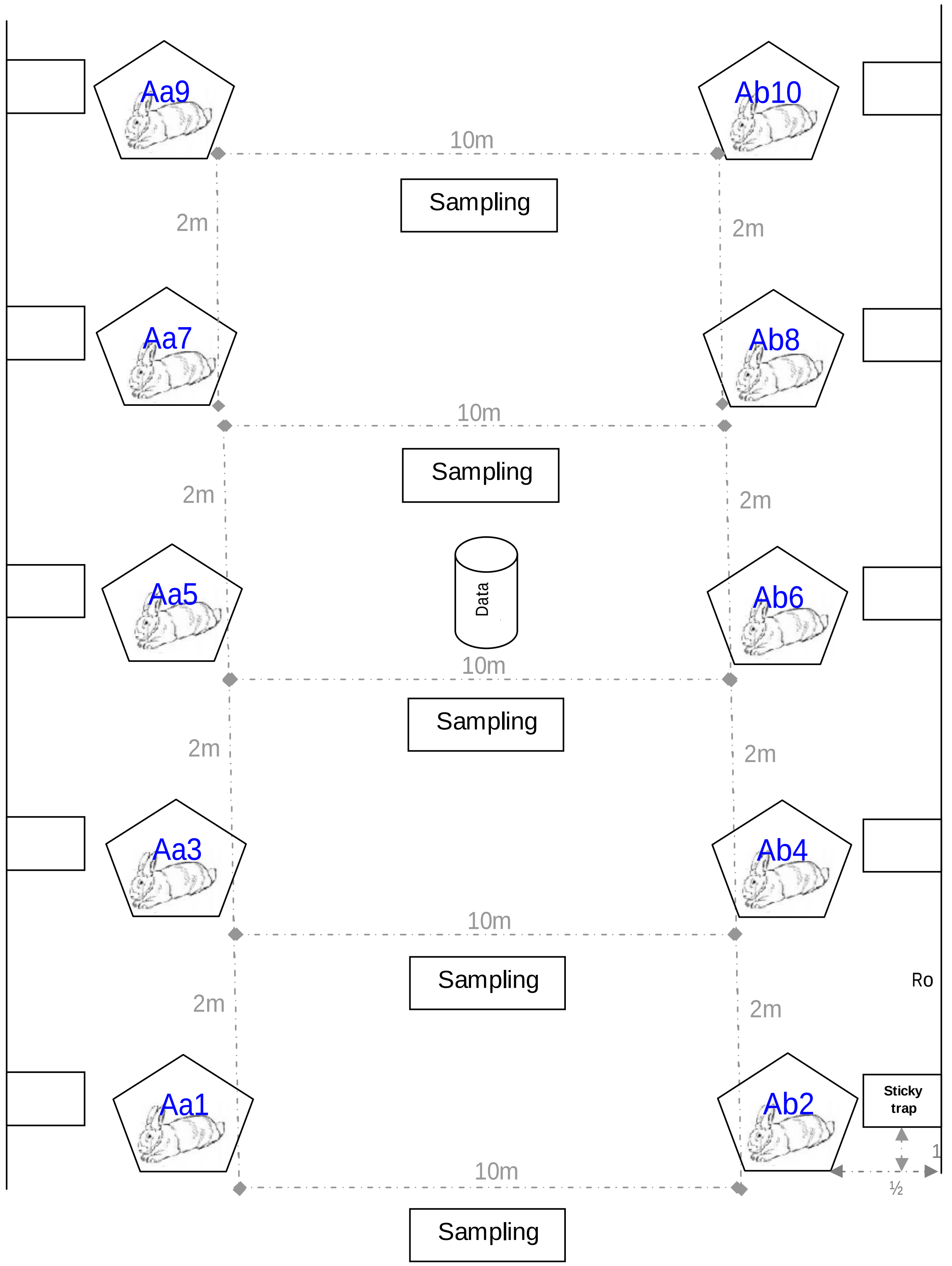


Fig. 3: Diagram shows the distribution of the cages and sticky traps and sampling time during the experiment at site A.

Fig. 4: ID cards with different colors representing different sites of the experiment.

Table 1: The study sites and the sampling days

Date	Site	Habitat: Area (Location)	Sampling A	Sampling B	Sampling C	Sampling D
16-Nov	**SITE A**	**Desert**: Amghara (Veterinary Laboratories)	**A0**=Placement of carcasses			
17-Nov						
18-Nov			**A2***=(Aa9+Ab10)			
19-Nov						
20-Nov			**A4**=(Aa7+Ab8)			
21-Nov						
22-Nov						
23-Nov			**A7**=(Aa5+Ab6)			
24-Nov						
25-Nov						
26-Nov			**A10**=(Aa3+Ab4)			
27-Nov						
28-Nov						
29-Nov						
30-Nov			**A14**=(Aa1+Ab2)			
10-Dec	**SITE C**	**Agriculture:** Sulaibeya (Cattle Farm)			**C0**=Placement of carcasses	
11-Dec	**SITE D**	**Costal:** Abo Al-Hasaneya (Police officers Club)				**D0**=Placement of carcasses
12-Dec	**SITE B**	**Urban**: Farwaneya (General Department of Criminal Evidence)		**B0**=Placement of carcasses	**C2**=(Ca9+Cb10)	
13-Dec						**D2**=(Da9+Db10)
14-Dec				**B2**=(Ba9+Bb10)	**C4=**(Ca7+Cb8)	
15-Dec						**D4=**(Da7+Db8)
16-Dec				**B4**=(Ba7+Bb8)		
17-Dec					**C7**=(Ca5+Cb6)	
18-Dec						**D7**=(Da5+Db6)
19-Dec				**B7**=(Ba5+Bb6)		
20-Dec					**C10**=(Ca3+Cb4)	
21-Dec						**D10**=(Da3+Db4)
22-Dec				**B10**=(Ba3+Bb4)		
23-Dec						
24-Dec					**C14**=(Ca1+Cb2)	
25-Dec						**D14**=(Da1+Db2)
26-Dec				**B14**=(Ba1+Bb2)		

*** A2 = Sampling at site A on 2nd day**

2.4. Laboratory work

In the laboratory, the bags were opened to take out the cages and sticky traps. The carcasses were removed from the cages carefully and weighed with a spring balance. Then they were dissected and examined for insects. The stage of decomposition, sites of colonization and insect developmental stages on carcasses were noted. Immature stages (eggs; 1st and 2nd instars) were collected and reared on 15g minced beef in plastic pots, covered with gauze. Daily, they were observed and moistened with water. When the 3rd instars reached full maturation, 15 larvae were placed in a bigger pot with sawdust and covered with gauze. The vials for rearing and pupation were placed in an air-conditioned room with controlled temperature in the laboratory. In addition, samples of each life stages were reared or pupated in the backyard of the laboratory (on the open) under ambient temperature and relative humidity. For identification samples of 3rd instars were placed in 70% ethanol after killing in boiling water. Larvae were identified to species or genus level to determine the abundance of different fly taxa in the samples. In addition, samples of each life stage present on the carcasses were collected and preserved in 70% ethanol. Egg masses, 1st and 2nd instars were collected using a paint brush, whereas 3rd instars and pupae were collected using entomological forceps. It should be noted that the portion collected from each life stage was representative to its relative abundance on the carcass. All adult flies attached to the sticky traps or trapped in the bottles (collection chambers) were removed carefully, and placed in vials for identification and counting. Live flies were killed using chloroform or refrigeration. The vials containing the preserved or pinned larvae or adult flies were labeled with the same ID of the cage or sticky trap from which they were collected. During rearing, the development of larvae or emergence of adult flies was noted in every day of observations.

The flies were identified using dichotomous and pictorial taxonomic keys by (James 1947; Zumpt 1965; Bei-Bienko 1988; Pont 1991; Erzinçlioğlu 1996; Spradbery 2002; Watson and Dallwitz 2003; Scudder and Cannings 2006; Krzysz 2010). For confirmation, some adult fly samples were sent to the school of Forensic and Investigative Sciences, University of Central Lancashire (UCLan), UK, for identification using molecular analysis. In addition, personal communication was made with Dr. M. Hall and N. Wyatt in the British Natural

History Museum (NHM) for consultation on identification of Sarcophagids and the other tiny flies as well as with Szpila K. for identification of Calliphoridae.

A photographic documentation using Dino-Eye USB (Digital Eyepiece/Camera adapter for microscope, model AM423) and Dino-Light (Digital Microscope/Camera, model Pro2 AD-413T) and video recording of all procedures of the experiment at the study sites and in the laboratory were made.

2.5. Environmental data

Since insect activity and carrion decomposition are influenced by temperature and relative humidity, continuously operating dataloggers (Lascar model EL-USB-2) were programmed to record the ambient temperatures and relative humidity every hour during the experimental duration at each study sites (habitats). Data loggers were attached to an upright post one meter above the ground in the middle of the study site. In sites B and D where the carcasses were placed in the open, the dataloggers were enclosed in a waterproof cover to prevent exposure to direct sunlight and rain. Daily mean temperatures and relative humidity were calculated from hourly datalogger recordings. In addition, weather records were obtained from the weather stations of the Directorate General of Civil Aviation located near the study sites.

2.6. Real case study

The main objective of this study was to collect information on the necrophagous Diptera colonizing human corpses to be compared with those from rabbit carcasses; the postmortem intervals of the cases were recorded from the medical and/or police reports, when the killer confessed or the victim was reported missing.

The study was conducted, after the agreement of the high authority of the Ministry of Interior, through the General Department of Criminal Evidence during investigations in cases of homicide, suicide, accident or unattended death. The material of the present study comprised 13 cases where human dead were located in different regions and habitats of Kuwait.

The crime scenes were visited to collect insect samples and data including the date of body discovery, types of habitat, sites of insect colonization on the body, stages of insect development, stages of decomposition, causes of death, circumstances of victims (nationality; sex; age; clothed; concealed; burned) and real intervals (when a victim was last seen alive or reported missing). In some instances, crime scenes were visited after the removal of corpses which then were examined at autopsy. Moreover, information were obtained from the medical and police reports. Climatic data were obtained from the stations of the Directorate general of Civil Aviation located near the crime scenes. The human remains and crime scene were photographed.

Samples of insects were collected on, around or under the corpses in the crime scene and/or at autopsy. The samples were representative to the relative abundance of each life stage and sites of colonization on the corpses. The methods of collection, preservation, identification and rearing of insect at all developmental stages were done as described for the samples from rabbit carcasses.

2.7. Statistical analysis

The decomposition rates of the carcasses at the four different locations were compared using ANCOVA (analysis of covariance). Successional data were summarized in kite diagrams, traditional in forensic entomology.

Differences in species at the locations (both qualitative and quantitative) were investigated using correspondence analysis which is an ordination technique. Statistical significant differences between groups were tested using ANOSIM (analysis of similarity; PAST version 1.94 b, Hammer *et al.*, 2001).

PAST is a comprehensive statistics package that is used not only by paleontologists, but also in many fields of life science, earth science, and even engineering and economics. It has a spreadsheet-like user interface where data are easily entered and analyzed.

A mixed effects Poisson model was used on rounded, logged data with species as the random effect. This was sensible as it takes into account the large number of zeros, as well as down-scaling the effects of abundant species. The analysis allowed comparisons of the decomposition stages.

Chapter 3: RESULTS

3.1. Necrophagous fly assemblages on decaying rabbit carcasses

Eleven families of necrophagous flies were collected at four sites (habitats) in this study. Of these, 13 species were identified: *Calliphora vicina, Chrysomya albiceps, Chrysomya megacephala, Lucilia sericata, Fannia canicularis, Musca domestica* Linnaeus 1758, *Musca sorbens* Wiedemann 1830, *Megaselia scalaris* Loew 1866, *Psychoda alternate* Say 1824, *Sarcophaga (Bercaea) africa (haemorrhoidalis), Parasarcophaga (Liopygia) ruficornis* (Fabricius), *Wohlfahrtia nuba, Physiphora alceae* (Preyssler 1791) (Table 2 and 3).

Table 2: The abundance scale of each fly species at the different sites (habitats)

Family	Species **Site: Habitats**	**Site A** Desert	**Site B** Urban	**Site C** Agriculture	**Site D** Coastal
Calliphoridae	*Calliphora vicina*	0	0	1	1
	Chrysomya albiceps	4	3	3	4
	Chrysomya megacephala	1	0	0	1
	Lucilia sericata	2	0	0	1
Carnidae	*Hemeromyia* sp.	0	0	0	2
Ephydridae		0	1	4	3
Fanniidae	*Fannia canicularis*	1	0	1	0
Muscidae	*Musca domestica* *Musca sorbens* *Musca* spp. *Stomoxys* sp.	4	5	5	5
Periscelididae	*Periscelis* sp.	0	0	0	2
Phoridae	*Megaselia scalaris*	1	0	1	1
Psychodidae	*Psychoda alternata*	0	0	1	0
Sarcophagidae	*Sarcophaga africa* *Sarcophaga* spp. *Parasarcophaga ruficornis* *Wohlfahrtia nuba*	1	3	2	2
Sphaeroceridae		0	0	4	2
Ulidiidae	*Physiphora alceae*	2	3	1	2

0 = (0), 1 = (1 – 5), 2 = (6 – 20), 3 = (21 – 100), 4 = (100- 500), 5= (>500)

Table 3: Total number of adult arthropods collected from rabbit carcasses in each habitat

Order	Family	Species (Site/Habitats)	Site A Desert	Site B Urban	Site C Agriculture	Site D Coastal	Total
Diptera Flies	**Calliphoridae**	*Calliphora vicina*	0	0	3	3	**6**
		Chrysomya albiceps	113	88	23	299	**523**
		Chrysomya megacephala	1	0	0	1	**2**
		Lucilia sericata	10	0	0	1	**11**
	Carnidae	*Hemeromyia* sp.	0	0	0	6	**6**
	Ephydridae	Not identified beyond family	0	1	230	42	**272**
	Fanniidae	*Fannia canicularis*	1	0	1	0	**2**
	Muscidae	*Musca domestica*, *Musca sorbens*, *Musca* spp., *Stomoxys* sp.	244	1737	1686	1951	**5618**
	Periscelididae	*Periscelis* sp.	0	0	0	6	**6**
	Phoridae	*Megaselia scalaris*	1	0	3	1	**5**
	Psychodidae	*Psychoda alternata*	0	0	1	0	**1**
	Sarcophagidae	*Parasarcophaga ruficornis*, *Sarcophaga* spp., *Sarcophaga africa*, *Wohlfahrtia nuba*	4	24	16	11	**55**
	Sphaeroceridae	Not identified beyond family	0	0	356	10	**366**
	Ulidiidae	*Physiphora alceae*	16	35	1	10	**62**
	Culicidae Mosquitoes	Not identified beyond family	0	0	7	4	**11**
Coleoptera Beetles	**Cleridae**, **Dermestidae**, **Histeridae**, **Silphidae**	Not identified beyond family	8	9	13	9	**39**
Hymenoptera	**Formicidae** Ants	Not identified beyond family	78	0	0	43	**121**
	Vespidae Wasps	Not identified beyond family	0	0	1	0	**1**
Lepidoptera	Butterflies	Not identified beyond order	0	0	1	0	**1**
	Moths	Not identified beyond order	0	0	1	0	**1**
Other Insecta		Not identified beyond order	8	0	1	1	**10**
Arachnida	Spiders	Not identified beyond order	0	0	4	2	**6**
	Mites	Not identified beyond order	1	4	1	1	**7**

Although these families: Muscidae, Carnidae, Ephydridae, Fanniidae, Periscelididae, Phoridae, Psychodidae, Sphaeroceridae and Ulidiidae were associated with the rabbit carcasses and in some instances they were abundant, not one of them bred; they were seemingly only attracted to the carcasses to feed. All families were represented in the coastal habitat (Site D) except family Fanniidae, which was found in agriculture and desert habitats, and family Psychodidae which was restricted to agriculture habitat. Similarly, families Carnidae and Periscelididae were restricted to the coastal habitat. Families Ephydridae and Sphaeroceridae are most abundant in agriculture habitat and family Muscidae was abundant in all habitats (Fig. 5).

Chrysomya albiceps from family Calliphoridae, *P. ruficornis* from family Sarcophagidae and *Musca domestica* from family Muscidae were the most abundant species on rabbit carcasses and representative in all habitats.

In addition to Diptera (flies), many other arthropods were reported in this study; however, they were identified only to the taxon 'order'. These arthropods included beetles and ants which are of forensic importance (Table 3).

Using analysis of similarities (ANOSIM) there was a significant difference in the diversity of fly species between different habitats (R=0.1761 and p=0.003). Also using the analysis to compare between pairs of habitats (0.05/n as the new significance threshold for rejecting the null hypothesis, i.e. 0.05/6 = 0.0083) indicates the greatest difference was between desert and agricultural habitats (R=0.468 and p=0.001), where as urban and coastal habitats were similar in terms of the insects found on rabbit carcasses (R=0.031 and p=0.5833) (Table 4).

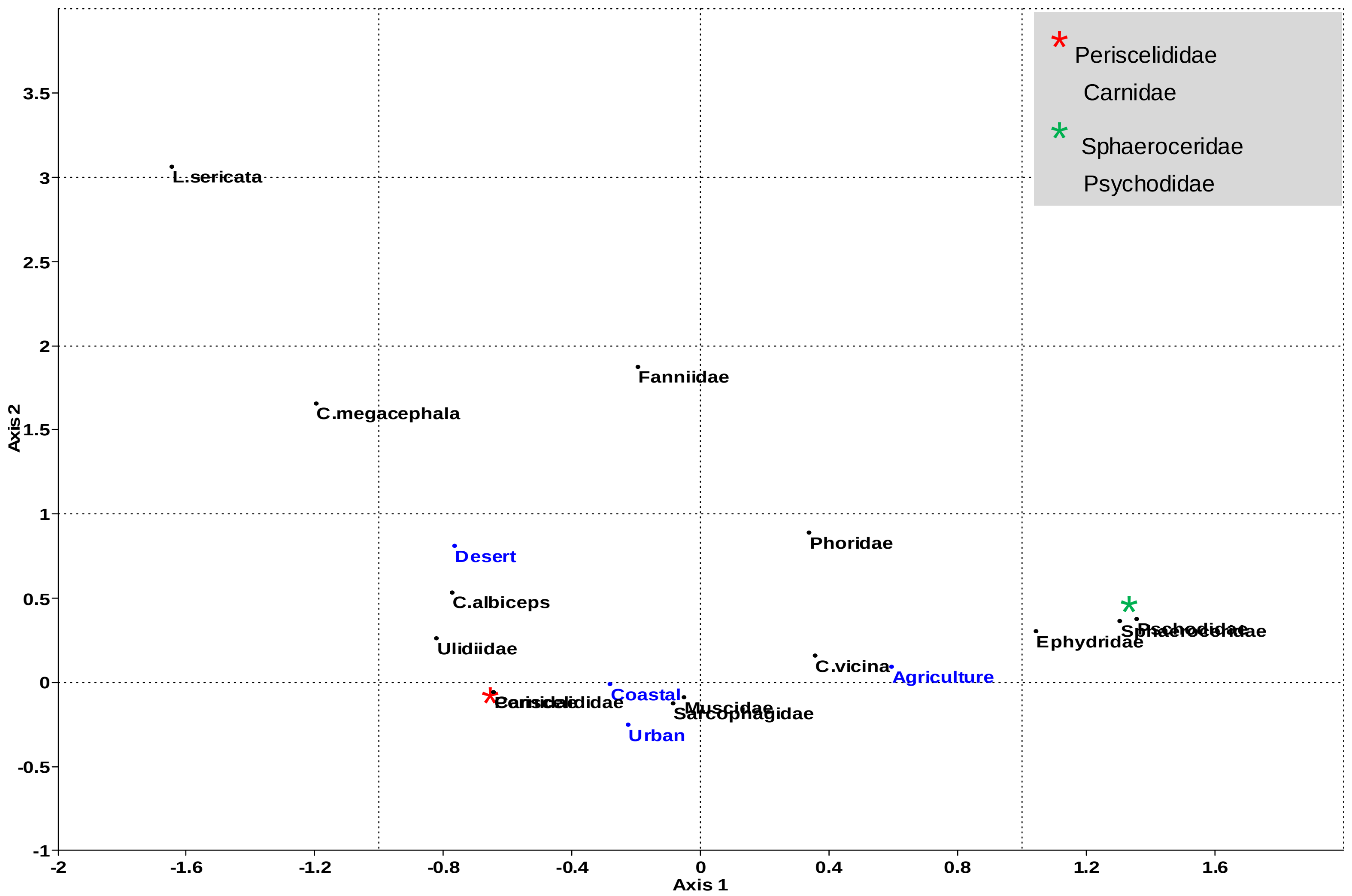


Fig. 5: Ordination of first two axes of correspondence analysis (based on presence/absence) showing relative similarities of fly species (and families) and habitats

Table 4: Analysis of similarities (ANOSIM) pairwise comparisons of species diversity across the four habitats: **p values** (R values)

SITE Habitat	**Site A** Desert	**Site B** Urban	**Site C** Agriculture	**Site D** Coastal
Site A Desert		**0.0218** (0.142)	**0.0001** (0.468)	**0.006** (0.2058)
Site B Urban	**0.0218** (0.142)		**0.0029** (0.2154)	**0.5833** (-0.031)
Site C Agriculture	**0.0001** (0.468)	**0.0029** (0.2154)		**0.0035** (0.222)
Site D Coastal	**0.006** (0.2058)	**0.5833** (-0.031)	**0.0035** (0.222)	

3.2. Decomposition stages and fly activity

Four stages of decomposition were observed, namely fresh (1-2 days), bloated (3-7 days), decay (8-14 days) and dry (10-14 days) (Table 5; Fig 6). Decay and dry stages were the most dissimilar followed by bloated and dry (Table 6).

