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SEASONAL CARRION DIPTERA AND COLEOPTERA COMMUNITIES FROM LISBON (PORTUGAL) AND THE UTILITY OF FORENSIC ENTOMOLOGY IN LEGAL MEDICINE

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Nota Prévia

Na elaboração da presente dissertação, e nos termos do nº 1 do Artigo 40, Capítulo V, do Regulamento de Estudos Pós-Graduados da Universidade de Lisboa, publicado no *Diário da República – II Série Nº 153*, de 5 de Julho de 2003, esclarece-se que foram integrados 5 artigos científicos já publicados em revistas internacionais com arbitragem, não indexadas, 2 artigos submetidos em revistas internacionais indexadas, bem como 1 manuscrito ainda em fase de preparação, os quais integram os capítulos da presente tese. Tendo os referidos trabalhos sido realizados em colaboração com outros investigadores, a candidata esclarece que participou integralmente no planeamento, na recolha, análise e discussão dos resultados e na elaboração de todos os trabalhos apresentados.

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CONTENTS

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RESUMO

A Entomologia forense baseia-se na sequência de aparição de insectos no cadáver e também no reconhecimento do grau de desenvolvimento dos estádios imaturos das diferentes espécies. Os insectos são usados em investigações forenses, especialmente para estimar o tempo decorrido após a morte ou intervalo *postmortem* (IPM). A vantagem deste método, em comparação com outros procedimentos usados em Medicina Legal é a sua precisão, mesmo em estados avançados de decomposição (depois de quatro a cinco dias *postmortem*), onde os métodos tradicionais falham.

Logo que um animal (ser humano incluído) morre, uma grande diversidade de dípteros são imediatamente atraídos para o corpo, através de odores específicos. A postura de ovos (oviposição) no cadáver marca o início de um relógio biológico que é usado por entomólogos forenses para estimar o IPM. Dois métodos podem ser utilizados: 1) a estimativa da idade dos insectos imaturos que se alimentam do cadáver, de acordo com seu grau de desenvolvimento, e 2) a análise da comunidade de insectos presentes. O segundo método requer uma base de dados precisa da comunidade de insectos específica do local e um bom conhecimento dos padrões de sucessão, baseados em estudos experimentais regionais, com carcaças de porcos.

As espécies de insectos e o seu padrão de colonização de cadáveres variam de acordo com vários factores, um dos mais importantes sendo a região geográfica ou zona biogeoclimática. Esta, define o habitat, vegetação, tipo de solo e condições meteorológicas da área que, obviamente, têm um enorme impacto nos tipo e espécies de insectos presentes, assim como na sua disponibilidade sazonal. Por este motivo, devem ser elaboradas bases de dados para cada região geográfica em que os insectos são usados em Entomologia forense.

A tese de doutoramento aqui apresentada baseia-se no estudo da fauna entomológica associada a cadáveres (porcos), com o objectivo de caracterizar a comunidade de dípteros e coleópteros e o seu padrão de sucessão nas diferentes estações do ano, em Lisboa.

O **capítulo 1** consiste numa introdução geral sobre vários aspectos relacionados com a Entomologia forense, nomeadamente a decomposição de cadáveres, a forma como

os insectos se relacionam com este recurso e a determinação do IPM. É feita uma breve retrospectiva histórica sobre esta ciência e o seu panorama actual, em diferentes linhas de investigação. São delineados os objectivos da presente tese e a metodologia utilizada.

No **capítulo 2** é descrita a metodologia utilizada neste trabalho. A armadilha usada para a captura dos insectos associados a cadáveres é baseada num modelo que foi previamente descrito; o facto de se terem introduzido diversas modificações e a simplificação da descrição da sua construção, levou a considerar-se relevante a elaboração do artigo apresentado.

O **capítulo 3** centra-se no estudo da composição específica, comunidade e padrão de sucessão dos dípteros capturados nos cadáveres animais. São focadas as diferenças no processo de decomposição, consoante as condições climáticas (estações do ano), a sazonalidade das espécies e a sua associação aos diferentes estádios de decomposição.

O conhecimento sobre dípteros, em particular dípteros associados a cadáveres, é muito escasso em Portugal. O trabalho realizado revelou-se extraordinariamente interessante a nível faunístico, pela quantidade de novas espécies para Portugal e inclusivamente para a Península Ibérica. No **capítulo 4** são registadas as novidades faunísticas relativas à família Calliphoridae (Diptera), o grupo de insectos de maior importância forense. Para cada espécie, é feita uma revisão da ecologia e distribuição geográfica. O **capítulo 5** diz respeito a uma outra espécie de Calliphoridae - *Chrysomya megacephala* (Fabricius, 1794), também registada pela primeira vez para Portugal. Enquanto que para as outras espécies da mesma família (capítulo 4), a sua presença no nosso território seria expectável, e em alguns casos óbvia (embora não pudesse ser apoiada com qualquer registo bibliográfico), o caso da *C. megacephala* é diferente, tratando-se de uma introdução recente na nossa fauna. É de prever que esta espécie, quando bem estabelecida, venha a ter um papel relevante a nível forense, dada a sua capacidade de expansão e carácter dominante, descrito noutras regiões geográficas.

No **capítulo 6** são descritas as novidades para Portugal, relativas à família Piophilidae (Diptera). Estes dípteros estão associados a fases tardias da decomposição, sendo ainda relativamente pouco estudados.

Durante o doutoramento, para além dos estudos de campo, com modelos animais, foi efectuada a análise de insectos recolhidos de cadáveres humanos, provenientes de

diversos casos forenses do Instituto Nacional de Medicina Legal. A presença da espécie *Piophila megastigmata* McAlpine, 1978 (Piophilidae), em dois desses casos marca o primeiro registo deste díptero em cadáveres humanos, a nível mundial. É este o tópico do **capítulo 7**.