In the fresh stage, the arrival of flies at the carcasses differed according to the site (habitat). At site A (desert habitat), *Calliphora vicina*, *Chrysomya albiceps*, *L. sericata*, sarcophagid flies (mainly *P. ruficornis*) were the first colonizers to breed on the rabbit carcasses, whereas at site C (agriculture habitat), the first colonizer was *P. ruficornis to breed*; *Chrysomya albiceps* was collected only in adult stage.

In the bloated stage, *Chrysomya albiceps* and sarcophagid flies (mainly *P. ruficornis*) were abundant as adults and immatures in the four habitats. Only at site C (agricultural habitat) was *Calliphora vicina* observed in adult and immature stages.

In the decay stage, when a strong odor was noted, the abundance of the carrion breeders from family Calliphoridae and Sarcophagidae increased in nearly all sites. Even the flies colonizing the carcass to feed increased in number as well.

Only one rabbit reached dryness at site B. In dry stage, when the odor disappeared and the nutrient matter became very little, all the flies left the carcasses at all sites except at site B where adult muscid, sarcophagid and *Chrysomya albiceps* adult flies were collected. In addition, prepupal stage of *Chrysomya albiceps* was found in this site.

Table 5: Diptera succession on rabbit carcasses

Decomposition stage / **Family** Species	Fresh	Bloat	Decay	Dry
Calliphoridae *Calliphora vicina*	108	250	1682	
Calliphoridae *Chrysomya albiceps*	70	2316	8650	320
Calliphoridae *Chrysomya megacephala*		2	31	
Calliphoridae *Lucilia sericata*	9		32	
Carnidae *Hemeromyia* sp.	1	4	1	
Ephydridae		27	246	
Fanniidae *Fannia canicularis*	1		1	
Muscidae *Musca domestica, Musca sorbens, Musca* spp., *Stomoxys* sp.	315	1535	2780	988
Periscelididae *Periscelis* sp.			6	
Phoridae *Megaselia scalaris*	1	3	1	
Psychodidae *Psychoda alternata*			1	
Sarcophagidae *Parasarcophaga ruficornis, Sarcophaga africa, Sarcophaga* spp., *Wohlfahrtia nuba*	125	1493	1738	3
Sphaeroceridae	129	247	10	
Ulidiidae *Physiphora alceae*	1	11	50	

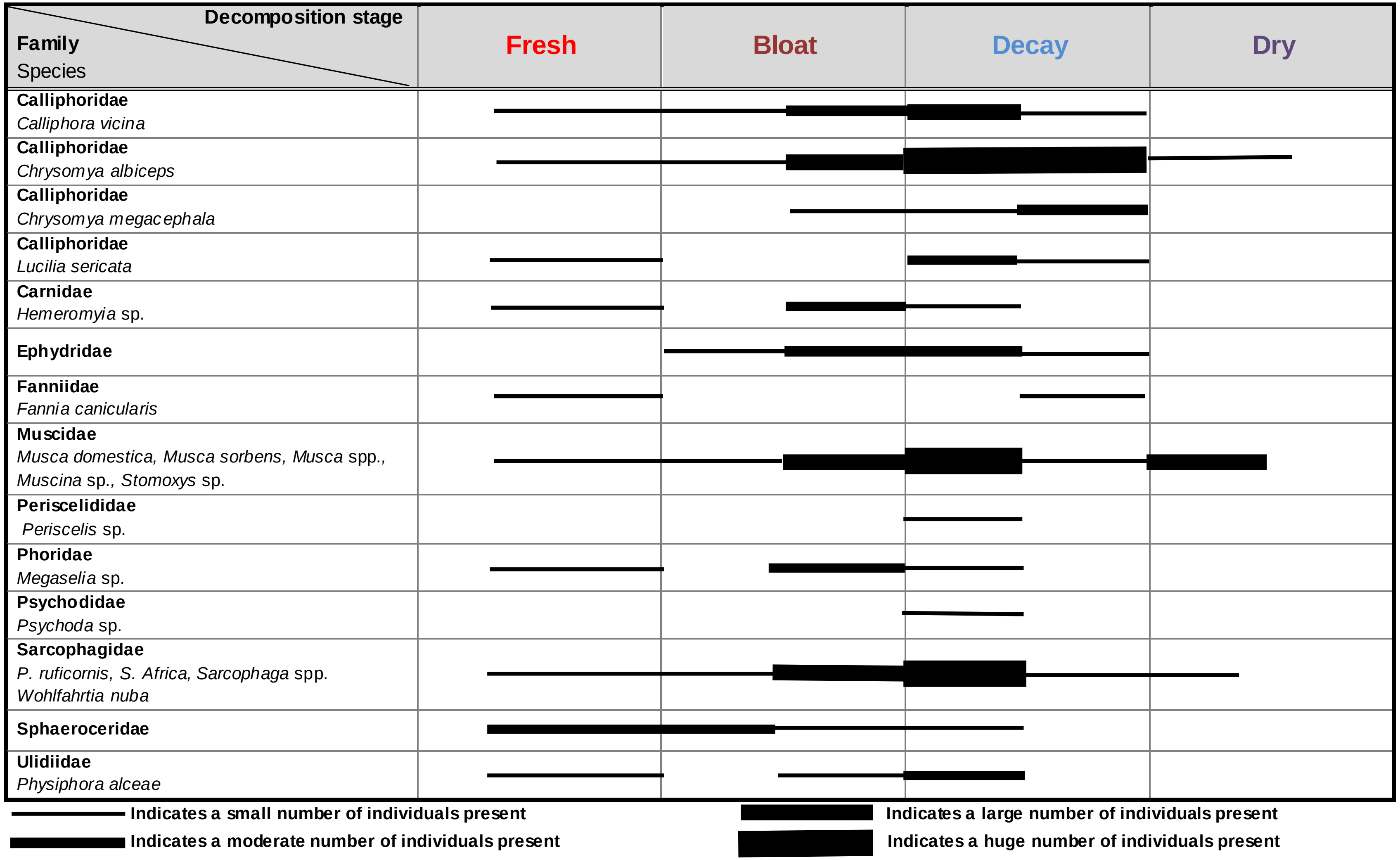


Fig. 6: Succession kite diagram of necrophagous flies on rabbit carcasses in different stages of decomposition

Table 6: Pairwise comparison of the decomposition stages of rabbit carcasses using "mixed effects poisson model"

Decomposition stage	Dry	Decay	Bloat
Fresh	0.0139	0.0109	0.166
Bloat	0.0003	0.2280	-
Decay	<0.0001	-	-

Decay and dry are the most dissimilar followed by bloat and dry

This study revealed that there was interaction between decomposition of carcasses and colonization with insects. Some carcasses were found to be without any insect colonization even after sufficient time had elapsed since death. The decomposition of these carcasses was delayed. Mainly, within two days, when the carcasses were in fresh stage, eggs or small larvae of the species of Calliphoridae and Sarcophagidae were found colonizing most of them. However, few carcasses, after four days were not shown any colonization of insects; even these carcasses were still in the fresh stage.

It was observed that the carcasses did not go through the stages of decomposition at the same rate; there were few exceptions for example, at site A, a carcass was still in fresh stage after 10 days from death. At examination, eggs and small larvae were found on its ears, neck, mouth and eyes. Similarly, the decomposition rate of two rabbit carcasses were delayed at both sites C and D. These carcasses were in bloated stage after seven days from the death. They were found to be colonized with 1^{st} and 2^{nd} instars of the sarcophagid sp.

3.3. Decomposition rates of rabbit carcasses

The decomposition rates of the carcasses at the four habitats were compared using ANCOVA (analysis of covariance), weight loss was used as a percentage of starting weight (the response variable), habitat type-4 levels; urban, desert, agricultural, coastal (a category explanatory variable). The results of the statistical analysis revealed that the decomposition rate of carcasses at the urban habitat was different from those at other habitats, which were similar.

Simplifying the analysis, we combine desert, agriculture and coastal since they are not significantly different. This tells us that there are differences in both the slope ($t=4.005$, $p=0.0003$) and intercept ($t=2.395$, $p=0.022$). The equations of the lines are:

Urban: % weight loss = -16.52 + 4.28 x day

Others: % weight loss = -0.16 + 1.10 x day

Thus decomposition appears to occur nearly four times faster in urban areas compared to other areas (Fig. 6, Table 7).

It is noted that the heavier rabbit carcasses were decomposed slower than lighter carcasses. i.e. the percentage of body weight loss is less in heavier carcasses than in lighter carcasses. In general, most carcasses dropped in weight as the decomposition advanced; however, in few cases, three carcasses in the fresh stage and two in the bloated stage increased little in weight then they decreased in the following advanced stage of decomposition.

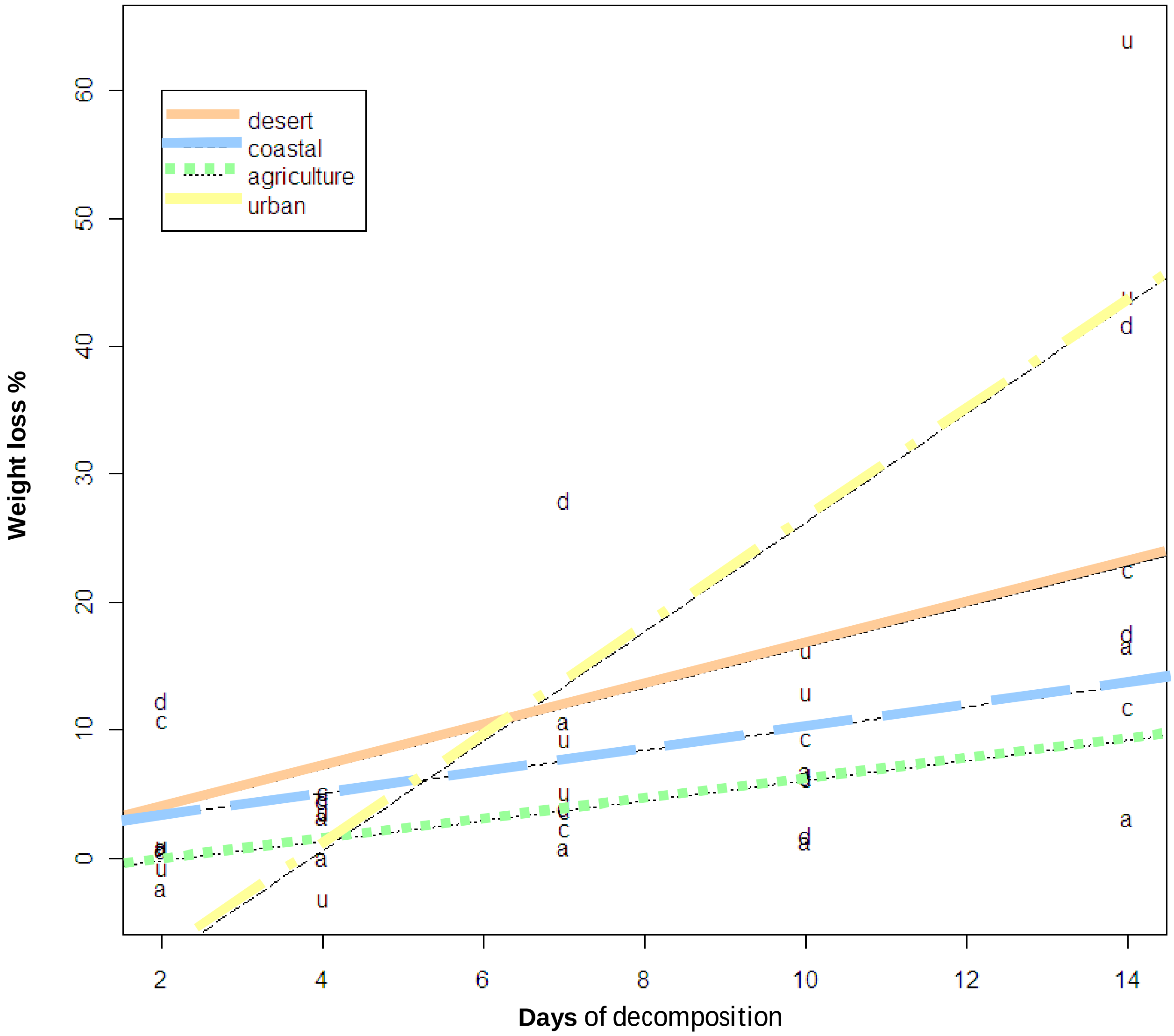


Fig. 7: Decomposition rate of rabbit carcasses at different habitats

Table 7: Analysis of covariance (ANCOVA) for the decomposition rates of rabbit carcasses at different habitats

Slope / **Intercept**	**Site B** Urban	**Site A** Desert	**Site C** Agriculture	**Site D** Coastal
Site B Urban		**0.009***	**0.001***	**0.002***
Site A Desert	**0.049***		**0.409**	**0.467**
Site C Agriculture	**0.077**	**0.774**		**0.951**
Site D Coastal	**0.046***	**0.911**	**0.696**	

*** Decomposition rate was significantly faster in the urban habitat**

3.4. Case studies

During the period of November 2008 to February 2010, 13 dead bodies were studied to collect data on the circumstances of death, identify of victims and decomposition stages as well as to collect insect samples in the crime scenes or at autopsy.

Table 8 showed the information on the cases studied. Of the 13 cases 10 were male and 3 females; their ages ranged from few days (newborn) to 53 years. Five cases were Indians, two Kuwaitis, one Pakistani, one Saudi, one from Congo and one from Nepal; two cases were not identified.

The cause of death was homicide in three cases, suicide in two cases, natural death in two cases, and drug abuse, toxicity and abortion in one case for each.

Concerning decomposition stages, one case was in fresh, six in bloated, one in decay and five in dry stages. All cases were clothed, except three, two of which were unclothed and one partially clothed. The cases were discovered several days or several weeks after death, probably the emanating odor called the attention to notify the police. All cases were in an urban habitat, except two cases where one was in an agricultural habitat and one in a desert habitat. The case in the desert was in dry stage and colonised by beetles; no necrophagous flies were recovered on the remains. In case No.3, the killer had divided the victim into many parts and thrown them in plastic bags in different areas. He burned the head and the hands to hide the evidence. The hands were found in the ground floor of an abundant building in Farwaneya after four days of death. The eggs of *Calliphora vicina* were collected from the two hands. After two days the head and trunk were found in a ditch in an urban area in Showaikh. The burned head was colonized by larvae of necrophagous flies, but no insects were found on trunk which was placed in tightly closed bags. The legs were thrown in the roof of an electric transformer in Farwaneya. They were colonized with *Chrysomya albiceps* and *Sarcophaga* sp. In case No.10, the feeding maggots on the remains were submitted to the toxicology and chemical analysis laboratory, the General Department of Criminal Evidence, to be tested for chemicals and toxins. Three chemicals were detected in their tissues: Butyl hydroxyl toluene which was used in manufacture of medicine, Petroleum products and cosmetics, and phosphoric acid and Diethyl phtalate which were used in manufacture of medicine and pesticide. The medical report of this case stated that the death was natural.

Table 8: Data collected on victim identity, death scene, decomposition stage, cause of death and insects recovered from 13 human cases

Case No.	Date	Area	Death scene	Sex	Age (Yrs)	Clothing	Nationality	Decomposition stage	Cause of death	Insect	Insect stage	Species identified	PMI (days)
1	11/10/2008	Jeleeb Al-Shoyoukh	Inside unusable bus	Male	-	Clothed	-	Mummified dry	-	Diptera	Pupa case	Not identified beyond order	_
											Adult	*Chrysomya albiceps* *Musca domestica* *Physiphora alceae*	
										Coleoptera	Pupa; Adult	Not identified beyond order	
2	12/1/2008	Jahra	Agriculture open area with dense trees; the corpse was found hanging from a tree	Male	53	Clothed	Indian	Dry	Suicide	Diptera	Adult	*Calliphora vicina* *Chrysomya megacephala* *Chrysomya albiceps* *Musca domestica* *Psychoda alternata* *Sarcophaga* spp.	_
										Coleoptera	Larvae	Not identified beyond order	
3	3/25/2009	Farwaneya	The body was divided into parts which were placed in plastic bags and thrown in different areas: in two districts	Female	33	Clothed	Pakistani	Bloated	Homicide	Diptera	Eggs	*Calliphora vicina*	4
	3/27/2009	Showaikh industrial area								Diptera	Larvae	*Calliphora vicina* *Chrysomya albiceps* *Sarcophaga africa* *Parasarcophaga ruficornis* *Wohlfahrtia nuba*	
										Coleoptera	Adult	Not identified beyond order	
	3/27/2009	Farwaneya								Diptera	Larvae	*Chrysomya albiceps* *Sarcophaga* spp.	
4	4/6/2009	Khaitan	Urban, abandoned building	Male	39	Clothed	Indian	Decay	Suicide	Diptera	Larvae; Pupa	*Chrysomya albiceps* *Sarcophaga* spp.	14
										Coleoptera	Larvae; Adult	Not identified beyond order	
5	4/13/2009	Rowdah	Urban building	Male	40	Clothed	Kuwaiti	Decay	Drug abuse	Diptera	Larvae	*Parasarcophaga ruficornis* *Chrysomya Albiceps*	8.5
6	5/8/2009	Kabd	Desert open area	Male	45	Clothed	Kuwaiti	Dry	-	Coleoptera	Adult	Not identified beyond order	21
7	6/13/2009	Salmeya	Urban building	Female	33	Clothed	Indian	Decay	Homicide	Diptera	Larvae	*Calliphora vicina* *Chrysomya albiceps* *Megaselia scalaris* *Musca domestica* *Psychoda alternata* *Sarcophaga* spp.	Several days
											Pupa case	Not identified beyond order	
										Coleoptera	Larvae	Not identified beyond order	
8	8/24/2009	Jabreya	Urban open area	Male	40	Clothed	-	Bloated	Pesticide toxicity	Diptera	Adult	*Chrysomya albiceps* *Musca* spp. *Musca domestica*	_
										Coleoptera	Adult	Not identified beyond order	
9	9/9/2009	Khaldeya	Female bathroom in police station	Male	Few days	Unclothed	Nepal	Bloated	Born dead or left without care after born	Diptera	Larvae	*Sarcophaga* spp.	Several days
10	11/6/2009	Mahboula	Urban building	Male	51	Partially clothed	Indian	Bloated	Natural death	Diptera	Larvae	*Chrysomya albiceps* *Sarcophaga* spp.	Several days
11	12/30/2009	Salmeya	Urban building	Male	58	Clothed	Saudi	Dry	Natural death	Diptera	Pupa case	Not identified beyond order	_
											Adult	*Chrysomya albiceps*	
										Coleoptera	Larvae; Adult	Not identified beyond order	
12	1/13/2010	Jeleeb Al-Shoyoukh	Urban building	Male	58	Clothed	Congo	Bloated	-	Diptera	Egg: rearing failed	Not identified beyond order	_
											Larvae	*Chrysomya albiceps* *Chrysomya megacephala* *Sarcophaga* spp.	
13	2/21/2010	Jeleeb Al-Shoyoukh	Urban building	Female	30	Unclothed	Indian	Fresh	Homicide	Diptera	Larvae	*Calliphora vicina* *Chrysomya albiceps* *Megaselia scalaris* *Sarcophaga* spp.	_
											Adult	*Calliphora vicina* *Chrysomya albiceps* *Megaselia scalaris* *Parasarcophaga ruficornis*	

The insect community recovered on the remains was composed of orders Diptera and Coleoptera. Families Calliphoridae, Sarcophagidae and Muscidae were the most encountered; three species from family Calliphoridae (*Chrysomya albiceps, Calliphora vicina, Chrysomya megacephala),* four species of family Sarcophagidae (*P. ruficornis, S. africa, W. nuba* and unidentified species), and two species of family Muscidae (*M. domestica* and unidentified species of *Musca*) were identified. *Physiphora alceae, Psychoda alternata* and *Megaselia scalaris* were collected less frequently.

It was noted that each stage of decomposition was associated with a certain developmental stage of necrophagous flies on the corpses. This means that there was chronological insect succession on the dead bodies. Case No.13, the slaughtered female victim, whose body was in fresh stage of decomposition, was colonized with 1st and 2nd instars of different species of flies on the cut wound on the neck. On bloated bodies (cases No.3, 8, 9, 10, 12) adults and/or immatures of flies were observed. Of these cases, two (No.3, 8), which were discovered in open areas, were colonized with beetles in addition to flies. The corpses in the decay stage were occupied with all developmental stages of flies including pupae. In this stage beetles started to appear. The dry stage characterized by occurrence of empty pupae and beetles, and disappearance of all other immature stage of flies. Adult flies were also observed on the remains.

3.5. Comparison between fly colonization on rabbit carcasses and human corpses

In comparing the insect colonization on the human corpses and rabbit carcasses, it was noted that all species of Calliphoridae and Sarcophagidae, the most forensically important flies, were found on both; except *L. sericata* which was not collected from the dead bodies. In general, the fly species richness was greater in rabbit carcasses than human corpses, as species of families Carnidae, Ephydridae and Fanniidae as well as *Musca sorbans* Wiedemann 1830 and *Stomoxys sp*. were not recorded on human bodies; however, these species are less forensically important. Although *Megaselia scalaris* and *Psychoda alternata* were found on both human and rabbit carrion, they bred only on the former; only adult stages of these flies were collected from rabbit carcasses. The insect succession pattern was comparable in both of them. On human bodies, which were in bloated and decay stages, large numbers of flies were observed with increasing putrefactive odors, whereas few flies were found on corpses which were in fresh and dry stages. The same trend was observed in rabbit carcasses. In the dry stage, adult flies visited both carrions but without breeding. Only, adult flies and beetles were found on the human bodies, which were in dry stage. The same finding was observed on dried rabbit carcasses (Table 9).

Table 9: Necrophagous flies collected from 40 rabbit carcasses and 13 human corpses in different habitat

Family	Genus and species	Insect stage: Rabbit carcasses (40 rabbits)								Human corpses (13 cases)			
		Site A Desert		Site B Urban		Site C Agriculture		Site D Coastal		Site B Urban		Site D Agriculture	
		Immature	Adult	Immature	Adult	Immature	Adult	Immature	Adult	Immature	Adult	Immature	Adult
Calliphoridae	*Calliphora vicina*	•		•		•	•	•	•	•	•		•
	Chrysomya albiceps	•	•	•	•	•	•	•	•	•	•		•
	Chrysomya megacephala		•			•			•	•			•
	Lucilia sericata	•	•					•	•				
Carnidae= Milichiidae	*Hemeromyia* sp.								•				
Ephydridae	Not identified beyond family				•		•		•				
Fanniidae	*Fannia canicularis*		•				•						
Muscidae	*Musca domestica*		•		•		•		•	•	•		•
	Musca spp.		•		•		•		•		•		
	Musca sorbens		•						•				
	Stomoxys sp.						•						
Periscelididae= Stenomicridae	*Periscelis* sp.								•				
Phoridae	*Megaselia scalaris*		•				•		•	•	•		
Psychodidae	*Psychoda alternata*						•			•			•
Sarcophagidae	*Parasarcophaga (Liopygia) ruficornis*	•	•	•	•	•	•	•	•	•	•		
	Sarcophaga africa = S. haemorrhoidalis	•	•			•	•			•			
	Sarcophaga spp.			•	•	•	•	•	•	•			•
	Wohlfahrtia nuba	•	•					•	•	•			
Sphaeroceridae	Not identified beyond family						•		•				
Ulidiidae= Otididae	*Physiphora alceae*		•		•		•		•		•		

3.6. Environmental data

Using ANOVA (analysis of variance), significant differences were found between the four habitats in temperature ($F_{3.56}$= 6.31, p=0.0009) and humidity ($F_{3.56}$= 5.70, p=0.0018). The mean temperature at site B (urban habitat) is higher than those at the other sites (19.56 ± 0.75) while humidity at site C (agriculture habitat) is higher than those at the other sites (78.15 ± 6.75) as shown in table 10.

Table 10: Environmental data recorded daily from dataloggers readings and meteorological stations on four sites (habitats) for 14 days (period of the study)

SITE	Temperature				Humidity			
	Mean*	SD	Min.	Max.	Mean*	SD	Min.	Max.
Site A Desert	**18.03**	**3.03**	**14.60**	**25.20**	**62.28**	**18.90**	**38.25**	**93.55**
Site B Urban	**19.56**	**0.75**	**17.40**	**20.55**	**73.43**	**4.08**	**66.20**	**79.70**
Site C Agriculture	**16.86**	**0.97**	**14.61**	**18.35**	**78.15**	**6.75**	**70.05**	**92.15**
Site D Coastal	**17.61**	**1.27**	**16.15**	**19.75**	**71.41**	**6.88**	**56.90**	**80.90**

*** Mean of datalogger readings plus mean of environmental station records**

Chapter 4: DISCUSSION

This study reported on necrophagous flies observed on decomposing rabbit carcasses at four locations (habitats) as well as on human remains, in real cases during criminal investigations. Beetles and other arthropods were not identified in spite of their occurrence on the studied carrions. The results of this study provide additional information to forensic entomologists by identifying fly species that are associated with carrion in Kuwait where no investigations on forensic entomology have been conducted before.

In this study it was found that the most important forensic flies belonged to families Calliphoridae and Sarcophagidae as the species of these families were abundant on both rabbit carcasses and human corpses and they were the first colonizers to breed. This finding is consistent with the results of other studies in different geographic areas (Payne *et al.* 1968; Smith 1986; Anderson and VanLaerhoven 1996; Hall 2001). But the species involved in this study differ from other studies in other habitats and geographic areas.

Four species from each family Calliphoridae and family Sarcophagidae were collected on carrion during this study. Of these the most frequent and abundant species were *Chrysomya albiceps* and *P. ruficornis.* In most European countries *Calliphora vicina, Calliphora vomitoria* and *L. sericata* are common primary colonizers of corpses (Bourel *et al.* 1999; Schroeder *et al.* 2003; Gennard 2007). In Hawaii, the tropical region of USA, it has been found that the initial colonizers are *L. cuprina, Chrysomya megacephala* and *Chrysomya rufifaces* in the family Calliphoridae and *S. africa* and *P. ruficornis, Sarcophagula (Sarcophaga) occidua* and *Helicoba morionella* in the family Sarcophagidae (Early and Goff, 1986). Whereas, in another habitat in USA, at high altitudes in Colorado, where temperatures are low, the most important primary colonizers are *Phormia regina, Protophormia terraenovae* (Robineau-Desvoidy 1830) , *L. sericata* and *Calliphora lilaea* (Walker, 1849) (De Jong and Chadwick 1999).

Chrysomya albiceps is a common first colonizer in Afrotropical regions, Oriental regions, Central South America and Southern Europe (Baumgartner and Greenberg 1984; Hall and Smith 1993). However, in some countries in Oriental and Australian regions, *Chrysomya megacephala* is widely distributed

(Aggarwal 2005). This fly species is the primary colonizer of human remains in Malaysia (Cheong *et al.* 1973; Salleh *et al.* 2007). In addition, Linhares (1981) considers it well known for its eusynanthropic and endophilic habitats in the New World being found in large numbers in urban areas. In contrast, in this study, *Chrysomya megacephala* is less important and a secondary colonizer; it was recorded only at desert and coastal locations in this study, in later stage of decomposition of rabbit carcasses. These observations were also found in other geographic areas; in southeastern Brazil, De Souza and Linhares (1997) have claimed that this calliphorid species is not primarily a carrion fly, for it bred in relatively small numbers.

Species of family Calliphoridae, particularly *Chrysomya albiceps*, were responsible for maximum rabbit carcass consumption although Sarcophagid species coexisted with them in the same carcasses. Early and Goff (1986) have stated that species of sarcophagid are frequently observed larvipositing on carrion at approximately the same time at the calliphorid species. This study confirms this observation; however, the numbers of sarcophagid maggots were much less than those of Calliphoridae. Thus they rank second regarding the reduction of carrion weight. The larvipositing sarcophagid female has much less fecundity than the ovipositting calliphorid female (Hanski 1987, Goff 1991).

Chrysomya albiceps is an aggressive predator feeding on other dipterous larvae present on carrions as well as decomposing tissues (Tantawi *et al.* 1996; Pérez *et al.* 2005). This explains the occurrence of relatively fewer numbers of maggots of other fly species, even Calliphoridae, when they coexisted with *Chrysomya albiceps* on the same carrions.

Tantawi *et al.* (1996) and Wolff (2001) recorded *Chrysomya albiceps* as secondary breeders on rabbit and pig carrions respectively. It has generally believed that Chrysomynae flies can act as primary species in the absence of Calliphorinae (Coe 1978; Braack 1981). However, Bharti and Singh (2003) reported that members of subfamily Chrysomynae can act as primary flies even when Calliphorinae is also available. This study showed *Chrysomya albiceps* was primary invader on many rabbits and human corpses despite the presence of species of Calliphorinae. It is suggested that when the fly species is abundant it had a greater chance to compete and arrive first at the carrion. O'Flynn and

Moorhouse (1979) found that Chrysominae are primary colonizers in warm seasons when they are abundant.

Calliphora vicina was amongst the first to colonize rabbit carcasses and its adults and larvae were collected from human remains although its abundance and frequency was less than *Chrysomya albiceps* and *P. ruficornis*. This calliphorid species prefers cooler temperatures (Gennard 2007). So, in subtropical regions, it is present in the cooler months of the year (Aggarwal 2005). Tantawi *et al.* (1996) recorded it on the rabbit carcass in winter only in Egypt.

Greenberg (1991) noted that while higher temperatures >30°C accelerate the development of feeding instars of *Calliphora vicina*, the post feeding larvae fail to pupariate and subsequently die. The present study was conducted in the cooler season when ambient temperatures ranged between 16-19°C (average 18°C). Further studies are needed to investigate carrion in different seasons.

Representatives of the family Sarcophagidae are found throughout the world, with most species occurring either in tropical or temperate regions (Byrd and Castner 2009). Flesh flies are primary invaders of carrion in warmer temperate and tropical regions (Payne 1965, Early and Goff 1986). Tantawi *et al.* (1996) found that sarcophagids were primary flies on rabbit carrion in fall when average ambient temperatures were 17°C comparable with those recorded in this study during the time of the experiment. This study shows that flesh flies were important components of carrion insects and the most important species was *P. ruficornis* in Kuwait. This species has a worldwide distribution in Oriental region, Afrotropical region and Australian region; also it is common in Saudi Arabia (Al-Misned 2002). Nolte *et al.* (1992) found that *P. ruficornis* is an early invader of remains and frequently encountered on the Island of Oahu. USA, this sarcophagid species is recorded in Kuwait for the first time and used in one case from Kuwait to estimate PMI (Al-Mesbah *et al.*, in press). Denno and Cothran (1975) have stated that Sarcophagidae are known to prefer smaller carcasses to avoid rich overlap with Calliphoridae. In this study, sarcophagid flies colonization on rabbit carcasses was as frequent as on human remains.

In this study, it was found that species of family Muscidae are abundant and attracted to carrion during all stages of decomposition. However, they were collected as adults and not larvae indicating that they visited carrions to feed not to breed. The same observation was recorded by Dear (1978) and De Souza and Linhares (1997). Similarly, *Fannia* sp. Did not breed on rabbit carcasses and occasionally their larvae found on human remains. De Jong and Chadwick (1999) found that adults of this species visited rabbit carcasses but they were uncommon. De Souza and Linhares (1997) also recorded no *Fannia* larvae on the pig carcasses although large numbers of adult *Fannia* sp. were collected.

Some tiny fly species of the families (Carnidae, Ephydridae, Periscelididae and Sphaeroceridae) which visited rabbit carcasses to feed not to breed seem to be of minor importance regarding legal investigations. However, restriction of family Psychodidae to agriculture habitat and families Carnidae and Periscelididae to coastal habitat may help to determine the location of death or whether the victim has been moved. Caution must be used in these cases as flies may extend their range to other habitats.

(Watson and Carlton 2003, 2005) found that sampling methods of adult flies using pitfall, hands, or aerial sweep were problematic as numerous species of small to tiny flies were either underrepresented or completely overlooked. In this study, using sticky traps and the designated cage with fly collection chamber were efficient and satisfactory to catch much tiny flies.

It has been found that there are differences in the arthropods community in various habitats in the same geographical area (Early and Goff 1986; Goff 1991; Isiche *et al.* 1992). In Kuwait, there was a diversity of fly species between the four habitats despite the close proximity of the studied locations. The same observation was noted by Tullis and Goff (1987).

The greatest difference was significantly between desert and agriculture habitats. This could be due to the differences of the ecological behavior of flies; those prevailing particularly tiny flies of families Ephydridae, Psychodidae and Sphaeroceridae in agriculture habitat could prefer shade and attracted to manure of animals.

Anderson and VanLaerhoven (1996) found that seven pig carcasses decomposed at the same rate, entering each stage synchronously and were colonized by the same insect species at the same time. However, in this study, while used larger number of animal models, In contrary, variation in the rate of decay was observed in this study although the carcasses were simultaneously exposed. This could be attributed to the large number of experimental animals, which increased the probability of greater individual variation.

It was observed that the percentage of body weight loss is less in heavier carcasses than light ones although they were sampled in the same habitat and sampled in the same day as well as they were colonized by larvae; no explanation can be given; Watson and Carlton (2003) noted that because of the large mass and unique fatty composition of a bear carcass, it remained in prolonged state of advanced and putrified decay. Keh (1985) has pointed out that of two people dying at the same time and due to the same cause, putrefaction may proceed more rapidly in one body than in another. The natural intrinsic variability, resulting from genetic and physiological differences between individuals of the same species, may affect the rate of decomposition (Turner 1991).

In the coastal habitat, the carcasses sampled after two and four days were in fresh stage and colonized by no insects. After seven days in the same habitat, the sampled carcasses were still in bloated stage and were exclusively colonized by 1st, 2nd, immature 3rd instars of Sarcophagidae.

It is obvious that the decay of the carcasses was slower when the arrival of insects at them was delayed. This lends support to the concept that there is inter-dependence between insect colonization and the decomposition rate. This observation has been recorded by many authors (Abell *et al.* 1982; Anderson and VanLaerhoven 1996; Simmons 2010). The explanation for the delayed colonization of carcasses at the coastal habitat may be due to the local weather conditions, as it was raining during the first two days of the experiment. Heavy rain and humidity influence the blowfly activity and delay their arrival at carrion (Smith 1986). In addition, Greenberg (1990) has stated that calliphorids do not fly in rain. In Contrast, sarcophagid flies are considered to be unimpeded by rain

(Erzinçlioğlu 2000); as a result, flesh flies may be the initial colonizers of the body if there is a long period of rainy weather.

The decomposition rate of carcasses at urban habitats was significantly faster than that of those at other habitats. The speed of decomposition was determined by insect colonization and climatic conditions particularly temperature (Anderson 2009).

No ants were collected from carcasses at the urban habitat, in contrary at other habitats; Ants play a determining rate in the time taken for decomposition. The process is slowed down by the removal of large quantities of dipteran eggs and larvae (Tullis and Goff 1987; Well and Greenberg 1994; Stoker *et al.* 1995; Pérez *et al.* 2005).

The mean temperatures, which were calculated from datalogger reading and the records of the nearest meteorological states, were significantly higher at the Urban habitat than those at other habitats. This could be another reason of faster speed of the carcass decomposition at urban habitat. In addition, at urban location, the carcasses were placed on a roof of a building with concrete ground, not soil ground as at other habitat; this microhabitat was relatively hotter as concrete reflect sun rays.

During 14 days, the period of the experiment, in November and December, when mean ambient temperatures were 18°C, the decomposition process of rabbit carcasses was studied. Though a continuous process, it is divided into various stages by different researchers (Goff 2010). In the present study the decomposition process was evident in the form of four succession stages (fresh, bloated, decay, dry). These observable stages of decomposition were defined also by Reed (1958), Rodriguez and Bass (1983), Braack (1986) and Tantawi *et al.* (1986). The final decomposition stage was not observed in this experiment except in one carcass at the urban location which reached dryness. It is not surprising to observe prolonged decomposition duration of carcasses in this time of the year and during a limited time period. Goddard and Lago (1985) stated that when temperatures are high, the decomposition process becomes short. Another important factor contributing to the decomposition of a carcass is the activity of necrophagous insects where arrival at carcasses and development affected also by climatic conditions (Gennard 2007). In the warm climate of the Kruger National Park, in South Africa, blowfly larvae were easily capable of reducing a medium sized mammal such as impala to the dry stage within five days in summer and within 14 days in winter (Braack 1986). Even using rabbit carcasses, Tantawi *et al.* (1996) found that carcasses in summer and spring decayed at a much faster rate than those in fall and winter. In summer, took only 4.5 days to reach dry stage when average daily temperatures were 28ºC. In contrast, during winter, 51.5 days were required for carcasses to reach the dry stage when average daily temperature ranged from 13.6 to 16.6°C.

In human carcasses, many corpses reached the dry stage as they were discovered after enough time to be decomposed to the last stage of the process. Some corpses were discovered in hot season and decomposed fast in few days.

Rodriguez and Bass (1983) noted that there is a direct correlation between the stage of decay and the succession of insect families and species arriving at a carcass. In general, the succession patterns in this study were similar to those described by Tantawi *et al.* (1966) and Anderson and VanLaerhoven (1996).

In the fresh stage, carcasses were colonized by few adult or immature flies particularly calliphorids and sarcophagids. The high peak of fly colonization was in bloated and decay stages as the odor become evident and the carcasses has been already occupied by eggs and larvae. Greenberg (1971) stated that flies oviposit on a carcass after putrefactive odors develop and prefer to deposit eggs in areas already colonized by calliphorid larvae. In the dry stage, no fly larvae and only a few adults were observed on the carcasses as nutrient resources diminished. Beetles began to appear on rabbit carcasses in the bloated stage; similarly, they were observed on two human bodies which were in bloated stage and discovered in the open.

Hall and Huntington (2009) analyzed succession data on arthropods on human cadavers from Rodriguez and Bass (1983) and showed that beetles are present mainly during bloated and dry stage of decomposition. However, the majority of their numbers were in the dry stage. In this study, a dried human corpse was exclusively occupied by beetles.

In case No.3, the body of the female victim was divided and some parts were burned. After 4 days postmortem her burnt hands were found to be colonized with eggs *Calliphora vicina*. Catts and Goff (1992) suggested burning of the flesh retarded its immediate attraction to adult blowflies. However, Avila and Goff (1998) observed immediate attraction of calliphorid flies to the burnt corpse and significant oviposition was evident on day 2. There are many reasons for limitation of access by insects including climatic conditions, burying concealment (Anderson, 2009). Hiding the hands for sometime before throwing them, prevent the access of blowflies and the colonization was delayed.

In case No.13, the female victim was in fresh stage of decay and colonized by 1^{st} and 2^{nd} instars of different fly species at a cut wound on her neck; no other sites on the body were colonized with insects. This gives an indication that injuries on a corpse are more attractive sites for fly colonization than the natural openings. Rodriguez and Bass (1983) have stated that a corpse may be more decomposed in the area of the body where there are injuries which result in exposure of blood and underlying tissue, thus providing access for carrion bacteria and insects. In this case, entomological evidence helped with other evidences to know the manner of death which was slaughtering of the victim with a sharp tool.

Carvalho *et al.* (2000) found that the adult flies that were attracted to human corpses were the same species that were collected on pig carcasses. Similarly, in this study, the attractiveness to necrophagous flies exerted by human remains and rabbit carcasses was the same. This gives support to the concept that a database of forensic insects collected from experimental animals can be used for human corposes in the same habitat in legal investigations. Arnaldos *et al.* (2005) used the entomological data obtained from experimental studies using chicken carcasses in order to determine PMI in real forensic cases in the same habitat.

In addition, the assemblages of necrophagous flies on human remains and rabbit carcasses revealed that the diversity of fly species of forensic importance and their succession patterns were the same regardless the size of carrion. Kuusela and Hanski (1982) and Hewadikaram and Goff (1991) found that the size of the carrion has no obvious effect on the composition of the insect fauna or succession pattern. No differences were observed between patterns of succession based on the use of the cat or pig carcasses in the same habitat (Early and Goff 1986).

Chapter 5: CONCLUSIONS

This study shows there are four species of Calliphoridae: *Chrysomya albiceps, Calliphora vicina, Chrysomya megacephala* and *L. sericata*, four species of Sarcophagidae: *P. ruficornis, S. africa Sarcophaga* sp. and *W. nuba*, that can be considered of potential forensic importance and can be used as indicators of PMI in Kuwait as they are able to breed on carrion. However, the most important of these are *Chrysomya albiceps* and *P. ruficornis* as they are the most frequent and abundant and the primary colonizers on decomposing human remains and rabbit carcasses in different habitats.

Although this study was conducted in one season and for a short period, the data obtained from it are useful for many reasons: it enriches the biodiversity of Kuwait as some families or species of Diptera are recorded for the first time in this country (*Chrysomya megacephala, Hemeromyia* sp.*, Fannia canicularis, Musca sorbens, Periscelis* sp.*,* and *Parasarcophaga ruficornis*). However, some flies were mentioned in unpublished medical and veterinary reports. The study provides database information on species composition and succession patterns of necrophagous flies, these data are valuable for forensic entomology analysis as no carrion data were previously available for this region. In addition, this study reveals that Kuwait has its own carrion Diptera composition and confirmed that insect succession on carrion is geographically specific.

The carrion Diptera composition and their succession patterns obtained from real forensic cases showed a number of basic similarities to those observed on rabbit carcasses as experiment animals. This supports the validity of the experimental data to be considered as representative of the circumstances occurring in a human cadaver as well as relieves the controversy about the validity of using certain animals (small sized) as experimental models. As the impossibility of using human corpses for those kinds of studies particularly in Kuwait and other Arab countries owing to religion considerations, it means that animal models are needed. The result of this study may be considered to be useful for forensic practice in the geographical area references.