O **capítulo 8** centra-se no estudo da composição específica, comunidade e padrão de sucessão dos coleópteros capturados nos cadáveres animais. A abordagem ao estudo dos coleópteros é similar à feita no capítulo 3, para os dípteros.

O **capítulo 9**, para além de registar 5 novas espécies de Staphylinidae (Coleoptera) para Portugal, providencia a listagem de espécies recolhidas desta família, que foi a mais abundante e biodiversa, de entre a ordem Coleoptera, no ecossistema cadavérico.

O **capítulo 10** apresenta uma discussão geral, integrando os resultados obtidos ao longo dos estudos referidos, terminando com sugestões para análises futuras, de forma a aprofundar o conhecimento nas diversas áreas abordadas, assim como em novas linhas de investigação.

Para além dos interessantes resultados faunísticos obtidos, neste trabalho, foram claramente identificadas as espécies de maior interesse forense na região estudada. No caso dos dípteros, foram espécies que usaram o cadáver para o seu desenvolvimento larvar, tratando-se portanto de espécies necrófagas; a maioria dos coleópteros comportaram-se como predadores – este comportamento justifica de forma geral a maior abundância e riqueza específica de dípteros em fases iniciais da decomposição e de coleópteros em fases mais avançadas. Nos dois grupos verificou-se uma alternância sazonal das espécies e respectivas abundâncias. Foi possível demonstrar, tanto nos dípteros como nos coleópteros, que as comunidades presentes nos cadáveres são claramente diferentes nos diferentes estádios de decomposição. Um aspecto que é discutido relativamente à divisão da decomposição em estádios, é que esta é uma divisão artificial, sendo a decomposição um processo contínuo; é portanto interessante que se tenha verificado que as comunidades destes insectos também "separem" os diferentes estádios de decomposição, para além da diferenciação feita visualmente, com base no aspecto do cadáver.

Os resultados obtidos, naturalmente revelam diferenças na fauna sarcosaprófaga, relativamente a outras regiões geográficas. Inclusivamente essas diferenças são notórias mesmo dentro da Península Ibérica; no caso dos dípteros, embora a composição específica das comunidades não seja muito diferente de outros locais, espécies muito relevantes em Lisboa, como *Lucilia caesar* (Linnaeus, 1758), *Lucilia ampullacea* Villeneuve, 1922 e *Hydrotaea ignava* (Harris, 1758) não o são, nomeadamente, na maior parte do território espanhol; relativamente aos coleópteros, à excepção de algumas espécies cosmopolitas, em particular as espécies das famílias Staphylinidae e Histeridae demonstraram ser de carácter geograficamente delimitado, sustentando a perspectiva de que estudos regionais são fundamentais nesta área do conhecimento.

Com este trabalho, através do estudo da comunidade de insectos, da sua sazonalidade e sucessão na zona de Lisboa, através da identificação de espécies indicadoras forenses, associadas aos diferente estádios de decomposição de um cadáver, pretendemos contribuir para a obtenção de informação biológica na qual o desenvolvimento sustentado da Entomologia forense se deve basear.

Palavras-chave: Diptera, Coleoptera, sucessão, entomologia forense, Portugal

ABSTRACT

The analysis of the community of insects present on a decomposing body can often provide valuable forensic insights, especially the estimation of the time of death, or *postmortem* interval (PMI). Due to the geographical variation of the communities of insects, Forensic Entomology is a locality-specific science; composition of local carrion fauna, its seasonal variations and patterns of succession, as well as the periods of time each life stage spends on a cadaver, comprise crucial information to be used in forensic cases of a particular region. In order to collect this information for Lisbon region, decomposition studies conducted with piglet carcasses were used to determine Diptera and Coleoptera activity during the four seasons of the year. A modified Schoenly trap was used to collect the entomofauna. In all seasons, Calliphoridae (Diptera) was the dominant family. *Calliphora vicina*, *C. vomitoria*, *Chrysomya albiceps*, *Lucilia ampullacea*, *L. caesar* and *L. sericata* were fundamental members of the community, voraciously consuming the cadaver in the initial stages of decomposition. *Hydrotaea ignava*, *Muscina prolapsa, Synthesiomyia nudiseta* (Muscidae), *Piophila megastigmata, Stearibia nigriceps* (Piophilidae) and *Nemopoda nitidula* (Sepsidae) were also relevant among the community. Coleoptera species were generally more associated to advanced decomposition stages and mostly acting as predators. *Anotylus complanatus*, *Atheta pertyi*, *Creophilus maxillosus*, *Oligota pusillima*, *Philonthus varians* (Staphylinidae), *Margarinotus brunneus*, *Saprinus* spp. (Histeridae), *Thanatophilus* spp. (Silphidae), *Necrobia* spp. (Cleridae) and *Dermestes* spp. (Dermestidae) were important members of the community as well. The present results highlight the particularities of our local fauna and its dynamics in the cadaver, compared with other regions and the need to have a deeper knowledge of regional carrion insects, in order to offer a committed contribute to forensic investigations. This work also marks the first record of more than 50 Diptera and 7 Coleoptera species in Portugal, an important contribution for the knowledge of this fauna in the country.

Keywords: Diptera, Coleoptera, succession, Forensic Entomology, Portugal

Chapter 1

General Introduction

GENERAL INTRODUCTION

1.1 - Insects, decomposition and Forensic Entomology

Three quarters of the animal species known today are insects; with this numeric importance, their role in the ecosystem functioning largely goes beyond the contribution of vertebrates (Wyss & Cherix, 2006). Among several functions that insects play in the natural environment, one of the most unnoticed, despite its importance is that of being recyclers of organic matter, consuming and decomposing both excrement and remains of vegetal or animal origin (González Peña, 1997). A dead animal is a temporary and changing food resource for a variety of organisms, from the microorganisms to vertebrate scavengers. Among the animal kingdom, the insects are the predominant group of this community attracted to the cadaver, both in terms of diversity and number of individuals present. They are the primary element involved in the decomposition process (Goff, 1993; Richards & Goff, 1997). Decomposition can be defined as the breakdown of organic matter accompanied by the release of carbon dioxide (Molles, 1999) and is one of the most important processes in ecosystem running. Insects play a very important role in organic matter recycling including cadavers and faeces. It is estimated that Diptera and Coleoptera account for 60% of the fauna associated with decomposing carcasses and, because of that, they are indeed very important in forensic science (Moretti *et al.*, 2008). As an example, a human cadaver exposed outdoors can house more than 2,5 million larvae of necrophagous flies during summer (Wyss & Cherix, 2006). Diptera, in particular, play an enormous role in the breakdown of dead organic matter and in nutrient cycling, thereby being central to the functioning of most ecosystems. The other large order of insects that includes many saprophagous species is the Coleoptera, but in most ecosystems Diptera are richer in species and have a higher biomass than other insect decomposers (McLean, 2000).

The decay of terrestrial vertebrates, dominated by the action of sarcosaprophagous insects is, in the case of human corpses, usually inhibited by hygienic measures such as burying or burning. Nevertheless, there are circumstances related to crimes or sudden deaths that may expose human bodies to the natural effects of the environment, including invasion by insects. Forensic Entomology is based on the sequence in which insects appear on the cadavers and also on the recognition of the developmental stage of the offspring of the different species (Nuorteva, 1977).

The term Forensic Entomology is generally used referring to Medicolegal Entomology; however its definition is, in fact, more comprehensive. Forensic Entomology is the broad field where arthropods, science and the judicial system interact (Hall & Huntington, 2010). Lord and Stevenson (1986) subdivided it into three areas: 1) Urban Entomology, which includes the legal proceedings involving the arthropods that affect diverse aspects of human environment and man-made structures (e.g. controversies involving termites, cockroaches, pest insects); 2) Stored products Entomology, that involves disputes over arthropods found infesting the food (e.g. plagues from stored products, fly maggots in sandwiches from restaurants), wood, tissues...; 3) Medicolegal Entomology, that uses the arthropod evidence in solving crimes, primarily to estimate the postmortem interval (PMI, i.e. the time since death). Forensic significant conclusions can often be achieved trough the examination and identification of arthropods collected from or near corpses, noting the state of successive colonization of a corpse by local arthropod fauna or by identifying the developmental stage of the insects collected (Hall, 1990).

The sequence of Diptera, other invertebrates and microorganisms colonizing and modifying decaying organic material, generates, via their feeding activities a great diversity of conditions over time. This represents many niches and opportunities for numerous species (McLean, 2000). In a decaying corpse, many species colonize it only during a limited period of time, arriving in a more or less predictable sequence, representing an ecological succession (Payne, 1965; Rodriguez & Bass, 1983; Smith 1986). The knowledge of the insect sequence of colonization for a given area allows, analyzing the arthropod fauna found in a human victim from a forensic case, the estimation of the elapsed time since death (Smith, 1986; VanLaerhoven & Anderson, 1999; Anderson, 2010). The advantage of this method of estimating the PMI, compared to other standard procedures in Forensic Medicine is its accuracy even in advanced stages of decomposition (after four to five days postmortem), where the traditional methods fail (Anderson & VanLaerhoven, 1996; Benecke, 2004).

1.2 - The postmortem interval

Estimating the PMI involves the setting of a minimal and maximal probable time interval between death and corpse discovery (Catts, 1990). Two methods for estimating postmortem intervals using insect evidence are used:

The first one, useful mainly during the earlier stages of decomposition, uses the information about the estimated age of immature insects that had fed on the body, usually, but not only, Diptera from the families Calliphoridae or Sarcophagidae. Since they are commonly the first insects to lay eggs on a body, even within minutes after death, the estimation of the age of the oldest present eggs, larvae or pupae will provide a minimum PMI (Catts, 1990; Catts & Goff, 1992; Goff, 1993; Anderson, 1995; VanLaerhoven & Anderson, 1999). Usually adult females do not deposit their eggs on a live host (Wells & LaMotte, 2010), so it can be assumed that a certain person died at least at the calculated period of time necessary to reach the developmental stage of the oldest insects studied. This approach does not estimate the maximum PMI, because an unknown period of time may elapse between death and the deposition of eggs or larvae (Wells & LaMotte, 2010). Depending on the insect species and conditions at the scene, the degree of development can indicate a PMI from less than 1 day to more than 1 month (Smith, 1986; Wells & LaMotte, 2010). Generally, previously existing laboratory experimental data of insect development are consulted in order to estimate PMI with this method (Wells & LaMotte, 2010).

The second method is based in the succession of arthropod species found in a body (Catts & Goff, 1992; Wells & LaMotte, 2010) and in the fact that a corpse is a rapidly changing ecosystem that in each stage of decomposition is attractive to different arthropod species (Anderson, 1995, 2010). The analysis of the composition of arthropod community provides information about the time elapsed between death related to the appearance of a particular arthropod species and stage. Therefore, it can be used to estimate both the minimum and maximum PMI (Wells & LaMotte, 2010). This method requires an accurate database of insect succession (VanLaerhoven & Anderson, 1999; Anderson, 2010), and is valuable for PMI calculations from a few weeks to a year, or in some cases several years after death (Anderson, 1995). Succession studies, usually made with animal models (nonhuman carcasses, such as pigs), provide the reference data used to compare with the taxa collected on human remains (Wells & LaMotte, 2010).