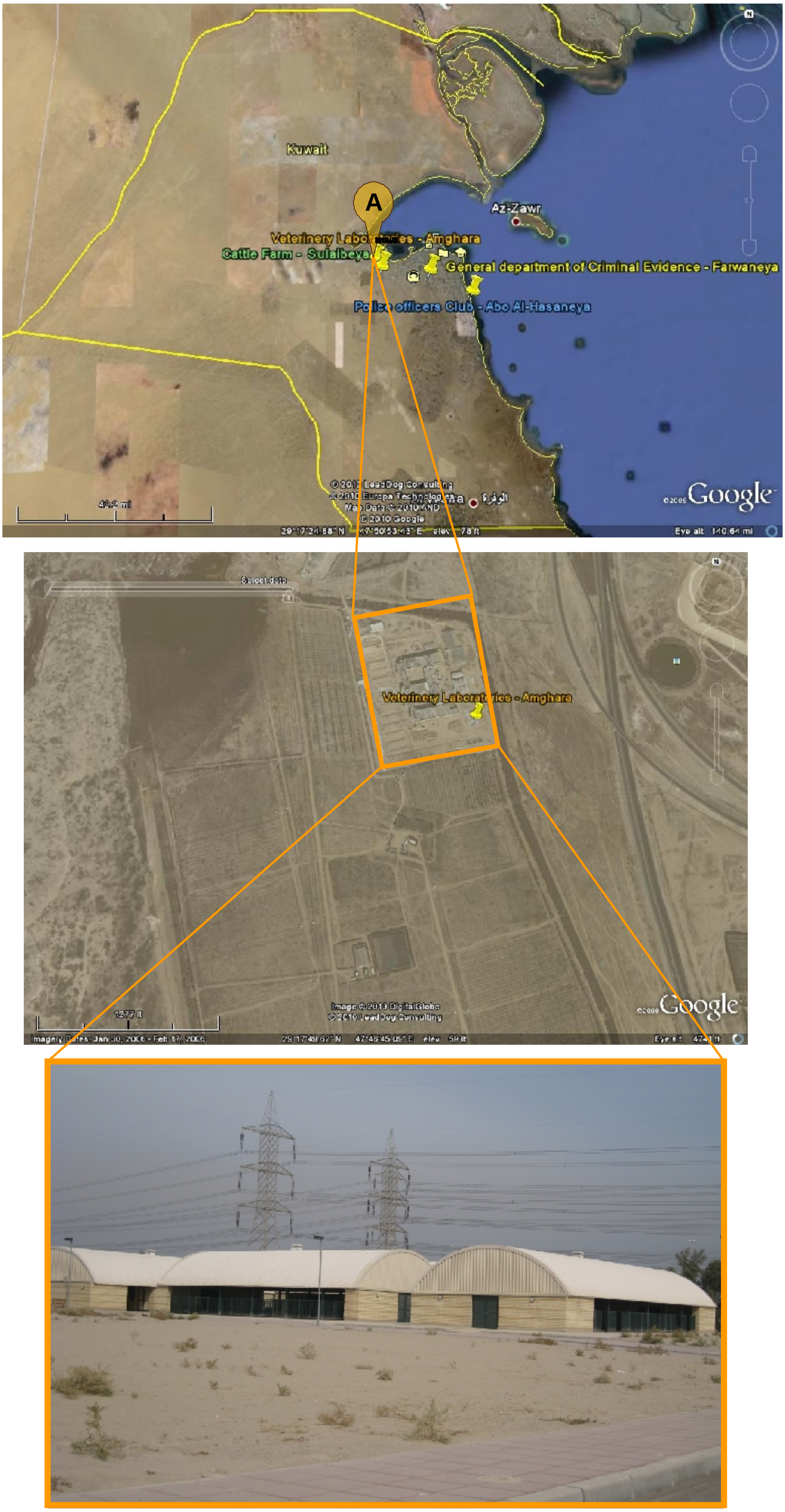
The involvement of this study in legal investigation enabled the officials and the investigators to be familiar with and aware of the importance of forensic entomology as well as initiated their interest in more information and further studies in this field.

This study also highlights the importance of forensic entomology in Kuwait. Using entomological tools helped with other evidences to know the manner of death in case No.13, estimated the PMI in case No.5 (Al-Mesbah *et al.*, in press) and provided evidence on the cause of death (toxicity) by chemical analysis feeding maggots in case No.10.

The study, it is hoped, will be the first step in presenting forensic entomology as a science and a new discipline in Kuwait as well as raising the role of the entomologist and the value of entomological information in legal investigations to be used as evidence in the court.

APPENDICES

APPENDIX A
Supplementary Figures

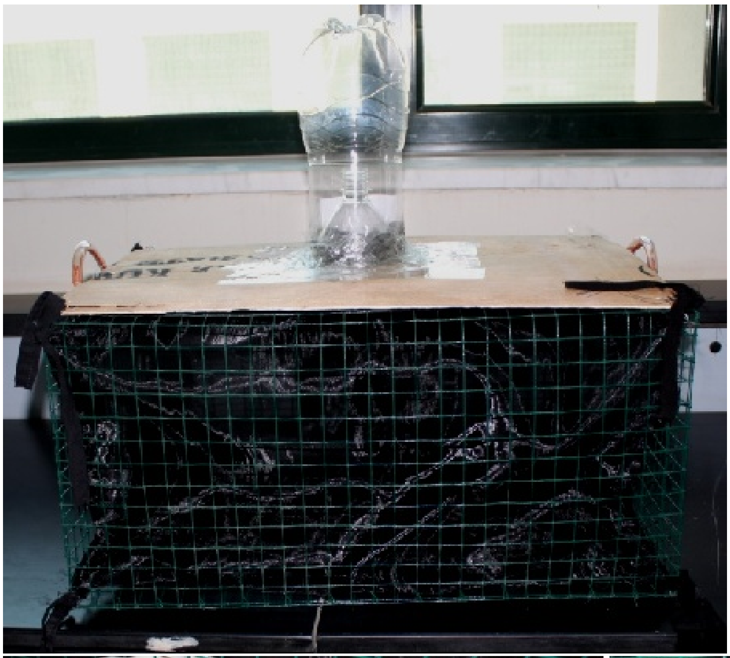


App. A-1: Site A (desert habitat).

App. A-2: Site B (urban habitat).

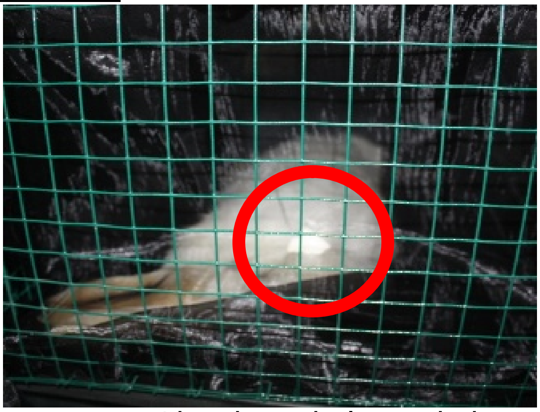
App. A-3: Site C (agriculture habitat).

App. A-4: Site D (costal habitat).



App. A-5: Showing a man made fabric cage.

App. A-6: Hinged opening at one side of the outer screen and inner cloth frame of the cage.



App. A-7: Showing a hole made in one side of the inner cloth frame of the cage.

App. A-8: Distribution of the cages and sticky traps at a site.

App. A-9: Ten rabbits after killing of site A.

App. A-10: Weighing of rabbits before placing cages.

App. A-11: Placing a rabbit in a cage at day 0 of the experiment.

App. A-12: Site A, group a, carcass no.1.

App. A-13:
A large bag used for transportation of the caged carcasses and corresponding sticky traps to the laboratory.

App. A-14:
Weighing a carcass in the laboratory.

App. A-15:
Desiccation and examination of a carcass for insects in the laboratory.

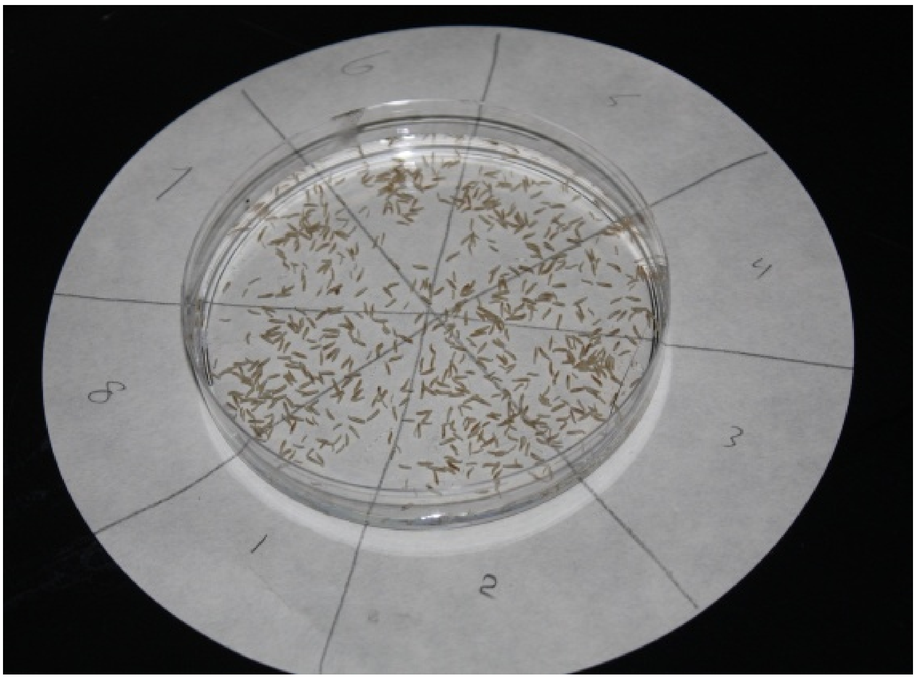
App. A-16: Rearing of immature larvae on beef minced meat.

App. A-17: The rearing containers are covered with gauze.

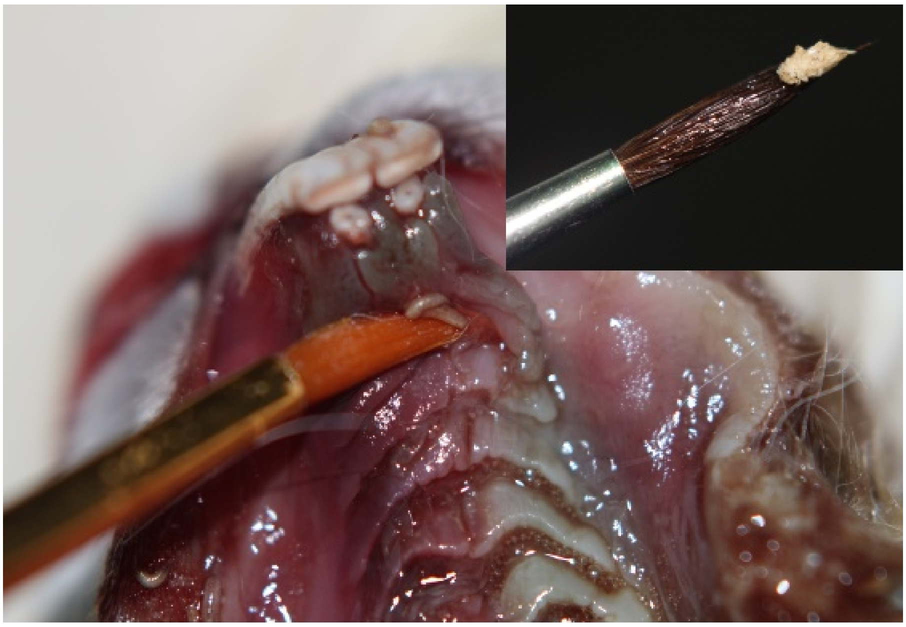
App. A-18: Sawdust for pupation of 3rd instars.

App. A-19: Observation of rearing and pupation of larvae in the laboratory backyard.

App. A-20: Killing the 3rd instar larvae in boiling water.

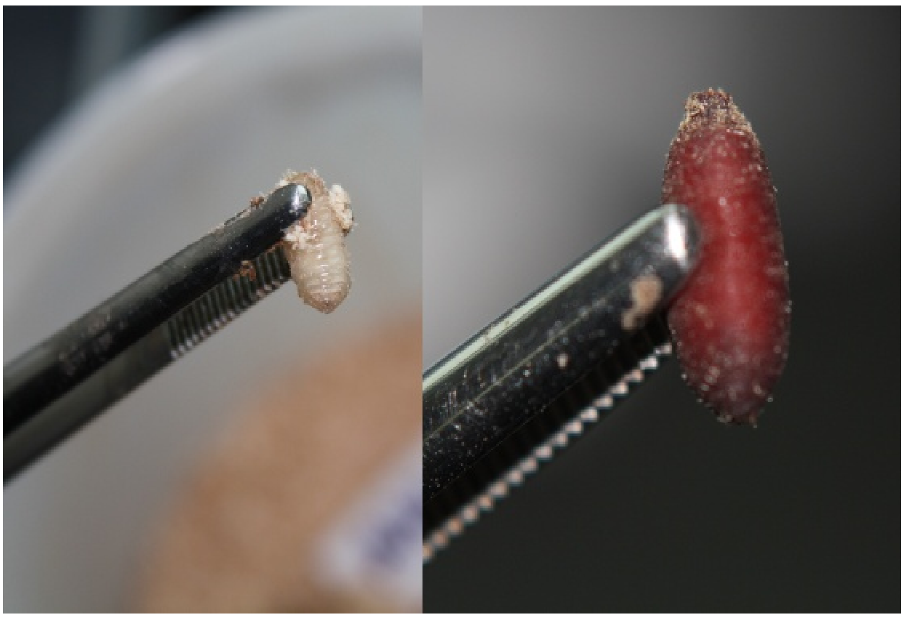


App. A-21: Identification and counting of larvae.



App. A-22: Collecting egg, 1st and 2nd instar larvae with paint brush.

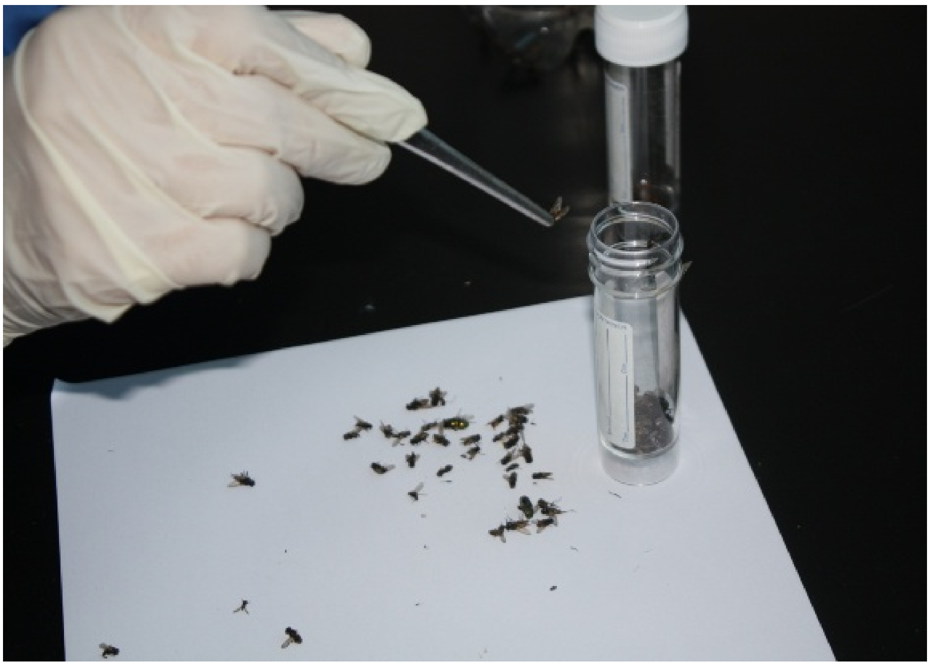
App. A-23: Collecting 3rd instar larvae with entomological forceps.



App. A-24: Collecting of pupae.

App. A-25: Adult flies attached to the sticky traps.

App. A-26: Adult flies trapped in the bottle (collection chamber).



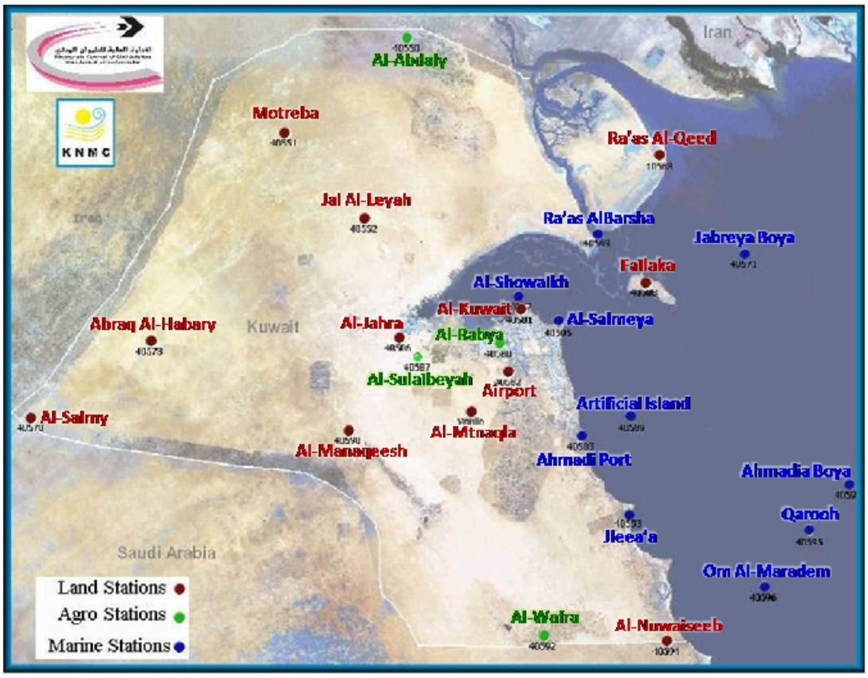
App. A-27: Adult flies removed carefully and placed in vials for identification and counting.

App. A- 28: (a-d): (a) Cage **(b)** Sticky traps **(c)** Pupation container **(d)** Vials are labeled with the same ID number and color.

App. A- 29 (a-b): The emergences of adult flies.

App. A-30: Placement of the datalogger at the site

App. A-31:
Enclosure of the datalogger in waterproof casting.



App. A-32:
Kuwait map shows the distribution of all the weather stations of the Directorate General of Civil Aviation.

APPENDIX B
Specimens Identified

App. B-1: Calliphoridae; ***Calliphora vicina*** Robineau-Desvoidy 1830. Urban blue bottle fly.

App. B-2: Calliphoridae; ***Chrysomya albiceps*** (Wiedemann 1819). Banded blow fly, hairy maggot blow fly.

App. B-3: Calliphoridae*;* ***Chrysomya megacephala*** (Fabricius 1794). Oriental latrine fly, big headed blow fly.

App. B-4: Calliphoridae; ***Lucilia sericata*** (Meigen 1826). English sheep blow fly, green bottle flies.

App. B-5: Carnidae; ***Hemeromyia*** **sp.**

App. B-6: Ephydridae.

App. B-7: Fanniidae; ***Fannia canicularis*** (Linnaeus 1761).

App. B-8: Muscidae; ***Musca domestica*** Linnaeus 1758. Common house fly; ***Musca sorbens*** Wiedemann 1830. Face fly; ***Musca*** **spp.**

App. B-9: Muscidae; ***Stomoxys*** **sp.**

App. B-10: Periscelididae*;* ***Periscelis*** **sp.**

App. B-11: Phoridae; ***Megaselia scalaris*** Loew 1866.

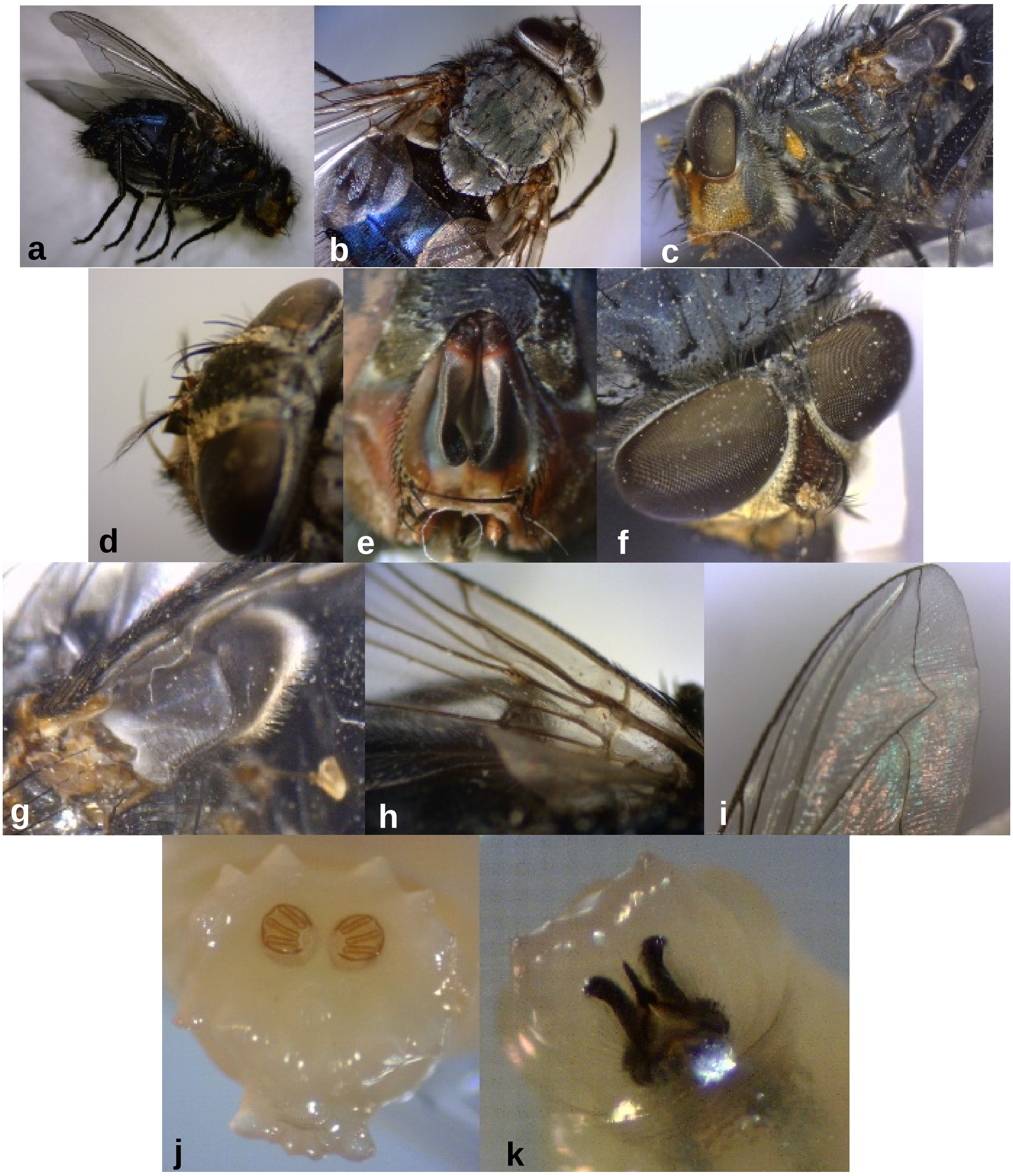
App. B-12: Psychodidae; ***Psychoda alternata*** Say 1824.