PMI estimation is the most important and recurrent contribution of insects in forensic cases, but there are other uses: e.g. indication of movement of the remains following death, linking suspects with crimes trough the DNA analysis of human tissues in the gut content of larvae, detection of drugs, revealing child and elderly neglect, etc. (Amendt *et al.*, 2004).

1.3 - Retrospective and state of the art

The first written document reporting a Forensic Entomology case is present in a Chinese Legal Medicine manual, from the 13th century. It describes a case in which a farm worker appeared dead. The investigator, thinking that the homicide weapon could have been a sickle, told all workers to lay down their working tools on the floor. One of those sickles, probably still having invisible traces of blood, attracted many flies, which lead to the tool's owner to confess the crime (Goff, 2002).

In Europe, the earliest application of Entomology in forensic science dates from the 19th century (1855), by Bergeret, in France. A mummified child's corpse was found in the bricked-up space behind a fireplace inside a house. By evaluating the insect fauna discovered on the remains, he could determine that the baby had been dead for about two years, which lead to the identification of the guilty as the residents of the house before the current inhabitants, who resided there over a short time. Bergeret identified the succession sequence of the different insect species on the cadaver, and understood the importance of the biological life cycles duration of the diverse carrion insects for solving the crime (Goff, 2002).

In the year 1894, J.P.Mégnin, also in France, published *La faune des cadavres: application de l'entomologie à la médecine légale*, which became a classic work in the field. He developed his theory of predictable, ecological waves of insects on corpses. Particularly, for freely exposed corpses, eight successional waves were reported (Benecke, 2001).

Despite the studies conducted by Mégnin, and co-workers, Medicolegal Entomology remained stationary until the middle of the 20th century. According to Magaña (2001), this could have been due primarily to: 1) Distance between entomologists and forensic medicine specialists; 2) The small number of cases in which entomologists were required; 3) The lack of specialized entomologists in the systematic-biological study of the cadaveric fauna.

Topics such as grave fauna, the skeletonizing of corpses or modification of corpses caused by insects were investigated in this period. However, data about biology, ecology and succession of necrophagous insects were not applied to forensic cases (Amendt, 2004).

A new cycle of advances in Forensic Entomology arose in the mid 1950's. It is worth of mention the pioneer works of Bornemisszа (1957), from Australia, Reed (1958) and Payne (1965), from USA, which studied arthropod succession in animal carcasses and associated the different stages of decay to characteristic groups of insects.

Between the 1960's and mid 1980's, Forensic Entomology was maintained primarily by Leclerq (1978), from Belgium, and Nuorteva (1967, 1974, 1977), from Finland, mainly focusing on casework. Smith published the first textbook devoted to Forensic Entomology in 1986. *A Manual of Forensic Entomology* is an excellent reference that brings together in one place all the relevant information on the subject (Hall, 1990), contained in the literature until that time. Since then, basic research and application of Forensic Entomology significantly developed in many countries, with an increasing amount of annual publications. Next sections mention some areas in which considerable work has been done:

1. Identification

The ability to identify arthropods accurately is the principal role played by forensic entomologists. This, more than any other factor provides the solid foundation for all subsequent inferences relative to that fauna (Hall, 1990). Since Mégnin (1894), some forensically oriented works provided information on the identification of cadaveric fauna (e.g. Easton & Smith, 1970; Reiter & Wollenek, 1982, 1983a, 1983b; Reiter, 1984; Smith, 1986). Recently it was published the first key for the identification of third instar larvae of European forensic important Calliphoridae (Szpila, 2010). This valuable contribution compiles information scattered in many papers and covers all European species. Vélasquez *et al*. (2010) also presented a larval identification key including several families and species of forensic important Diptera for the Iberian Peninsula.

Adults of most forensic important Diptera families (Calliphoridae, Sarcophagidae, Muscidae, Piophilidae, Sepsidae...) are well known in comparison with immatures. Even so, it is curious that, within Piophilidae family, one species (*Piophila megastigmata* McAlpine, 1978), present in Europe, has been unnoticed and/or confused with the forensically important and well-known *Piophila casei* (Linnaeus, 1758), during an unknown period of time (Martín-Vega *et al*., 2011; chapter 7).

Because species-diagnostic anatomical characters are not known for the immature stages of many forensically important insects, and an existing key maybe incomplete or require very specialized taxonomic knowledge, in the mid 1990's, DNA methods started to be used by forensic entomologists for species determination (Wells & Stevens, 2008). Calliphoridae is, by far, the most studied group (e.g. Harvey *et al*., 2003, 2008; Wells & Williams, 2007; Cainé *et al*., 2009), not only because of the importance of its species in PMI estimation but probably because they are relatively easy to identify in adult stage, to be used for DNA analysis. Many young immature stages of Muscidae and all immatures of Sarcophagidae species, frequently found in cadavers are difficult or even impossible to identify. Fewer DNA data exist for these groups (e.g. Wells *et al*., 2001; Zehner *et al*., 2004; Tan *et al*., 2010) and here, DNA methods may be a powerful tool to identify specimens not distinguishable based on morphology (Amendt *et al*., 2010).

2. Insect succession

In these studies, the fauna associated to decomposing vertebrates is analysed in order to establish the chronology of the colonizing insects, in a certain area (e.g. Payne, 1965; Early & Goff, 1986; Tullis & Goff, 1987; Anderson & VanLaerhoven, 1996; Tantawi *et al.,* 1996; Richards & Goff, 1997; Arnaldos *et al.,* 2001, 2004; Grassberger & Frank, 2004; Matuszewski *et al*., 2008, 2010, 2011; Battán Horenstein *et al*., 2010; Segura *et al*., 2011). Diptera and Coleoptera are the predominant insects associated with cadavers, being therefore the most studied groups. The species involved in the sequential colonization of the remains and their times of arrival vary from region to region, so databases should be developed for every geographic region in which insects are used to estimate time of death (Anderson, 2010). Additionally, insect colonisation of burnt, buried or submerged bodies has been focused in some studies (e.g. Avila & Goff, 1998; VanLaerhoven & Anderson, 1999; Hobischak & Anderson, 2002, Anderson & Hobischak, 2004), as in these conditions, decomposition and insect succession can be completely different.

3. Insect development

Laboratory rearing of forensically important species is conducted at different temperatures (usually constant) in order to obtain data pertaining to the duration of development of immature stages (Amendt *et al*., 2011). Three developmental models are used to summarize the data: isomorphen diagrams (Grassberger & Reiter, 2001, 2002a, 2002b; Grassberger *et al*., 2003; Richards *et al*., 2008, 2009), isomegalen diagrams (Reiter, 1984; Grassberger & Reiter, 2001; Richards *et al*., 2008, 2009) and thermal summation models (e.g. Kamal, 1958; Greenberg, 1991; Anderson, 2000; Marchenko, 2001; Grassberger & Reiter, 2002a, 2002b; Ames & Turner, 2003; Donovan *et al*., 2006; Richards *et al*., 2008, 2009). In the isomorphen and isomegalen diagrams, the development of insects is visualized in growth curves that illustrate morphological (size or stage) changes during development, depending on the time and temperature. The thermal summation model or accumulated degree hour/day model (ADH/ADD) presents the total thermal input required for an insect to develop from time of oviposition to any stage of development, until adult emergence (Amendt *et al*., 2007).

4. Entomotoxicology

Entomotoxicology is a branch of Forensic Entomology that deals with the use of toxicological analysis of carrion-feeding insects in order to identify toxic substances and to investigate the effects caused by these substances on insect lifecycles (Goff & Lord, 2010). In the absence of body samples to perform toxicological analysis (e.g. cadaver greatly decomposed, lack of appropriate tissue, blood or urine), insects, mainly larvae have frequently been used (Carvalho *et al*., 2010). Knowledge of larval growth rates on substrates altered by various substances is applied in the dating of cadavers, since it is known that, for instance, drugs such as cocaine, heroin and methamphetamine have been found to increase the rate of development of Sarcophagidae larvae (Goff *et al.*, 1989, 1991, 1992).

Insects found on corpses, used for toxicological analysis, besides detecting that a substance has been present in the cadaver's tissues, reveal the risk of calculating an incorrect PMI because of a modified rate of development of the immature stages (Amendt *et al.*, 2004).

1.4 - Forensic Entomology in Portugal

Insects associated to cadavers never received much attention from Portuguese entomologists. The Diptera catalog recently elaborated (Carles-Tolrá, 2002) is very clear on that. Within Calliphoridae family, no species of *Calliphora* Robineau-Desvoidy, 1830, *Chrysomya* Robineau-Desvoidy, 1830 or *Lucilia* Robineau-Desvoidy, 1830 were recorded to be present in mainland Portugal. Very little was known about Sarcophagidae species; only 11 species recorded (131 in comparison for Spain). Identical situation was found for Piophilidae, with only one species recorded for continental Portugal. Therefore, no records existed for most of the usually considered forensically important species. In addition, logically no data existed on the distribution of species in our territory or information about the phenology of species. Regarding the Coleoptera, the second most important insect group in Forensic Entomology, the faunistic information about species most related to cadavers (belonging to families Cleridae, Dermestidae, Histeridae, Silphidae and Staphylinidae) is far more complete (Barros, 1929; Seabra, 1943; Yélamos & Ferrer, 1988; Costas, 1990; Prieto Piloña *et al.*, 2002; Bahillo de la Puebla & López-Colón, 2006a, 2006b; see chapter 9 for Staphylinidae). A list of Coleoptera species associated with different animal carcasses was recently compiled by Grosso-Silva & Soares-Vieira (2009).

Forensically oriented insect succession studies were performed for the first time, using piglet carcasses, in Coimbra (Prado e Castro, 2005) and after, in Lisbon. As a result, fundamental ecological and also faunistic information was obtained, essentially for some groups of Diptera and Coleoptera (Faria e Silva *et al.*, 2009; Prado e Castro & García, 2009, 2010; Prado e Castro *et al.*, 2010a, 2010b, 2010c, 2011a, 2011b), but also for Arachnida (Crespo *et al.*, 2010). Some of the abovementioned works are presented in this thesis (chapters 4, 5, 6 and 9).

It is also worth of mention that Cainé *et al*. (2006, 2009) identified, with DNA methods, several species of Calliphoridae from human cadavers in the north of Portugal.

1.5 - Objectives

The succession of insects in a cadaver, if studied, becomes more or less predictable; data from succession studies are mostly used in cases where corpses are found in an advanced stage of decomposition. The comparison of the fauna collected in a cadaver with the fauna collected in controlled studies, in a similar environment, gives the entomologist information to estimate the PMI in forensic cases. Succession studies are usually performed with animal models, being the pig the most recommended because decomposition of its carcass approaches the pattern of human decomposition, providing the most accurate extrapolation of results to human corpses (Catts & Goff 1992; Goff 1993; Anderson & VanLaerhoven 1996; Richards & Goff 1997).