App. B-13: Sarcophagidae; ***Parasarcophaga ruficornis*** (Fabricius 1794); ***Sarcophaga africa*** (Wiedemann) = *S. haemorrhoidalis* (Fallen) Red-Tailed Flesh Fly***; Sarcophaga*** **spp.** Flesh Flies.

App. B-14: Sarcophagidae; ***Wohlfahrtia nuba*** (Wiedemann 1830) checkerspot fly.

App. B-15: Sphaeroceridae.

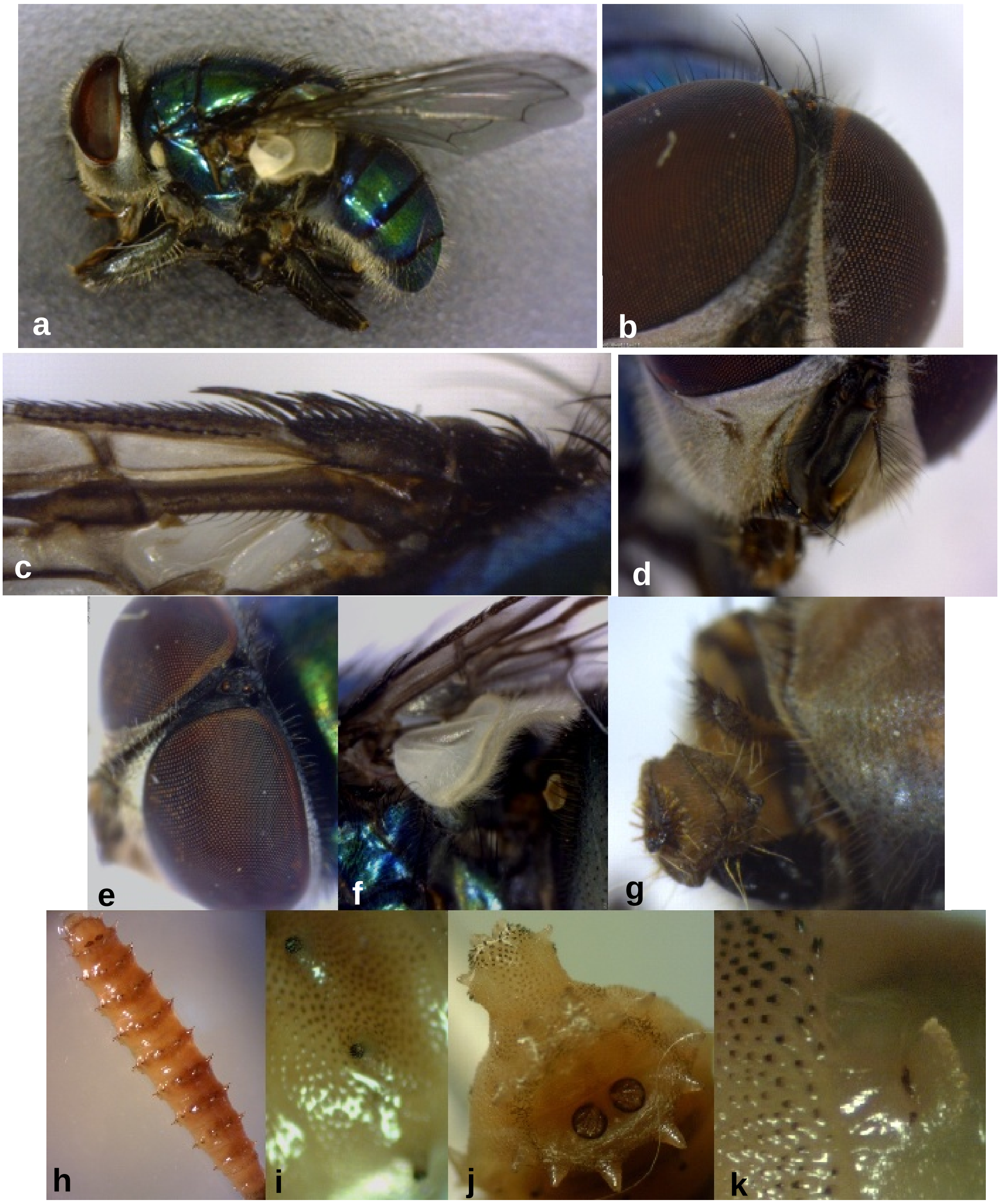
App. B-16: Ulidiidae; ***Physiphora alceae*** (Preyssler 1791).



App. B-1: Calliphoridae; *Calliphora vicina* Robineau-Desvoidy 1830 (Urban blue bottle fly) (a-k):

Adult: (a) general appearance (b) dorsal view: thorax dull, pruinose with complete transverse suture, abdomen; abdomen shiny metallic blue to blue-black (c) lateral view: postgena & lower posterior corner of genal dilation & back of head with long yellowish/reddish setae, yellow/orange thoracic anterior spiracle, basicosta dull yellow/orange (d) plumose aristae (e) facial ridges, mouth edge and anterior part of genal dilation, bucca (mouth) orange/reddish (f) orbital bristles, postocular setae (g) well developed lower calypter setose (h) wing stem vein bare (i) wing vein 4 has sharp bend.

Larva (3rd instar): (j) posterior spiracle small, relatively close to each other (distance between spiracles ≥ width a single spiracle, linear slits, peritreme complete, tubercles (papillae) (k) pseudocephalon, oral sclerite well sclerotised along the whole length.



App. B-2: Calliphoridae; *Chrysomya albiceps* (Wiedemann 1819) (Banded blow fly)/ (Hairy maggot blow fly) (a-k):

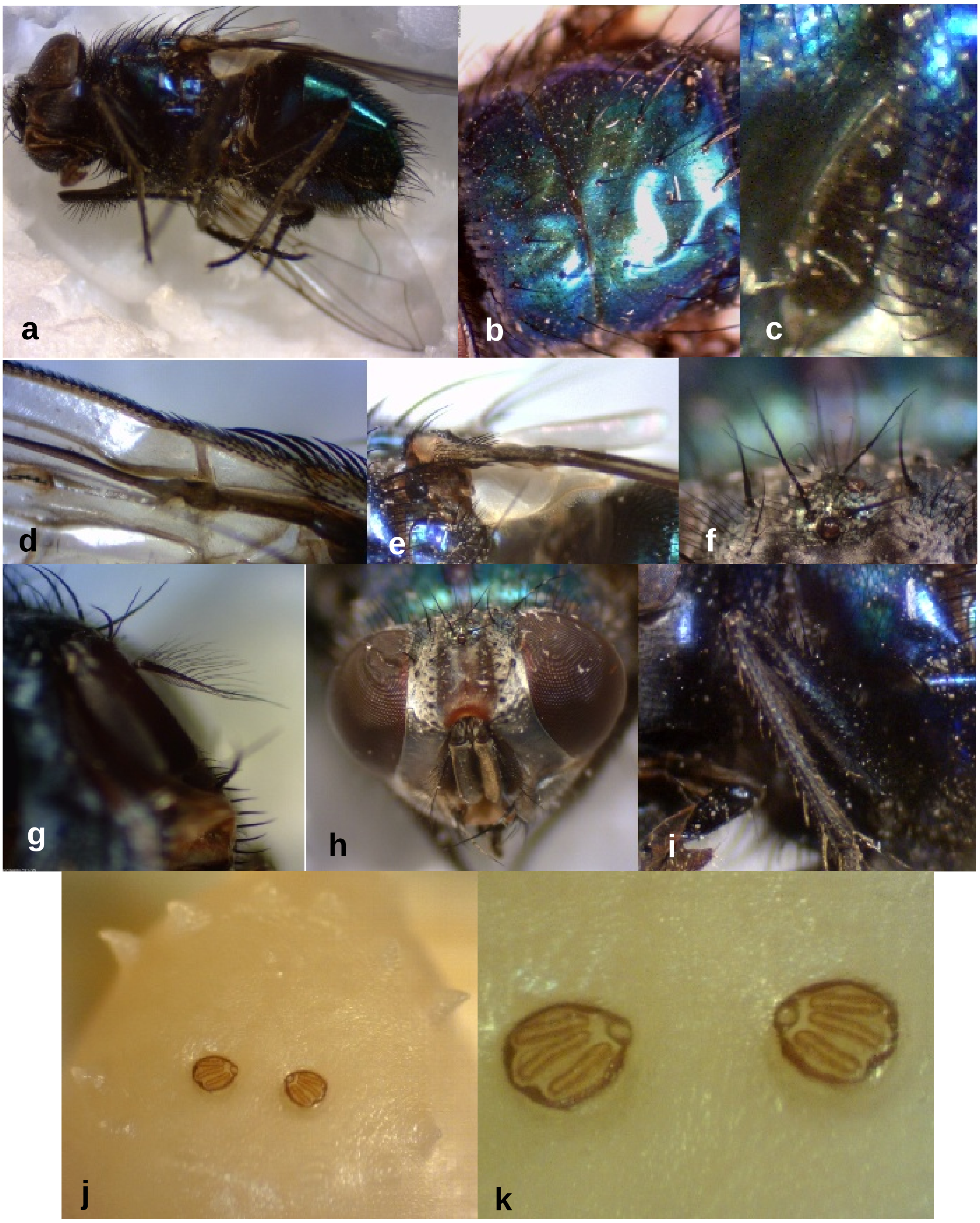
Adult male: (a) lateral view: body bright metallic green colored, thorax with complete transverse suture, abdomen segments has narrow dark line on rear edge, no prostigmatic bristle, white/pale yellow thoracic anterior spiracle, wing vein 4 has sharp bend (b) ocellar bristles (c) wing basal stem vein setose (d) plumose aristae, head extensive silverish/white color with a lot of white hairs (e) 3 ocelli present, postocular setae (f) well developed lower calypter (g) mouth parts.

Larvae (3rd instar): (h) with numerous fleshy prominent projections (protuberances) laterally and dorsally (i) detail of apical spines on tubercles, strongly pigmented on the apex, spines absent from stalk of all papillae (j) posterior segment showing posterior spiracles with linear slits, tubercles (papillae), anal protuberances (k) anterior spiracles with 9 lobes and cuticular spines (papilla).

App. B-3: Calliphoridae; *Chrysomya megacephala* (Fabricius 1794) (Oriental latrine fly)/ (Big headed blow fly) (a-j):

Adult male: (a) lateral view: stumpy box-like body (b) dorsal view: shining metallic blue abdomen and thorax, thorax with complete transverse suture, wing vein 4 has sharp bend (c) large red eyes, male frons very narrow (eyes nearly touching), genal dilation and bucca with golden/orange ground color with orange setae (d) male eye with upper facets much enlarged and sharply demarcated from facets in lower third (much smaller), plumose aristae (e) setose stem vein (f) blackish/brown thoracic anterior spiracle (g) well developed lower calypter, very dark, white only at the base.

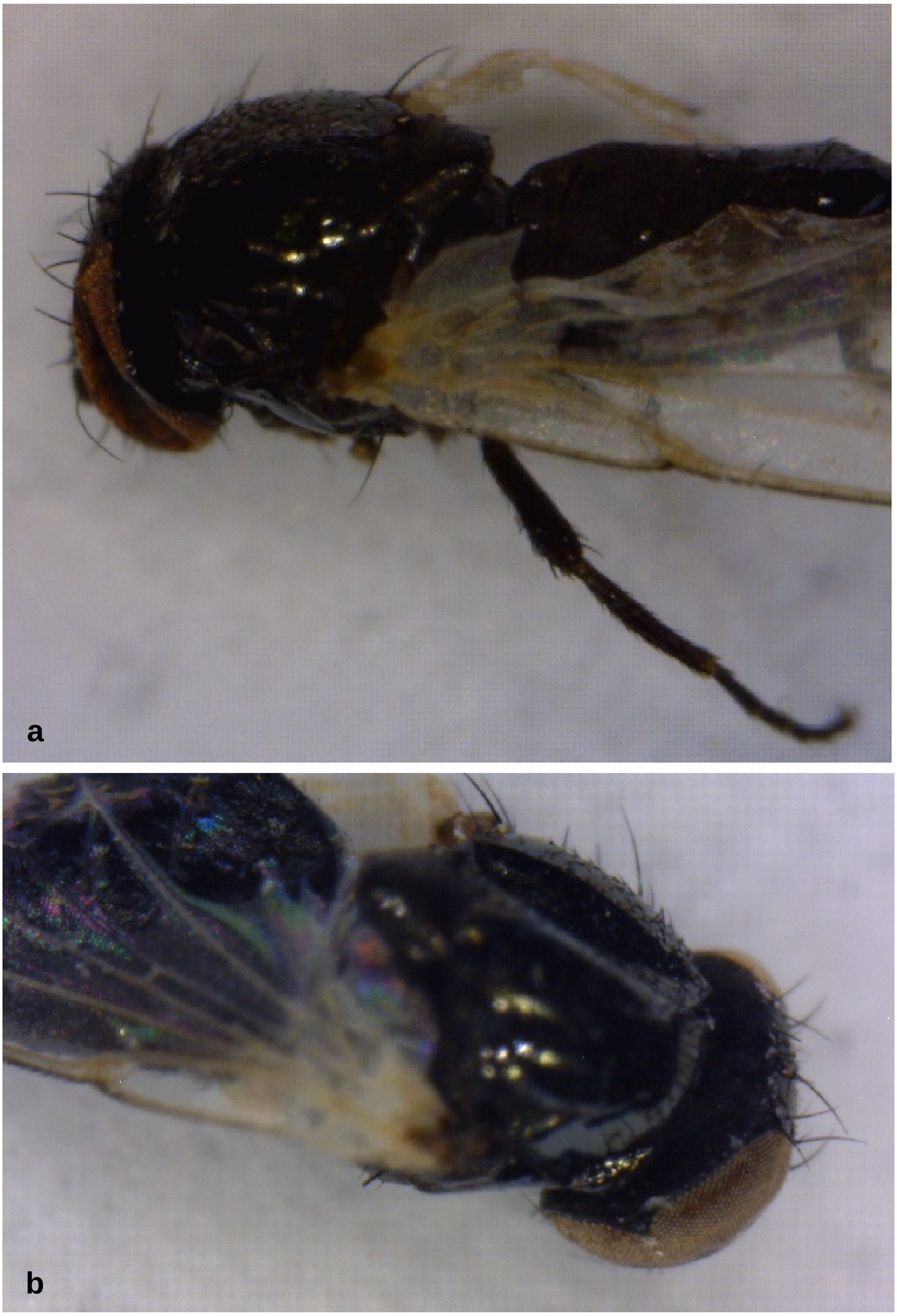
Larvae (3rd instar): (h) posterior spiracles close to each other with incomplete peritreme and linear slits, anal protuberance (i) pseudocephalon, sclerotised part of the oral sclerite (small, circular) (j) anterior spiracle with >9 lobes (short branches), cuticular dorsal spines are big, robust with serrated tips (thoracic segments).



App. B-4: Calliphoridae; *Lucilia sericata* (Meigen 1826) (English sheep blow fly)/ (Green bottle fly) (a-k):

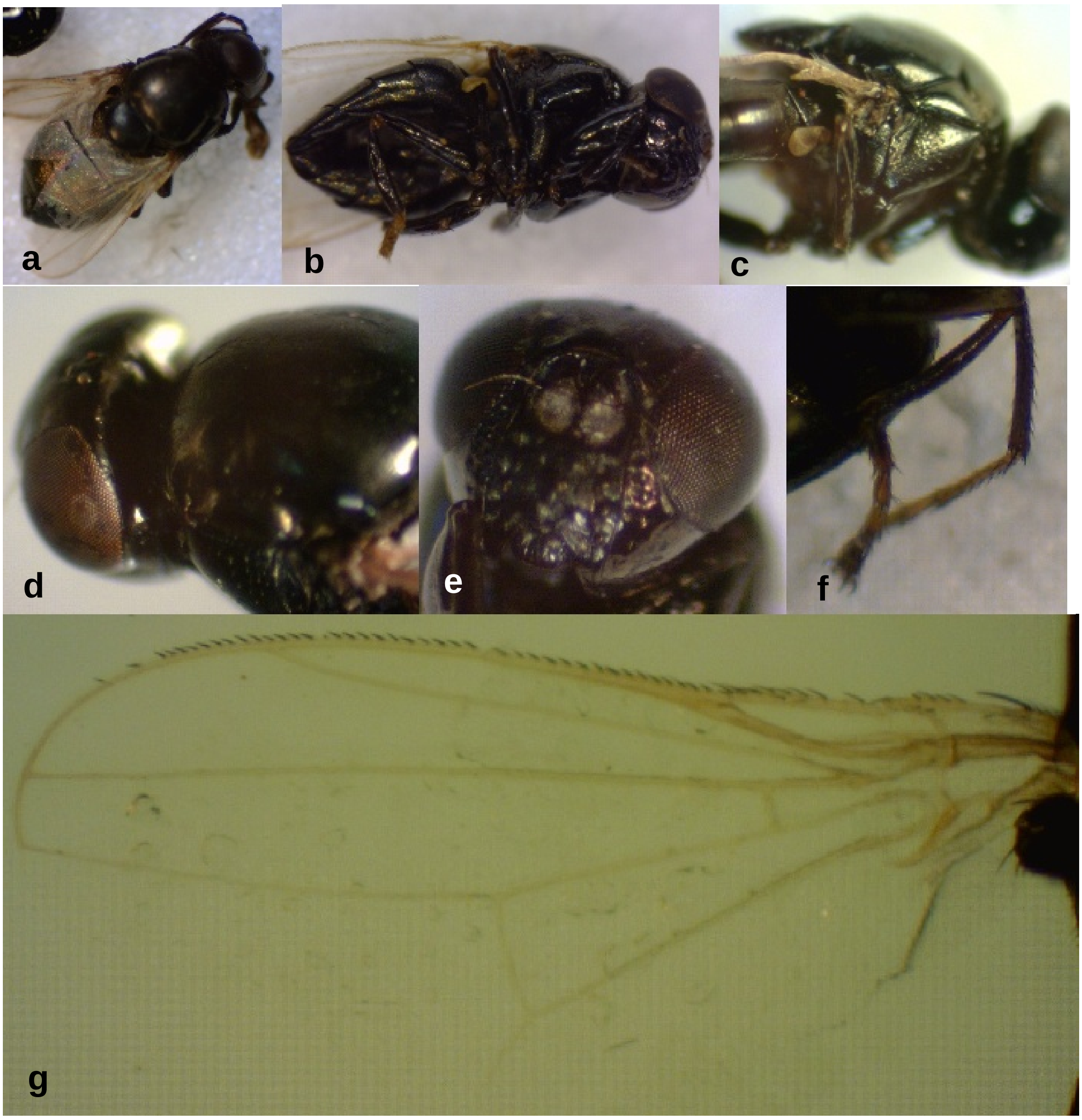
Adult female: (a) lateral view: thorax and abdomen shiny metallic green/blue with coppery/bronze reflections, wing vein 4 has sharp bend (b) dorsal view: thorax with complete transverse suture, strong acrostichal bristles (setae) (c) black thoracic anterior spiracle (d) wing stem vein without setae (bare) (e) basicosta pale yellow, subcostal sclerite bare, well developed bare lower calypter (f) ocellar seta (g) plumose aristae (h) female, interfrontal stripe (space between eyes) > twice width of ⅓ antennal segment (i) very dark bluish black front femur.

Larvae (3^{rd} instar): (j) posterior spiracles and tubercles (papillae), distance between each P1 = distance between P2 and P2 (k) close view: pear-shaped (length > width), peritreme complete, thin and narrow, linear slits (long and thin).