Insect species associated with carrion and their times of colonization vary according many factors, one of the most important being the geographic region or biogeoclimatic zone. The biogeoclimatic zone defines the habitat, vegetation, soil type, and meteorological conditions of the area that, obviously, have a major impact on the types and species of insects present, as well as their seasonal availability (Anderson, 2010). Because of that, databases should be developed for every biogeoclimatic zone in which insects are being used in Forensic Entomology (Anderson 1995, 2010; Anderson & VanLaerhoven 1996).

In Portugal, only the studies from Prado e Castro (2005) and Prado e Castro *et al.* (2011a, 2011b) focused on Diptera succession on cadavers, particularly in a spring/summer period, in central Portugal. More studies were clearly needed to gather relevant information for forensic practice.

The main objectives of this thesis are:

1. Study the entomosarcosaprophagous faunistic composition in Lisbon area, focusing on Diptera and Coleoptera associated with piglet carcasses, in the 4 seasons of the year;
2. Recognize the patterns of faunistic entomological succession in cadavers, during the different seasons of the year, in Lisbon;
3. Determine the duration of the different decomposition stages of a cadaver in each season of the year, as well as the most important indicator species of each stage, in each season;
4. Create a database useful for forensic purposes, in the studied area and in other regions with similar environmental characteristics.

1.6 - General Methodology

The experimental study, conducted at a small patchy woodland park inside urban perimeter (Instituto Superior de Agronomia, Tapada da Ajuda, Lisbon) consisted in four experiments of 8 to 10 weeks, each covering a season of the year. In each experiment, a domestic piglet, *Sus scrofa* L., of 7.5 to 8 Kg weight was used. A modified version of the trap designed by Schoenly (Prado e Castro *et al*., 2009) (Chapter 2), baited with the freshly killed piglet was used to collect the entomofauna.

The carcass was visited daily during the first 23 days and afterwards at alternate days. Samples were collected from the trap at each visit, removing the arthropods (immersed in a 40% ethylene glycol solution) from the bottles of the trap and substituting it with new solution. Manual sampling was performed as well, to collect immature stages of Diptera, when present. These were fed with pork meat and reared in small containers until adult stage. Physical appearance of the carcass, odours, quantity and type of insect species present was recorded in each visit. Ambient temperatures were continuously registered with a HOBO Data logger placed into the trap.

In the laboratory, using a stereomicroscope, the arthropods were separated in orders. Diptera and Coleoptera were further identified to family level. Several families were selected for species identification, based on its abundance on the cadaver, breeding capacity, but also on the individual expertise.

1.7 - Thesis Structure

This dissertation is organized in 10 Chapters. The first chapter is the General Introduction, where I give an overview about Forensic Entomology and refer the purpose of this study. The following 8 chapters correspond to scientific papers which are either published (5), submitted (2) or to be submitted (1). In Chapter 2, I present the methodology used in the experiments. This work was published in *Anales de Biología*. Chapter 3 presents the information on seasonal activity of Diptera and successional patterns in Lisbon area, data that may serve as model to areas with similar environmental characteristics. This paper was submitted to *Medical and Veterinary Entomology*. Chapters 4, 5 and 6 include papers already published in *Boletín de la Asociación española de Entomología* and *Graellsia*, mainly of faunistic nature. Several species belonging to Calliphoridae and Piophilidae (Diptera) families were recorded for the first time for Portugal from specimens collected during the experiments. In chapter 7, I report, for the first time, the presence of *Piophila megastigmata* (Diptera: Piophilidae) in human corpses, in Portugal, during the course of forensic investigations. The species becomes a useful new tool for Legal Medicine in Europe. This work has been submitted to *Forensic Science International*. Chapter 8 presents the information collected about seasonal activity of Coleoptera and successional patterns in Lisbon area. In chapter 9, a list of Staphylinidae species collected in carcasses is presented, of which five species were recorded for the first time in Portugal. This work has been published in *Boletín de la Asociación española de Entomología*. Finally, in Chapter 10, I present a General Discussion of the major findings of this work, their implications, as well as future directions for further work.

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Chapter 2

A MODIFIED VERSION OF SCHOENLY TRAP FOR COLLECTING SARCOSAPROPHAGOUS ARTHROPODS. DETAILED PLANS AND CONSTRUCTION

Prado e Castro, C., Chichorro, D., Serrano, A. & García, M.D. (2009) A modified version of Schoenly trap for collecting sarcosaprophagous arthropods. Detailed plans and construction. *Anales de Biología*, **31**, 1-6.

A modified version of Schoenly trap for collecting sarcosaprophagous arthropods. Detailed plans and construction

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Resumen

Versión modificada de la trampa Schoenly para capturar artópodos sarcosaprófagos. Planos detallados y construcción.

Este trabajo intenta simplificar la construcción de una trampa tipo Schoenly (Schoenly 1981), en la que se han incorporado algunas modificaciones. Este tipo de trampa resulta muy útil para estudiar la sucesión faunística entomosarcosaprófaga, uno de los temas de interés de la Entomología Forense. Se aportan planos detallados y medidas exactas de los distintos componentes de la trampa.

Palabras clave: Entomología forense, Fauna sarcosaprófaga, Sucesión de insectos, Trampa Schoenly

Abstract

This paper attempts to simplify the task of building Schoenly's insect trap (Schoenly 1981), with some modifications to the original. The trap is very useful in Forensic Entomology works, in order to study sarcosaprophagous insect succession. Detailed plans of the trap with exact measures are supplied.