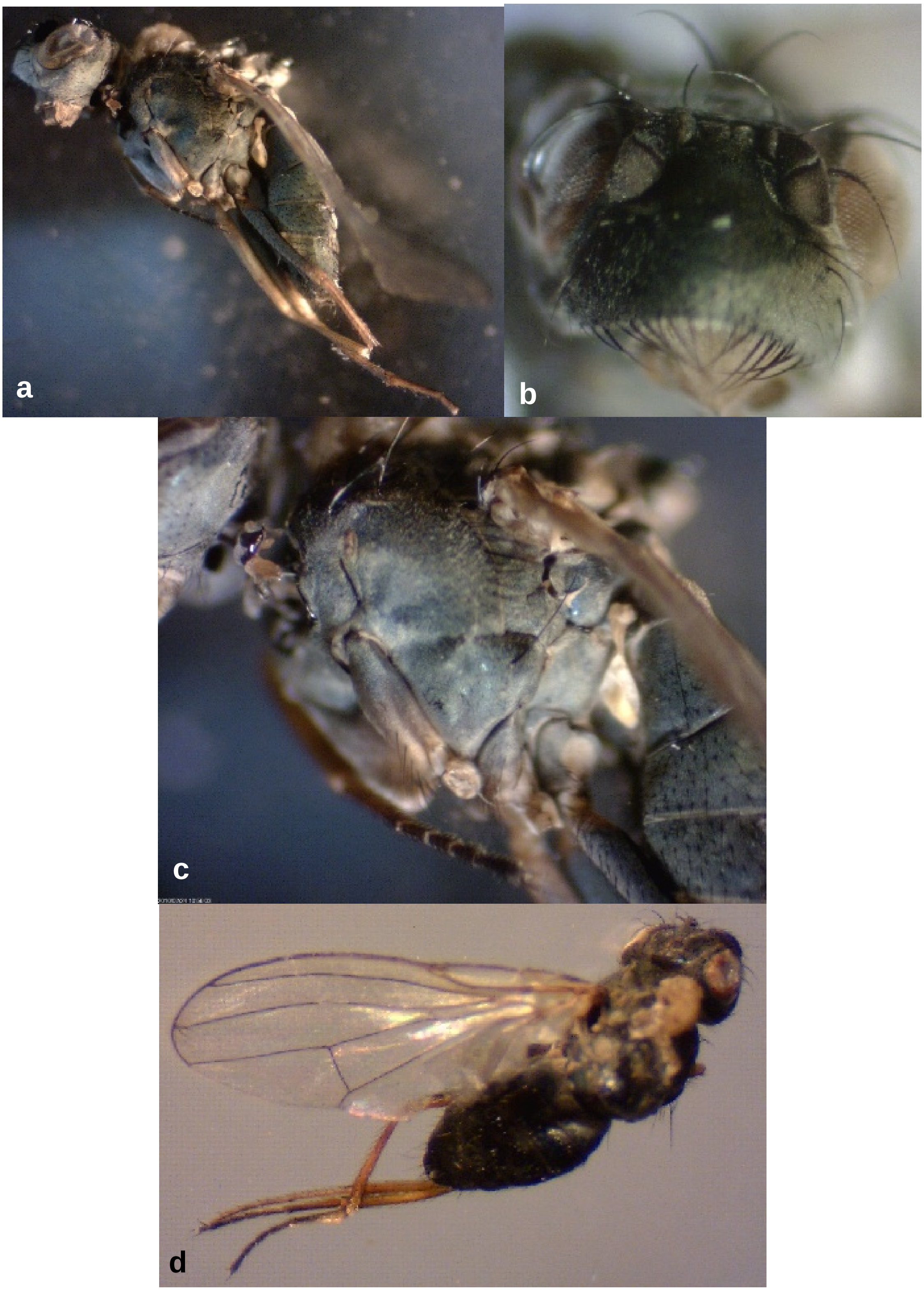
App. B-5: Carnidae; *Hemeromyia* sp., size less than 4mm (a-b):

Adult: (a) lateral view: black highly polished, reduced head chaetotaxy (bristles short and mostly pale), hind tibia without spike, wing costa with 2 breaks, mid-tibia without periapical dorsal bristles (b) dorsal view: thorax with incomplete transverse suture, 3 ocelli present, presence of 2 medioclinate "frontal" setae and 2 lateroclinate orbital setae, parallel postvertical bristles, mesonotum has one row of acrostichal bristles.



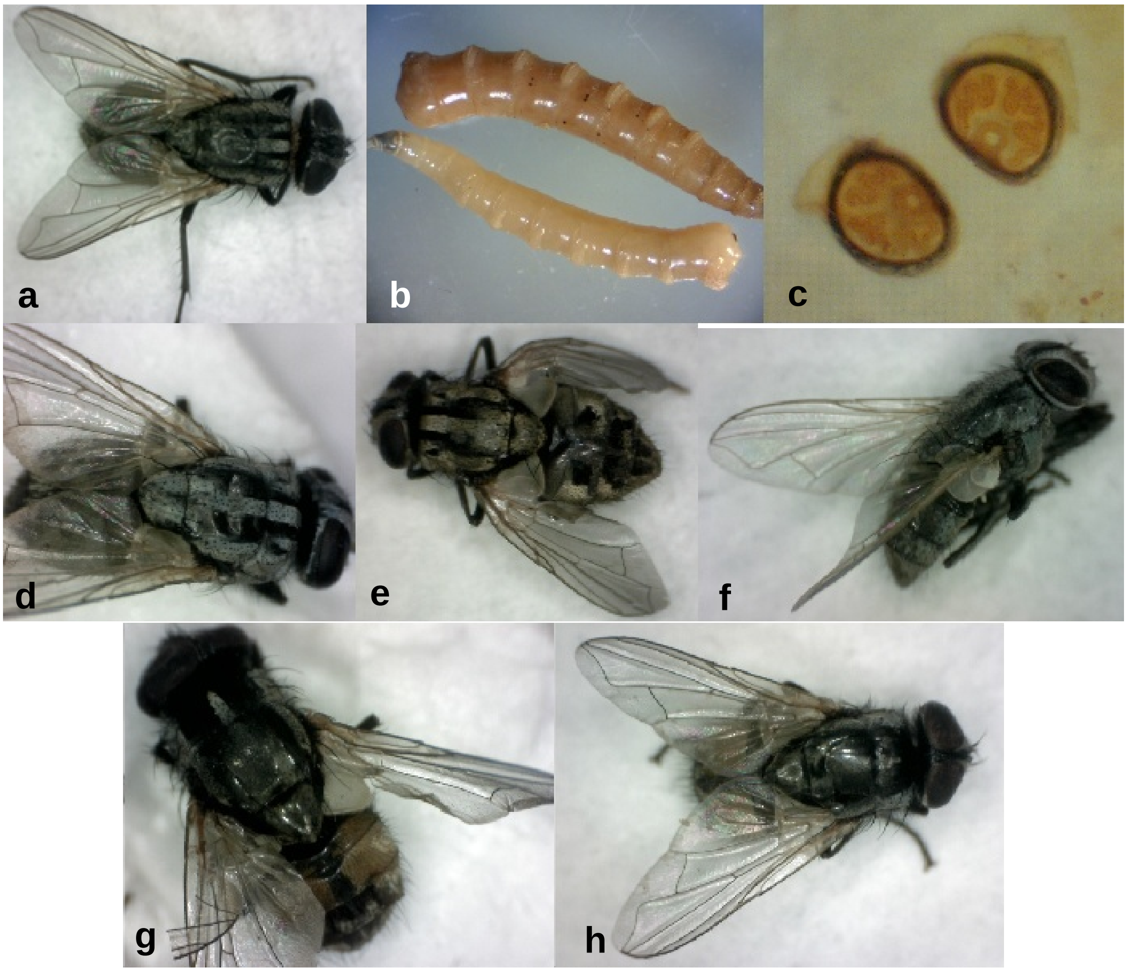
App. B-6: Ephydridae; size 2.5mm long (a-g):

Adult: (a) dorsal view: thorax without transverse suture (b) ventral view: acalypterate (c) lateral view: robustly-built, dorsal thorax bare (d) 3 ocelli present (e) mouth parts functional, ptilinal segment clearly defined, frons large, 2nd antennal segment not grooved and the 3rd segment rounded, not apical aristae (downwardly) (f) tibiae without dorsal pre-apical bristle (g) wing costa with 2 breaks (near humeral costal vein and the end of vein 1), small bristles around the edge, discal, anal cell and sub-apical cell absent.



App. B-7: Fanniidae; *Fannia canicularis* (Linnaeus 1761) (Lesser house fly) (a-d):

Adult: (a) general appearance: female grey thorax with 3 faint brown stripes, yellow color in the abdomen base (b) head, silvery color in front of the eye, female has one outcurved bristle on each side of frons (parafrontalia) (c) lateral view (d) wing: the smallest vein 2 in lighter color and strongly curved, 4th vein straight, 6 vein short.



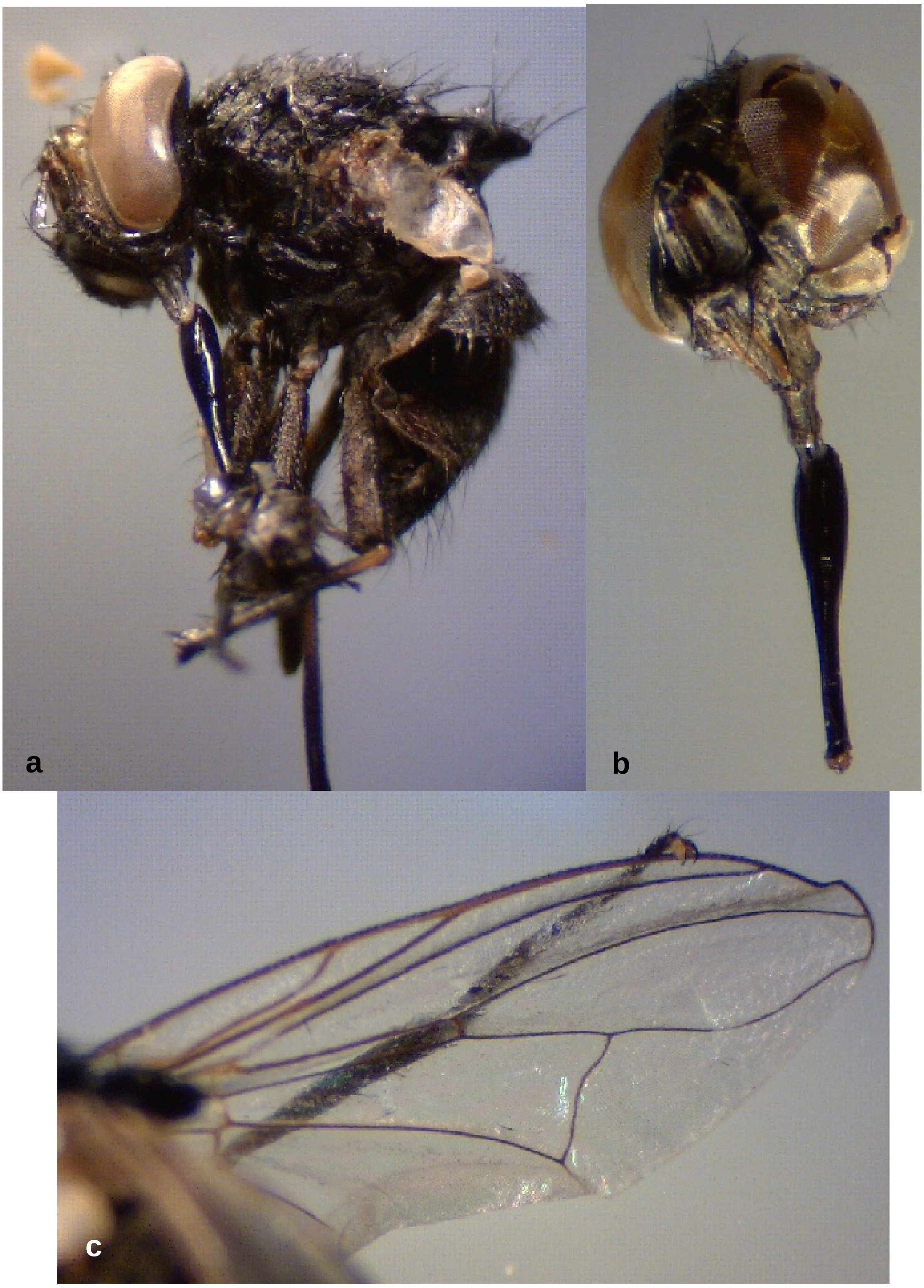
App. B-8: Muscidae: (a-h).

Musca domestica **Linnaeus 1758 (Common house fly):**
Adult female: (a) dorsal view, thorax grey with 4 black stripes, complete transverse suture, female has widely separated eyes (frons), dull grey head with black hairs, short dark bristles in propleural depression, yellow base abdomen (below the wings), wing 4th vein angled, with discal cell, subapical cell and a closed anal cell (short).

Larvae (3rd instar) of *Musca* sp.: (b) lateral view: locomotory pads, inner surface of anal plate surrounded by small spines (c) thick peritremes, posterior spiracles with kidney-shaped strongly sinuous respiratory slits.

Musca sorbens **Wiedemann 1830 (Face fly) (d-f): Adult:** (d) general appearance, complete transverse suture (e) dorsal view: thorax grey with 2 Y-shaped stripes, yellow abdomen (f) wing with discal cell, subapical cell and a closed anal cell (short).

Musca **sp. (g-h):** (g) dorsal view of adult male: complete transverse suture, wing with discal cell, subapical cell and a closed anal cell (short) (h) dorsal view of adult female: thorax grey with 2 wide black stripes, variation in abdomen appearance (yellow areas and checkerboard pattern).

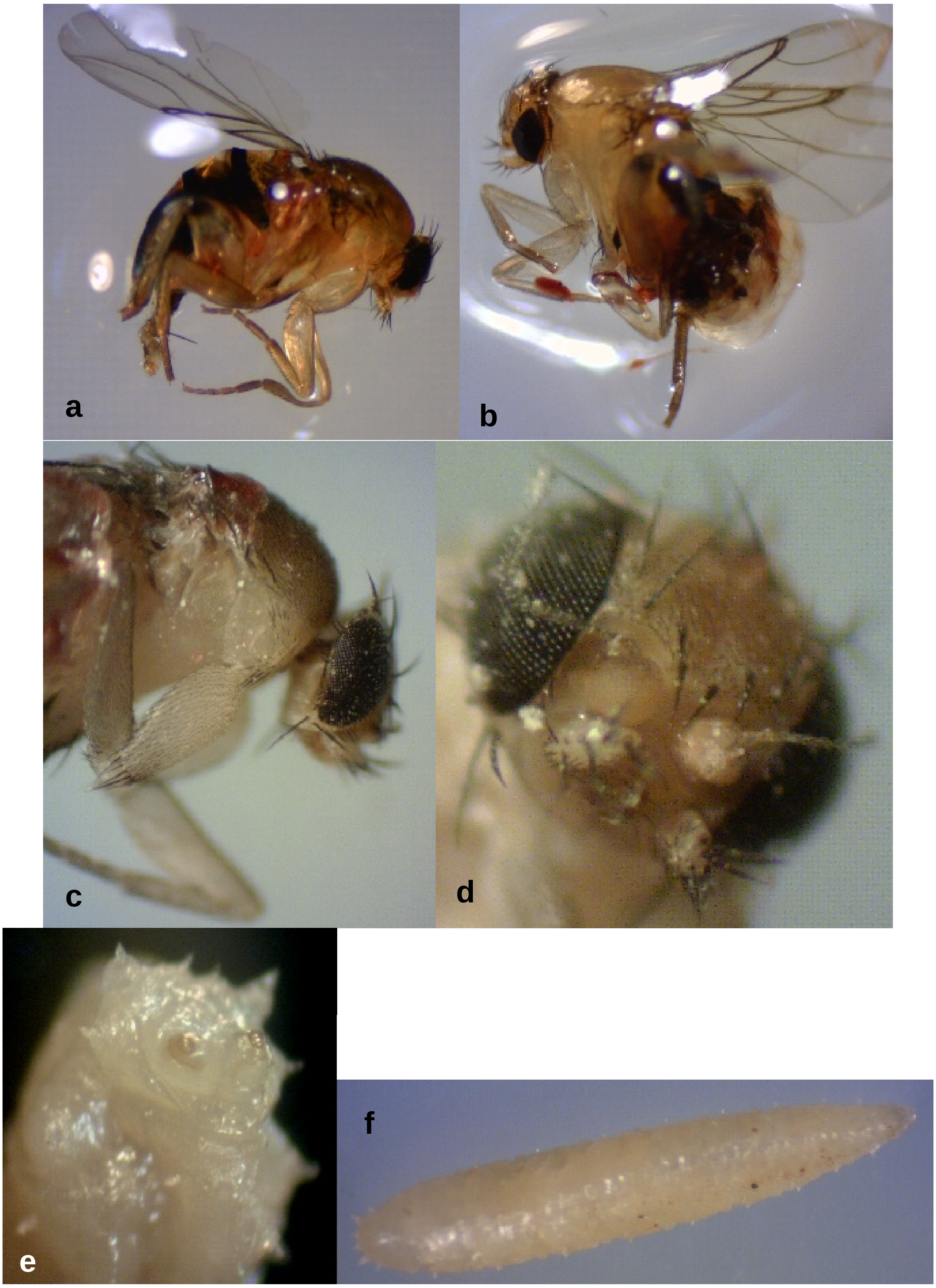


App. B-9: Muscidae; *Stomoxys* sp. (a-c);

Adult female: (a) dorsal view: thorax with pale spot behind head (b) mouth parts functional: proboscis elongate stiff, non-retractile for piercing and blood-sucking, antennae plumose arestate, ptilinal suture clearly defined (c) vein M1+2 only slightly curved forward towards vein R4+5, wing with discal cell, subapical cell and a closed anal cell (short).

App. B-10: Periscelididae; *Periscelis* sp. (a-e):

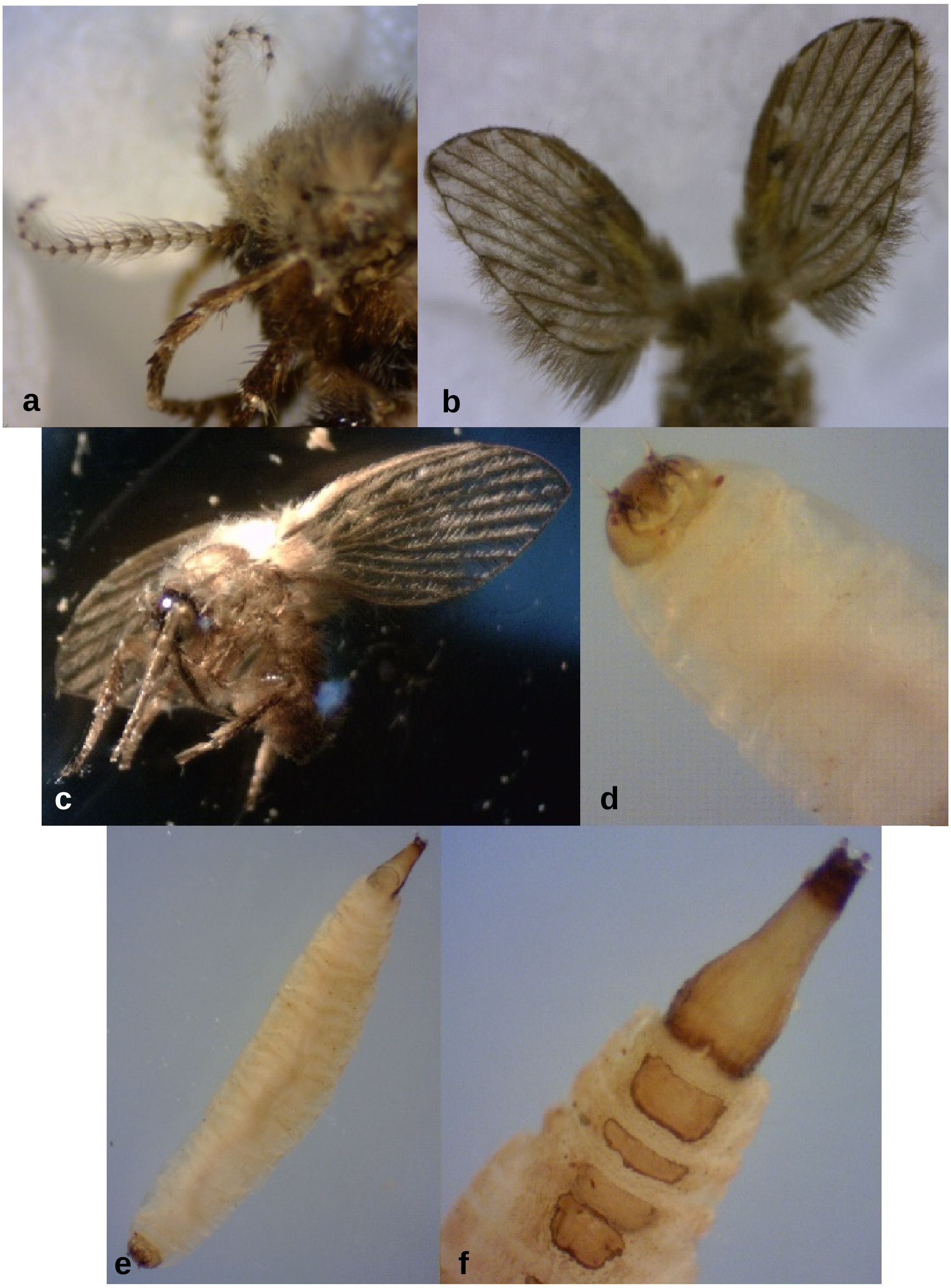
Adult: (a) general appearance (b) lateral view: acalypterate (c) dorsal view: incomplete transverse suture, 3 ocelli present (d) functional mouth parts, ptilinal suture clearly defined (e) lateral view: wings without closed anal cell and discal cell; tibia with dorsal pre-apical bristles.



App. B-11: Phoridae; *Megaselia scalaris* Loew 1866 (Scuttle fly), size 4mm long (a-f):

Adult: (a) dorsal view: abdomen orange-brown with extensive black areas (b) dorsal view: thorax orange-brown, wings without closed anal cell and sub-apical cell, the leading edge veins markedly stronger than the rest (crowded basal, posterior veins fine) without cross veins (c) arched thorax, (d) antennae modified, long aristate, ptilinal sutures weakly defined.

Larva: (e) posterior spiracles on brown, sclerotised tubercles, each with narrow opening (f) lateral view: white color, slightly flattened, body segments with short fleshy processes on the dorsal and lateral surfaces.



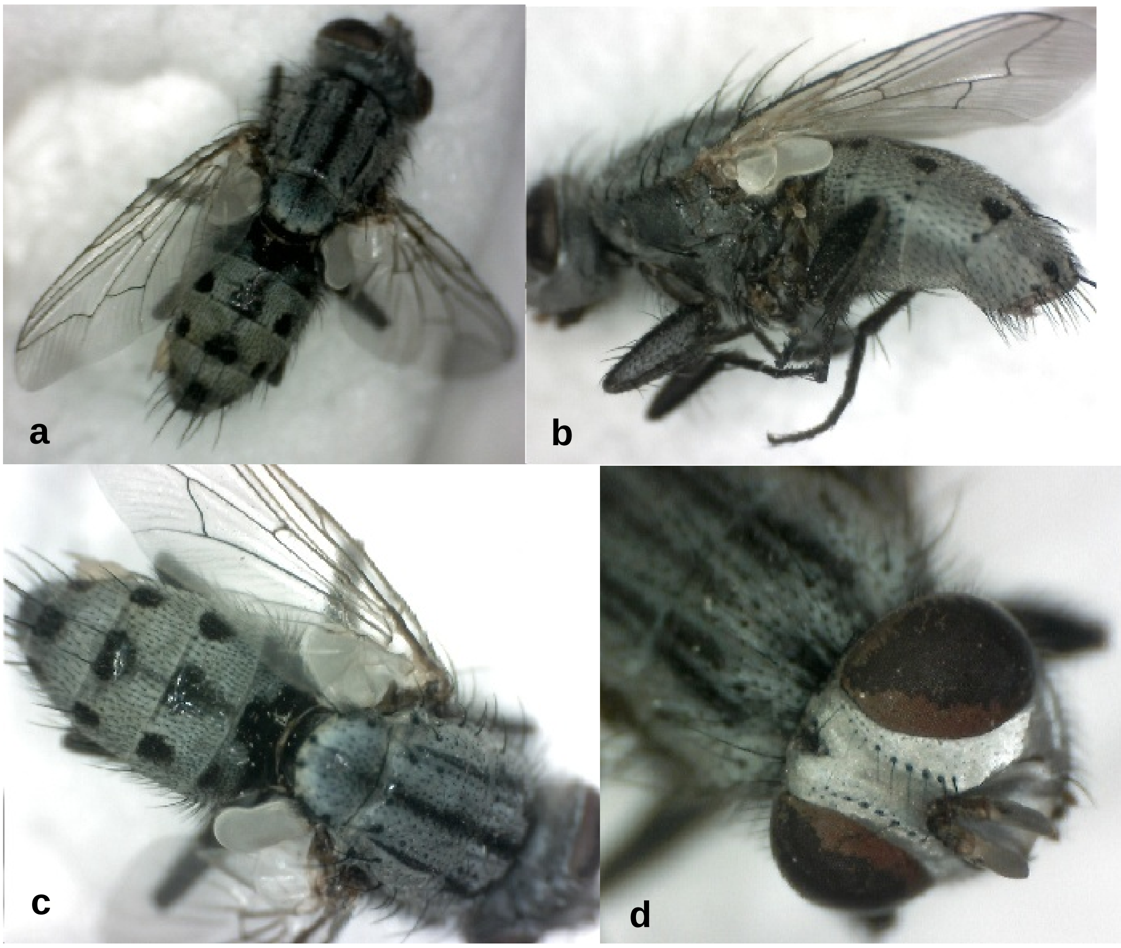
App. B-12: Psychodidae; *Psychoda alternata* Say 1824 (Moth fly), size very small 2 to 3mm (a-f);

Adult: antennae elongated with a whorl of setae (b) wing with costa continued around apex, thickly clad with hairs, dark patches around the wing, with numerous veins, longitudinal veins prominent, without cross veins beyond the basal third, anal vein reduced, wings pointed apically (c) ventral view: body densely setose and wings held tent-like over abdomen, pale yellow abdomen, black eye facing abdomen.

Larvae: (d) grub-like, brown hard head capsule (e) ventral view (f) apical posterior dorsal segment with narrow, sclerotized strap- like transverse bands (on a siphon, short apical tube).

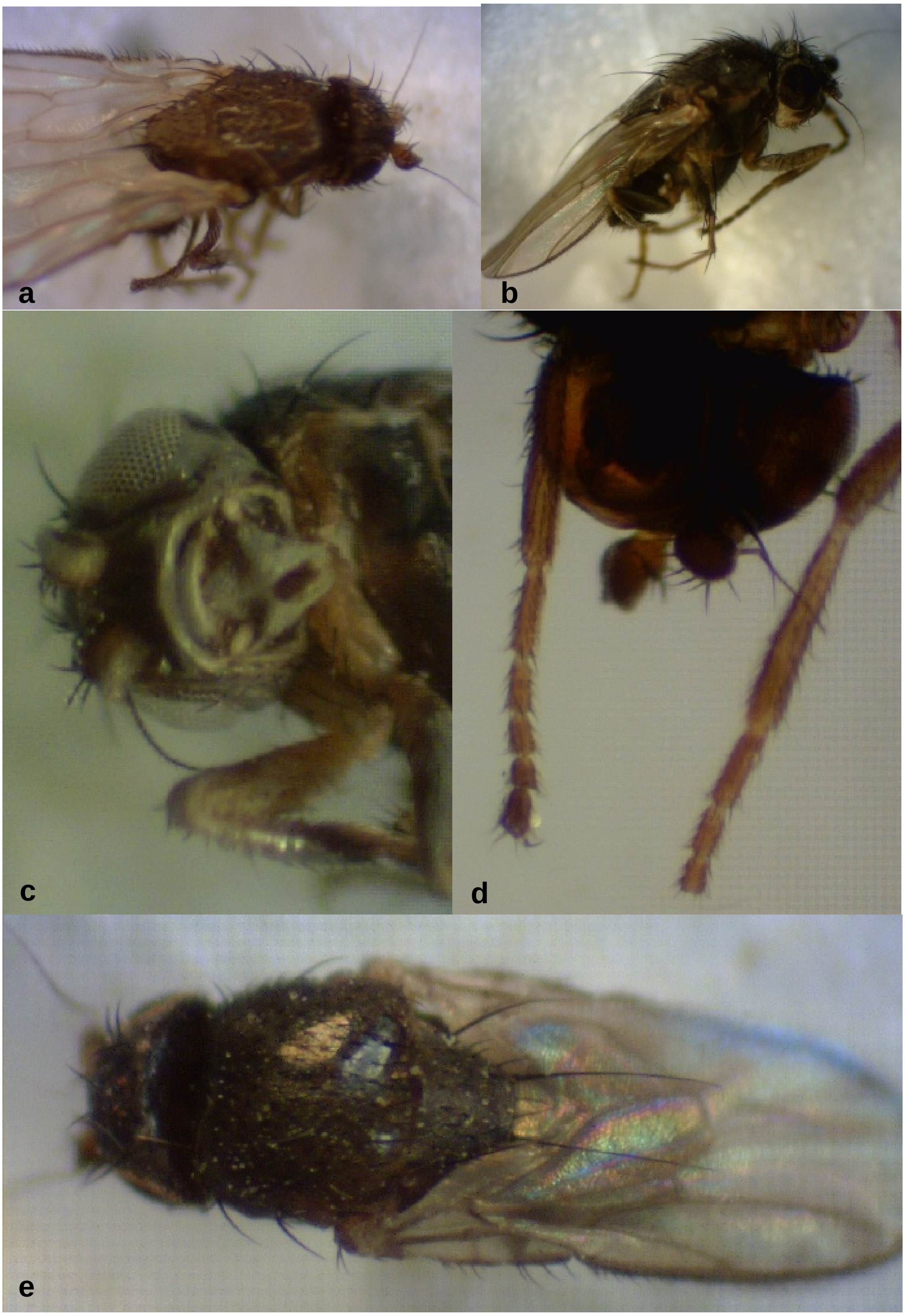
App. B-13: Sarcophagidae; *Sarcophaga* spp. (Flesh fly) (a-l):

Adult: (a) general appearance: abdomen with gray/ black checkerboard (dark and pattern, scutum with 3 conspicuous black stripes on a silver or yellowish-grey (gold) background (b) lateral view: female has red 6th tergite, separated dorsally with row of strong bristles at the edge, wing with sharp curve (angled) 4th vein (c) orange brown epandrium, long bristles on hind margin of protandrial segment (d) aristae with long hairs (e) ***P. ruficornis* (Fabricius 1794)** with red antennae, white hairs on the genae below the eyes (f) ***S. africa* (Wiedemann) = *S. haemorrhoidalis* (Fallen) (Red-tailed flesh fly)**, with black antennae, head with white hairs, dark hairs on genae (g-i) unidentified ***S.* spp.**
Larvae (3rd instar) of Sarcophagida: (j-l) different posterior segments with posterior spiracles, open peritremes (button indistinct), long thin vertical slits, not straight, anterior spiracles in deep cavity (spiracular/stigma cavity).



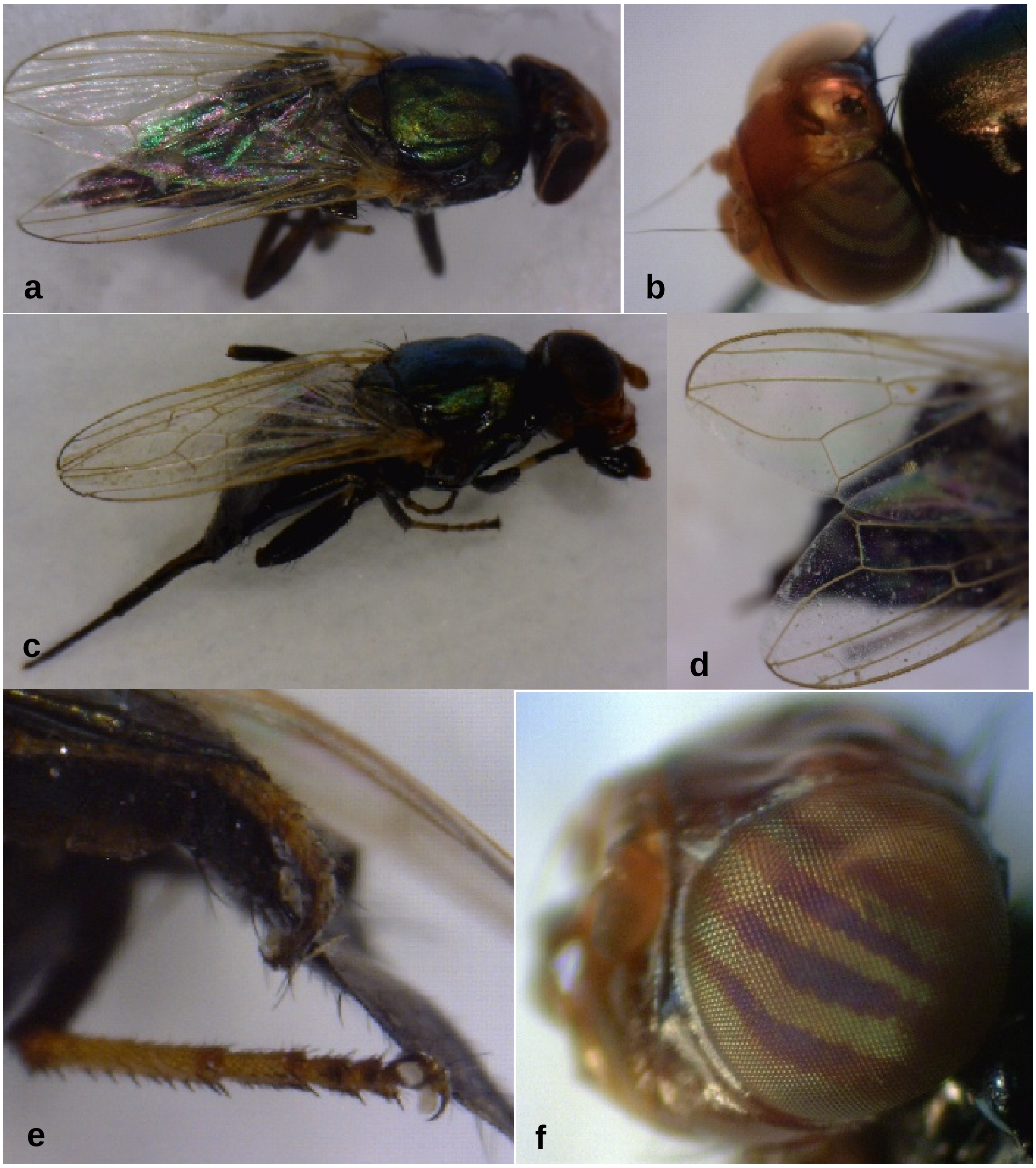
App. B-14: Sarcophagidae; *Wohlfahrtia nuba* (Wiedemann 1830) (Checkerspot fly) (a-d):

Adult female: (a) general appearance: wing venation, grey thorax with 3 black stripes (b) lateral view: calypter (c) dorsal view: gray abdomen with black 3 round spots at the end of each segment (d) head with bare aristae (very short hair), head has less hair than other sarcophagids.



App. B-15: Sphaeroceridae; size less than 4mm long (a-e):

Adult: (a) general appearance showing antennae and aristae (b) lateral view (c) modified antennae, with dorsal aristae, terminal segment not annulated (d) legs (e) dorsal view: thorax transverse suture incomplete, 3 ocelli present.



App. B-16: Ulidiidae; *Physiphora alceae* (Preyssler 1791) (a-f):
Adult: (a) general appearance, dark green thorax with incomplete transverse suture, lower calypters much reduced or absent (b) head with 3 ocelli (c) lateral view: tipped abdomen, functional mouth parts (d) wing with pulvilli claws, with a discal cell, a closed anal cell and a subapical cell, without a "vena spuria", unbroken costa (e) legs: hind tibia without strong bristles in the basal 4/5 (without a dorsal pre-apical bristle) (f) eye with colored striped appearance, modified antennae, vibrissae absent, plinital suture clearly defined

APPENDIX C
Tables

App. C-1: Succession of necrophagous flies on rabbit carcasses in different stages of decomposition

Decomposition Stage / **Family** *Species*	Stage	Fresh				Bloat				Decay				Dry			
		A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D
Calliphoridae *Calliphora vicina*	E							•		•							
	I	•						•		•	•	•	•				
	A							•	•				•				
Calliphoridae *Chrysomya albiceps*	E	•				•	•		•	•			•				
	I	•				•	•	•	•	•	•	•	•		•		
	A	•		•		•	•	•	•	•	•	•	•		•		
Calliphoridae *Chrysomya megacephala*	E																
	I							•				•					
	A									•			•				
Calliphoridae *Lucilia sericata*	E																
	I	•								•			•				
	A	•								•			•				
Carnidae *Hemeromyia* sp.	E																
	I																
	A				•				•				•				
Ephydridae	E																
	I																
	A							•	•		•	•	•				
Fanniidae *Fannia canicularis*	E																
	I																
	A			•						•							
Muscidae *Musca domestica, Musca sorbens, Musca* spp. *Stomoxys* sp.	E																
	I																
	A	•		•		•	•	•	•	•	•	•	•		•		
Periscelididae *Periscelis* sp.	E																
	I																
	A												•				
Phoridae *Megaselia scalaris*	E																
	I																
	A	•						•					•				
Psychodidae *Psychoda alternata*	E																
	I																
	A											•					
Sarcophagidae *P. ruficornis, S. africa, Sarcophaga* spp. *Wohlfahrtia nuba*	E																
	I	•		•		•	•	•	•	•	•	•	•				
	A	•		•		•	•	•	•	•	•		•		•		
Sphaeroceridae	E																
	I																
	A			•				•	•			•	•				
Ulidiidae *Physiphora alceae*	E																
	I																
	A	•				•		•	•	•	•		•				

App. C-2: Rabbit carcasses weights at four habitats in 14 days

Rabbit ID	Site A: Desert, Amghara area (Veterinary Laboratories) Rabbit weight in kg Day 0	Sample day	Difference* kg	%	Stage	Rabbit ID	Site B: Urban, Farwaneya area (MOI: General Department of Criminal Evidence) Rabbit weight in kg Day 0	Sample day	Difference* kg	%	Stage	Sample Day
Aa1	3.26	1.90	1.36	41.72	Adv. decay	Ba1	1.72	0.62	1.10	63.95	Dry	Day 14
Ab2	2.84	2.34	0.50	17.61	Decay	Bb2	2.28	1.28	1.00	43.86	Decay	Day 14
Aa3	2.16	2.12	0.04	1.85	Fresh	Ba3	2.02	1.76	0.26	12.87	Decay	Day 10
Ab4	2.82	2.64	0.18	6.38	Adv. decay	Bb4	2.22	1.86	0.36	16.22	Decay	Day 10
Aa5	1.78	1.28	0.50	28.09	Decay	Ba5	1.98	1.88	0.10	5.05	Sever bloat	Day 7
Ab6	2.00	1.92	0.08	4.00	Sever bloat	Bb6	2.18	1.98	0.20	9.17	Sever bloat	Day 7
Aa7	2.16	2.06	0.10	4.63	Decay	Ba7	2.82	2.72	0.10	3.55	Bloat	Day 4
Ab8	3.46	5.60	-2.14	-61.85	Bloat	Bb8	1.84	1.90	-0.06	-3.26	Bloat	Day 4
Aa9	1.78	1.56	0.22	12.36	Fresh	Ba9	2.22	2.20	0.02	0.90	Fresh	Day 2
Ab10	2.96	2.94	0.02	0.68	Fresh	Bb10	2.10	2.12	-0.02	-0.90	Fresh	Day 2

Rabbit ID	Site C: Agriculture, Sulaibeya area (Cattle Farm) Rabbit weight in kg Day 0	Sample day	Difference* kg	%	Stage	Rabbit ID	Site D: Costal, Abo Al-Hasaneya area (Police officers Club) Rabbit weight in kg Day 0	Sample day	Difference* kg	%	Stage	Sample Day
Ca1	2.42	2.02	0.40	16.53	Adv. decay	Da1	2.40	1.86	0.54	22.50	Decay	Day 14
Cb2	2.68	2.60	0.08	2.99	Bloat	Db2	2.90	2.56	0.34	11.72	Decay	Day 14
Ca3	2.40	2.24	0.16	6.67	Decay	Da3	2.30	2.16	0.14	6.09	Sever bloat	Day 10
Cb4	3.18	3.14	0.04	1.26	Bloat	Db4	2.36	2.14	0.22	9.32	Decay	Day 10
Ca5	2.74	2.72	0.02	0.73	Bloat	Da5	2.24	2.16	0.08	3.57	Bloat	Day 7
Cb6	2.82	2.52	0.30	10.64	Bloat	Db6	2.62	2.56	0.06	2.29	Bloat	Day 7
Ca7	2.50	2.42	0.08	3.20	Fresh	Da7	3.10	2.94	0.16	5.16	Fresh	Day 4
Cb8	3.06	3.06	0.00	0.00	Fresh	Db8	2.14	2.04	0.10	4.67	Fresh	Day 4
Ca9	2.44	2.50	-0.06	-2.46	Fresh	Da9	2.80	2.50	0.30	10.71	Fresh	Day 2
Cb10	2.46	2.44	0.02	0.81	Fresh	Db10	2.62	2.90	-0.28	-10.69	Fresh	Day 2

* Rabbit carcasses weights are standardized to 100% and weight loss given as percentage using the calculation: % = differences in kg x 100 /weight in day 0.