Key words: Forensic entomology, Sarcosaprophagous fauna, Insect succession, Schoenly trap

Introduction

In Forensic Entomology, when studying arthropod fauna that colonizes a cadaver, it is desirable that collection of samples is easy and efficient. Most of the works focusing on insect succession in cadavers and inventories of this type of fauna have been done using manual sampling as methodology (Reed 1958, Payne 1965, Early & Goff 1986, Anderson & VanLaerhoven 1996, Tantawi et al. 1996, Richards & Goff 1997, Grassberger & Frank 2004, Matuszewski et al. 2008). This traditional method of collection consists in laying the dead animal in the study site (eventually protected inside a vertebrate scavenger-exclusion cage) and periodically visiting it. In each visit, part of the organisms found on, in, under and around the carcass is sampled with commonly used entomological material, as aerial nets and tweezers. The problem with this methodology is that only the fauna present in the moment of the visit is collected, and so, a considerable part of it is ignored. Another disadvantage is related with the dependence in the collector's ability and experience. The data obtained may lead to incomplete and biased inventories (Ordóñez et al. 2008) and cannot be easily compared with other works.

An alternative to face this problem is the use of specific traps that allow the collection of all the arthropod fauna that enters the trap and that emerges from the decaying remains.

Schoenly (1981) described a trap invented to collect arthropod fauna from baits (such as small-animal carrion, dung or rotting fruit). Schoenly et al. (1991) described another trap with the same mechanisms, but somewhat improved, and sized for human and pig cadavers. These traps are designed to collect adult arthropods that are attracted to a carcass. Some of them are immediately captured as they enter the trap and another part enters and reaches the carcass. These uncollected arthropods will eventually leave the corpse, being also captured; the same way, second generations emerging inside the trap are also collected, as well as migratory larvae that leave the carcass. With this method, all insects that are attracted to the carcass and enter the trap are captured, thus giving a total census, not only samples. Physical contact with the bait during sampling is not needed, arthropods are collected continuously and automatically, reducing the disturbance in the colonization and collector's bias is not introduced. Even thought, only a few works refer Schoenly traps as methodology for collecting arthropods

Figura 1: Fotografía del exterior de la trampa.
Figure 1: Photo of trap, exterior.

Figura 2: Fotografía del interior de la trampa.
Figure 2: Photo of trap, interior.

attracted to cadavers (Arnaldos et al. 2001, 2004, Prado e Castro 2005, Battán Horenstein et al. 2007). Recently, Ordóñez et al. (2008) proved the efficiency of Schoenly trap in the collection of adult sarcosaprophagous dipterans, where it is described as a superior methodology to perform inventories of Diptera associated with carcasses and recommended for the study of sarcosaprophagous succession.

Although Schoenly (1981) and Schoenly et al. (1991) have descriptions and careful details of trap construction, in fact it is not very simple to build it.

The objective of this work is to provide plans with the precise dimension of the trap designed by Schoenly (1981), but with some modifications to the original. Since we wanted to study insect succession in a piglet carcass, it was decided to use the smaller Schoenly trap. However, this one was prepared for animals such as mice and rats, so we had to design it much bigger. Another modification introduced to the trap was the use of underground conduits to collect beneath the ground bottles, so the trap didn't need to be raised, in the way described in Schoenly et al. (1991). The exact dimension of each piece that constitutes the trap is given, so that trap construction becomes much simpler.

General trap characteristics

The trap consists of 12 lateral holes connected with funnels, 8 of them are 'entry holes' and 4 are 'exit holes' (Figs. 1 & 2). From the 8 entry openings, 4 of them give the arthropods direct access to the bait (a dead piglet or other animal). The other 4 are connected with collecting channels that lead to bottles that immediately collect the portion of the fauna attracted to the carcass. The 4 exit holes plus the one on the top of the trap, connect to bottles with preservative solution. All the fauna that leaves the body is captured.

Schoenly`s trap allows a total census of the arthropods that enter it and that develop on the cadaver, with a minimum interference in the natural decomposition process and its faunistic succession.

Construction details and functioning

The trap is dodecahedral, measuring 99 cm diameter at its base and stands 61 cm high. It is appropriate for animals until 10 kg weight.

The figure 3 shows trap structure, constructed in marine plywood, which is a light material, very resistant to water and humidity and so, convenient for outdoors studies. Exact dimensions are given for each piece of the trap, as well as indications of the holes that should be made in some pieces.

During the process of properly gluing and nailing all the pieces, a fine mesh plastic net was nailed in the superior part of the trap, coupled to pieces D, E and G (Figs. 1 & 3), and a large mesh plastic net was fastened with a screw between pieces A and B of trap base and pieces F (Figs. 2 & 3). The wood surfaces of the trap were coated with water resistant varnish.

To the 12 lateral holes in the trap, 12 funnels (7 cm diameter, with the end cut off) were glued. Eight of them serve as entry for the insects in the trap and 4 as exit, so, alternately, funnels are turned inside and outside (Figs. 1 & 2). In the top of the trap another funnel (with 8 cm diameter and end cut off) was glued to piece D, also serving as exit hole to arthropods. To this funnel a tube and a bottle were connected (Fig. 1). To the 4 lateral funnels turned outside (exit funnels), 4 bottles were connected. Four of the 8 funnels turned inside the trap give the arthropods direct access to the carcass. To the other 4, "tubes" of fine mesh plastic net were coupled, making the connection between the funnels and the 4 holes in the base of the trap (Fig. 2). Bottles were coupled to these holes in the base of the trap (pieces B). Since these 4 bottles will be positioned beneath the ground, a