Weights in red color were little increased

App. C-3a: Meteorological data obtained from the nearest meteorological station at site A

Station 40506	Date	Precipitation (mm)	Evaporation (mm)	Maximum temperature (˚C)	Minimum temperature (˚C)	Relative humidity (%)	Wind speed (m/s)	Wind direction (degrees)
Jahra	15/11/2009	0.1		32.8	16.7	89	2.1	214
Jahra	16/11/2009	0		29	17.3	57	0.2	25
Jahra	17/11/2009	0		32.9	17	44	1.6	316
Jahra	18/11/2009	0		24.9	16.8	37	3.5	348
Jahra	19/11/2009	0		24.5	12.3	55	1.5	81
Jahra	20/11/2009	0		23.8	11.3	37	0.8	312
Jahra	21/11/2009	2.1		18	11.6	47	0.7	337
Jahra	22/11/2009	0		21.9	11	82	0.9	350
Jahra	23/11/2009	0		24	10	69	1.4	295
Jahra	24/11/2009	0		22.4	10.2	50	1.5	315
Jahra	25/11/2009	0.7		23.9	12.3	47	1.1	318
Jahra	26/11/2009	2.8		20.2	15.3	88	3.1	74
Jahra	27/11/2009	20.7		17.9	14.3	96	0.6	347
Jahra	28/11/2009	33.6		18	12.9	95	3.2	89
Jahra	29/11/2009	0		19.9	10.9	83	5.2	325
Jahra	30/11/2009	0		20.4	9.8	86	2	324
Jahra	01/12/09	0		20.5	9.6	73	3.9	324
Jahra	02/12/09	0		19.4	9.9	81	4.1	324
Jahra	03/12/09	0		20.2	9.9	73	4.1	323
Jahra	04/12/09	0		19.3	11.4	76	1.5	310
Jahra	05/12/09	0		24.1	14.5	87	0.9	310
Jahra	06/12/09	0.1		26.1	16	90	2.3	150
Jahra	07/12/09	0		22.5	16.2	89	5.3	228
Jahra	08/12/09	0		21.1	14.3	90	4.9	340
Jahra	09/12/09	0		20.1	12.6	84	2.1	322
Jahra	10/12/09	0		19.1	10.7	71	2.4	324
Jahra	11/12/09	2.7		16.9	12.1	73	1.4	280
Jahra	12/12/09	0		20.5	11.5	88	0.6	29
Jahra	13/12/2009	0		21.6	15	85	4.3	96
Jahra	14/12/2009	0		21.5	12.4	81	1.7	23
Jahra	15/12/2009	0		22.6	11.3	83	1.4	322
Jahra	16/12/2009	0		23.4	11	70	1.7	311
Jahra	17/12/2009	15.3		22.2	13.1	76	3.3	208
Jahra	18/12/2009	0		22.4	13.6	84	6.4	192
Jahra	19/12/2009	0		23.3	11.7	78	1.6	268
Jahra	20/12/2009	0		23.8	11.7	76	0.8	302
Jahra	21/12/2009	0		25.3	12.2	73	1.6	168
Jahra	22/12/2009	0		24.9	11.7	74	1.4	263
Jahra	23/12/2009	0.1		23.5	13.4	92	0.8	221
Jahra	24/12/2009	0.1		23.9	14.4	89	0.7	102
Jahra	25/12/2009	0		23	12.6	86	1.5	332
Jahra	26/12/2009	0		22.4	13.3	70	3.4	353
Jahra	27/12/2009	0		23.5	12.3	67	3.4	322

App. C-3b: Meteorological data obtained from the nearest meteorological station at site B

Station 40580	Date	Precipitation (mm)	Evaporation (mm)	Maximum temperature (˚C)	Minimum temperature (˚C)	Relative humidity (%)	Wind speed (m/s)	Wind direction (degrees)
Rabyah	15/11/2009	0	3.4	32.1	16.6	76	2.7	144
Rabyah	16/11/2009	0	2.2	28.5	16.6	82	0.1	13
Rabyah	17/11/2009	0.2	4	32.6	16.8	81	0.6	169
Rabyah	18/11/2009	0	3.8	25.6	17.8	37	3.2	288
Rabyah	19/11/2009	0	2.8	24.7	13.3	59	1.5	28
Rabyah	20/11/2009	0	2.6	23.7	11.4	50	0.4	16
Rabyah	21/11/2009	1	1	19.4	11.3	63	0.7	343
Rabyah	22/11/2009	0	2	21.9	11.3	72	1.5	352
Rabyah	23/11/2009	0	2.2	24	9.1	74	0.4	191
Rabyah	24/11/2009	0	2	22.4	9.3	69	0.9	198
Rabyah	25/11/2009	1	0.8	23	11.4	61	0	95
Rabyah	26/11/2009	4.4	0.2	21.8	16.7	83	1.5	53
Rabyah	27/11/2009	14.6	0.2	18.5	14.7	93	1	337
Rabyah	28/11/2009	37.4	0.2	17.1	13.7	94	2.9	26
Rabyah	29/11/2009	0	1.6	19.7	10.8	76	1.8	262
Rabyah	30/11/2009	0	1.6	19.9	8.4	86	0.8	221
Rabyah	01/12/09	0	1.8	20.7	7.2	81	1.2	240
Rabyah	02/12/09	0	1.6	19.5	9.1	84	1.2	225
Rabyah	03/12/09	0	1.6	20.6	7.7	82	0.6	227
Rabyah	04/12/09	0	1	20	9.2	87	1.2	211
Rabyah	05/12/09	0	1.6	23.7	13.5	89	0.4	156
Rabyah	06/12/09	0	2.2	26.2	17	83	2.1	131
Rabyah	07/12/09	0	1.2	22.6	17.4	83	3	155
Rabyah	08/12/09	0	1.6	20.4	13.2	86	1.9	292
Rabyah	09/12/09	0	1.6	19.9	12.2	89	0.3	214
Rabyah	10/12/09	0	7.8	21.2	9.5	70	0.4	250
Rabyah	11/12/09	0.5	0.6	18.1	11	67	2.3	245
Rabyah	12/12/09	0	0.8	21.6	10.8	93	0.6	356
Rabyah	13/12/2009	1.5	1	21	15.5	81	1	11
Rabyah	14/12/2009	0	1.4	21.1	11.7	82	1.3	327
Rabyah	15/12/2009	0	1.6	22.4	9.2	93	0.4	217
Rabyah	16/12/2009	0	1.6	23.4	8.9	80	0.6	239
Rabyah	17/12/2009	9.4	1.8	22	13.9	62	1.7	146
Rabyah	18/12/2009	0	2	22.2	13.3	67	4.8	136
Rabyah	19/12/2009	0	1.6	23	12.4	65	1.6	225
Rabyah	20/12/2009	0	1.6	23.3	10.1	88	0.5	165
Rabyah	21/12/2009	0	1.6	26.3	10.6	84	0.7	232
Rabyah	22/12/2009	0	1.6	25	10.3	76	0.6	191
Rabyah	23/12/2009	0	0.6	23.6	13.7	87	0.9	171
Rabyah	24/12/2009	0.1	1	22.2	13.1	85	0.6	138
Rabyah	25/12/2009	0	1	22.8	11	95	0.7	172
Rabyah	26/12/2009	0	2	22.7	12	71	1.6	291
Rabyah	27/12/2009	0	1.6	22.9	9.6	74	1.3	232

App. C-3c: Meteorological data obtained from the nearest meteorological station at site C

Station 40587	Date	Precipitation (mm)	Evaporation (mm)	Maximum temperature (˚C)	Minimum temperature (˚C)	Relative humidity (%)	Wind speed (m/s)	Wind direction (degrees)
Sulaibeya	15/11/2009	0	3.8	34.1	15.1	90	3.4	170
Sulaibeya	16/11/2009	0	3.2	29.6	15.6	63	2.7	321
Sulaibeya	17/11/2009	0.1	7.6	33.1	15.9	57	1.3	246
Sulaibeya	18/11/2009	0	5	27.2	16.1	37	5	311
Sulaibeya	19/11/2009	0	3.8	26.6	11.1	62	5.4	28
Sulaibeya	20/11/2009	0	3.8	24.7	9.8	40	2.2	331
Sulaibeya	21/11/2009	1.5	0.8	18.7	9.6	51	2.5	252
Sulaibeya	22/11/2009	0	2.4	22	9.3	88	2.2	331
Sulaibeya	23/11/2009	0	3.6	25.1	8.3	78	2.5	228
Sulaibeya	24/11/2009	0	3.2	22.6	7.4	62	2.2	246
Sulaibeya	25/11/2009	0.6	1.2	23.7	11	57	1.6	253
Sulaibeya	26/11/2009	3.9	0.6	20.6	15.4	89	3.3	18
Sulaibeya	27/11/2009	16.7	0.2	18.2	14.4	97	2.3	327
Sulaibeya	28/11/2009	34.9	0.8	17.6	12.7	92	4.8	5
Sulaibeya	29/11/2009	0	2.6	19.3	10.7	86	6.5	271
Sulaibeya	30/11/2009	0.2	2.8	20	9.6	90	2.9	256
Sulaibeya	01/12/09	0	2.2	20.3	9.4	79	2.5	242
Sulaibeya	02/12/09	0	2.4	19.1	9.1	85	3.6	268
Sulaibeya	03/12/09	0	2	19.8	8.6	77	4.3	270
Sulaibeya	04/12/09	0	1	19.6	10.6	85	2.3	234
Sulaibeya	05/12/09	0.2	1.8	24.5	13.4	87	1.5	296
Sulaibeya	06/12/09	0	2.2	26.2	15.6	93	1.9	115
Sulaibeya	07/12/09	0	1.4	23.1	16.3	89	5.2	164
Sulaibeya	08/12/09	9.9	2.6	21	13.9	91	7.8	279
Sulaibeya	09/12/09	0	2.6	20.1	12.1	89	2.9	269
Sulaibeya	10/12/09	0	1	19.9	10.1	80	2.3	272
Sulaibeya	11/12/09	1.1	0.4	17	11.8	75	1.7	257
Sulaibeya	12/12/09	0	1	21.5	10.5	92	1.4	326
Sulaibeya	13/12/2009	0.2	0	22.5	14	90	2.8	22
Sulaibeya	14/12/2009	0	1.8	22	11.6	88	2.9	287
Sulaibeya	15/12/2009	0	2.2	22.9	10.4	91	3.1	260
Sulaibeya	16/12/2009	0	2	23.9	9.5	78	1.7	238
Sulaibeya	17/12/2009	6.9	2.2	22.7	13.6	79	3.3	153
Sulaibeya	18/12/2009	0	2.4	22.1	13.3	83	6.9	135
Sulaibeya	19/12/2009	0	2	24.1	11.8	76	2.8	217
Sulaibeya	20/12/2009	0	2	24.4	10.7	90	2.1	261
Sulaibeya	21/12/2009	0	2.2	26.7	11.7	78	3.7	246
Sulaibeya	22/12/2009	0	2.2	26	10.6	84	2	263
Sulaibeya	23/12/2009	0	1	24.4	12.5	94	1	148
Sulaibeya	24/12/2009	0.2	1.2	24.1	13.4	95	0.3	25
Sulaibeya	25/12/2009	0	2	23.9	11.3	91	2.4	220
Sulaibeya	26/12/2009	0	3.4	23.1	12.7	75	3.5	296
Sulaibeya	27/12/2009	0	3	23.4	11.6	77	2.5	224

App. C-3d: Meteorological data obtained from the nearest meteorological station at site D

Station 40583	Date	Precipitation (mm)	Evaporation (mm)	Maximum temperature (˚C)	Minimum temperature (˚C)	Relative humidity (%)	Wind speed (m/s)	Wind direction (degrees)
Ahmadi port	15/11/2009	0		26.2	22.7	77	4.7	175
Ahmadi port	16/11/2009	0		26.1	20.6	64	3.4	265
Ahmadi port	17/11/2009	0		28.6	22.3	73	3.8	175
Ahmadi port	18/11/2009	0		24.7	19.3	34	8.9	308
Ahmadi port	19/11/2009	0		23.2	19.1	49	4.6	3
Ahmadi port	20/11/2009	0		22.7	17.2	32	5.1	7
Ahmadi port	21/11/2009	0		21	16.9	44	4.4	310
Ahmadi port	22/11/2009	0		20.8	14.4	68	7.5	360
Ahmadi port	23/11/2009	0		22.6	14.8	46	3.6	311
Ahmadi port	24/11/2009	0		20.8	15.8	47	4.3	316
Ahmadi port	25/11/2009	0.2		23.7	17.7	45	2.8	307
Ahmadi port	26/11/2009	4.2		23.4	19.5	68	4.7	77
Ahmadi port	27/11/2009	7		19.9	16.4	86	4.9	357
Ahmadi port	28/11/2009	29.9		18	14.4	82	9.2	355
Ahmadi port	29/11/2009	0		19.5	12.9	73	6.5	293
Ahmadi port	30/11/2009	0		19.2	12.2	65	4.4	291
Ahmadi port	01/12/09	0		18.9	12.8	52		
Ahmadi port	02/12/09	0		18.6	13.1	63		
Ahmadi port	03/12/09	0		18.8	12.9	58		
Ahmadi port	04/12/09	0		20.8	15	57	1.9	283
Ahmadi port	05/12/09	0		21.5	16.6	59	1.3	149
Ahmadi port	06/12/09	0		21.7	18.6	87	2.5	156
Ahmadi port	07/12/09	0		22.2	17.8	84	6.1	178
Ahmadi port	08/12/09	0		20.4	14.9	84	4.9	293
Ahmadi port	09/12/09	0		19.3	15.3	75	1.9	259
Ahmadi port	10/12/09	0		17.3	14.1	62	4.1	299
Ahmadi port	11/12/09	0		17.4	14.5	65	3	318
Ahmadi port	12/12/09	2.6		21.1	16.8	75	4.3	25
Ahmadi port	13/12/2009	17		20.7	16.5	81	2.3	67
Ahmadi port	14/12/2009	0		19.4	15.1	81	3.2	292
Ahmadi port	15/12/2009	0		20	15.1	72	2.9	308
Ahmadi port	16/12/2009	0		20.5	13.7	61	3	245
Ahmadi port	17/12/2009	0		21	15.8	68	4.4	174
Ahmadi port	18/12/2009	0		21.9	16.7	76	5.3	164
Ahmadi port	19/12/2009	0		20.7	14.4	56	3.1	265
Ahmadi port	20/12/2009	0		22.5	14.4	60	1.5	288
Ahmadi port	21/12/2009	0		24.3	15.4	58	1.5	253
Ahmadi port	22/12/2009	0		22.5	16.2	59	1.3	159
Ahmadi port	23/12/2009	0		20.5	18.9	82	3.8	139
Ahmadi port	24/12/2009	0.1		20.9	16.5	64	4.1	171
Ahmadi port	25/12/2009	0		22	15.8	71	3.3	305
Ahmadi port	26/12/2009	0		22	15.2	64	5.7	296
Ahmadi port	27/12/2009	0		22.1	13.7	54	5	293

App. C-4a: Datalogger readings at site A

Date Time, Celsius (°C), Humidity (%rh), Dew point (°C), Serial Number

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App. C-4b: Datalogger readings at site B

Date Time, Celsius (°C), Humidity (%rh), Dew point (°C), Serial Number

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App. C-4c: Datalogger readings at site C

Date Time, Celsius (°C), Humidity (%rh), Dew point (°C), Serial Number

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312,23/12/2009 07:56:29,15.0,92.5,13.8
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App. C-4d: Datalogger readings at site D,
Date Time, Celsius (°C), Humidity (%rh), Dew point (°C), Serial Number

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307,24/12/2009 03:49:14,15.5,90.5,14.0
308,24/12/2009 04:49:14,15.5,90.0,13.9
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331,25/12/2009 03:49:14,13.5,94.0,12.6
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336,25/12/2009 08:49:14,17.0,84.5,14.4
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App. C-4e: Datalogger readings at open,
Date Time, Celsius (°C), Humidity (%rh), Dew point (°C), Serial Number

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APPENDIX D

App. D-1: Article acceptance letter: Al-Mesbah H, Al-Osaimi Z, El-Azazy OME (in press) Forensic entomology in Kuwait, the first case report. Forensic Sci Int. (accepted in July 2010).

App. D-2: Curriculum vitae

App. D-3: DVD: A study of forensically important necrophagous Diptera in Kuwait, Produced in 30 December 2009

App. D-1: ARTICLE ACCEEPTANCE LETTER

> From: fs.international@elsevier.com
> To: El_Azazy@hotmail.com
> Date: Wed, 7 Jul 2010 13:23:26 +0100
> Subject: Provisional Accept - FSI-D-09-00519R2
>
> Ref.: Ms. No. FSI-D-09-00519R2
> Forensic Entomology in Kuwait: the first case report
> Forensic Science International
>
> Dear Professor El-Azazy
>
> I am pleased to tell you that your work has now been preliminarily accepted for publication in Forensic Science International.
>
> Your paper has been forwarded to our language Editor for final review before being submitted for typesetting.
>
> Comments from the Editor and Reviewers can be found below.
>
> Thank you for submitting your work to this journal.
>
> With kind regards,
>
> Martin Hall
>
> Martin J.R. Hall, PhD
> Associate Editor
> Forensic Science International
>
> Comments from the Editors and Reviewers:
>
> Reviewer #1: I am very satisfied with the corrections the author/s have made to the manuscript. The language is much improved from the original submission, although I would still recommend it be sent to a language editor for review. The science within the manuscript is very sound and I'm happy for this to be accepted for publication.
>
> ***
> For further assistance, please visit our customer support site at http://epsupport.elsevier.com. Here you can search for solutions on a range of topics. You will also find our 24/7 support contact details should you need any further assistance from one of our customer support representatives.
>

App. D-2: CURRICULUM VITAE

PERSONAL DATA

Name: Hanadi A. J. M. AL-Misbah

Birth: 1967

Nationality: Kuwaiti

CONTACT INFORMATION

Address: P.O.Box: 1069 Al-Surra, Zip code: 475111

E-mail: tulip2lily@hotmail.com

Fax: 965-25333543, Phone (Mobile): 965-66640286

HIGHER EDUCATION

1993 Kuwait University - BSc (Major: Botany, Minor: Microbiology).

EMPLOYMENT HISTORY

1993-2000 General Department of Criminal Evidence (GDCE), Department of Forensic Laboratories, Section of Drugs and Psychotropic Substances - Chemical Analyst.

2000-2003 (GDCE), Research, Development and Training Unit - Forensic Chemist and the Director of the Library of (GDCE).

2003-present (GDCE), Research, Development and Training Section, Forensic Studies and Research Division - Senior Criminal Laboratory Specialist and the Head of the Forensic Studies and Research Division.

PRESENTATIONS AND LECTURES

December 2008 Presented a lecture about "applications of forensic entomology and their importance as evidence" to the Undersecretary of the Interior Ministry.

November 2009 Presented a lecture on "forensic entomology in Kuwait: applications and accomplishments" in the symposium held under the auspices of Naif Arab University for Security Sciences and the Federation of forensic Syrian Arab Association of Forensic Medicine, Tartous, Syrian Arab Republic.

December 2009 Presented a lecture titled "an introduction to forensic entomology" during the cultural season in the Public Authority for Applied Education and Training.

December 2009 Presented a lecture on "applications of forensic entomology in forensic medicine" during a training course about forensic medicine.

May 2010 Presented a series of theoretical and practical lectures on "essentials and applications of forensic entomology" during a training course for crime scene investigators and crime scene laboratory technicians.

ARTICLES AND POSTERS

Al-Mesbah H, Al-Osaimi Z, El-Azazy OME (in press) Forensic entomology in Kuwait, the first case report. Forensic Sci Int. (Accepted in July 2010).

Al-Mesbah H, Moffat C, El-Azazy OME, Al-Osaimi Z (June 2009) Forensic entomology in Kuwait. Poster presented at European Association of Forensic Entomology EAFE meeting, Uppsala, Sweden.

Al-Mesbah H, Moffat C, El-Azazy OME, Al-Osaimi Z (July 2009) Forensic entomology in Kuwait. Poster presented at North America Forensic Entomology Association NAFEA meeting, Miami, USA.

CONFERENCES AND WORKSHOPS

December 1998 Attended the 8th Meeting on Psychotropic Substances, Precursors & Essential Chemicals, Pretoria, South Africa

June 2009 Attended the conference of the European Association of Forensic Entomology EAFE, Uppsala, Sweden

July 2009 Attended the conference of the North America Forensic Entomology Association NAFEA, Miami, USA.

November 2009 Attended the symposium held under the auspices of Naif Arab University for Security Sciences and the Federation of forensic Syrian Arab Association of Forensic Medicine, Tartous, Syrian Arab Republic.

RESEARCH INTERESTS

- Forensic entomology.
- Forensic botany.
- Forensic microbiology.

TRAIINIG

December 2008 Attended a lecture on forensic entomology presented in Kuwait (GDCE) by Prof. Ian Dadour, University of Western Australia.

May 2009 Attended a theoretical and practical intensive two-week training course in forensic entomology in the British Natural History Museum (NHM).

Attended and completed many local training courses, lectures, seminars on variety skills including information technology skills and soft-skills besides different theoretical and practical skills in forensic sciences.

SKILLS

Computer skills: Microsoft word, powerpoint, excel, access, adobe photoshop, database management systems for organizing resources in libraries and taxonomic applications for identifying & classifying insects such as: intkey and Luicid.

Soft-skills: Leadership, decision making, planning, team building, team management, meeting management, motivating, delegation, presenting.

Language skills: Arabic & English.

PROFESSIONAL ACTIVITES IN (GDCE)

2001 Member of the committee of the officers jurisdiction program in the (GDCE).

2003 Member of the (GDCE) administrative team for researching and identifying prisoners (POWs) and missing persons.

2005 Member of the technical committee for the planning for the new building of the (GDCE).

2005 Member of the committee of the health and safety officers.

2007 Member of the committee for preparing training course manuals.

2008-present Member of the technical committee for supervising and applying of forensic entomology in Kuwait.

OTHER EXPERIENCES AND ACTIVITIES

1996-2000 Trained students of the Public Authority for Applied Education and Training in drug laboratory, (GDCE).

1999 Participated in opium program and was nominated to represent the State of Kuwait (GDCE) according to the recommendation of the meeting of interior ministers of the Gulf Cooperation Council of Drugs.

2001-present Coordinated and supervised local training courses in (GDCE).

2003 Contributed in the development programs of chemistry and applied physics in Centre for Development of Programs and Curricula, General Authority for Applied Education and Training.

2003 Developed foundations and protocol of scientific research in (GDCE).

2005-2006 Developed foundations and procedures of the training programs in (GDCE).

2008-present Contributed with the colleague Mr. Zarraq A. Al-Osaimi in preparing and designing many posters, brochures and handouts about forensic entomology, besides a guidance booklet about dealing with entomological evidence in Arabic language (in press).

2010 Appeared in an interview with Kuwait TV about forensic entomology and its applications in (GDCE).

2010-present Contributed with the colleague Mr. Zarraq A. Al-Osaimi in preparing and building a website (CSIArabia.net) and a blog (ForensicArthropodology.com) about the forensic sciences including forensic entomology.

App. D-3: DVD

A study of forensically important necrophagous Diptera in Kuwait, Produced in 30 December 2009 (attached).

Researcher: Hanadi A. Al-Mesbah

Supervisor: Dr. Colin Moffatt, director of the study

Dr. Osama M. El-Azazy, second supervisor

Photography, video recording & video editing: Zarraq A. Al-Osaimi

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