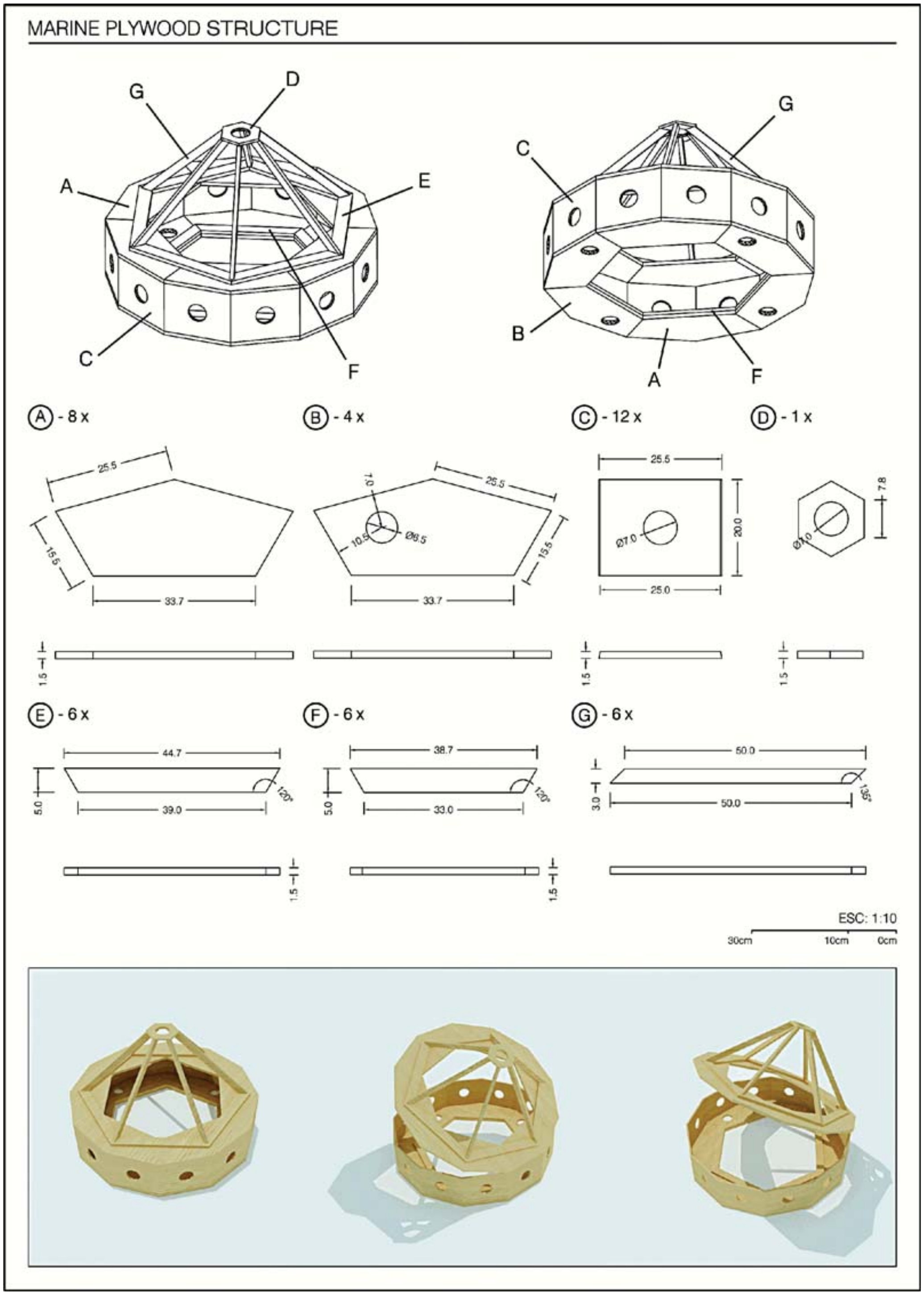

Figura 3: Estructura de la trampa con los detalles de sus piezas constituyentes.

Figure 3: Trap structure with details of each piece that constitutes it.

tube in which an arm can enter should be used, in order to be possible the access to these bottles. The trap is installed in the field by digging holes to accommodate those 4 tubes and then, using soil to fill spaces around them. The trap should settle for 1 or 2 weeks before experiments begin (Schoenly et al. 1991).

To sum up, the trap has 4 unobstructed openings for the arthropods to enter and access the carcass and a total of 9 bottles with a solution that kills and preserves the arthropods (4 bottles underneath the ground connected to collector channels for incoming organisms, 4 bottles and 1 top bottle for outgoing organisms).

The bottles should be filled approximately ¼ of its capacity with Leech's solution (Leech 1966), which is usually used in pitfall traps (Morril 1975). This solution serves as general arthropod killing agent and temporary preservative and is a mixture containing 600 ml water, 400 ml ethylene glycol (anti-freeze coolant), 5 ml formalin and 1 ml detergent. This solution preserves specimens for up to a week (Morril 1975), is odorless, rate of evaporation is low (Schoenly 1981, Schoenly et al. 1991) and the specimens stay in good conditions.

At last, the carcass can be placed inside the trap that is ready to work. The regularity of collections is up to the investigator, however, due to the large amount of insects (mainly Diptera) that are attracted to the cadaver, especially in the first days we recommend it to be done, at least, in a daily basis.

The trap can easily be opened (Fig. 3, bottom) to perform temperature measurements in the carcass, to observe or make manual collections, e.g. immature stages, if desired.

Final considerations

As it was demonstrated by Ordóñez et al. (2008), Schoenly trap is very effective in the collection of entomosarcosaprophagous fauna and suggested as the best methodology for making inventories of this type of fauna associated with a decaying carcasss. The trap we describe is suited for animal carcasses up to 10 kg. It was modified in order to be bigger than the one in which is based (Schoenly 1981), for the purpose of using larger animals and because a bigger size increases the possibility that pupation occurs in trap area, leading to 2nd generation collections. It is smaller than the one described in 1991 (Schoenly et al. 1991) and has different shape, but includes some of its improvements. Above all, the plans supplied with exact measures of trap pieces will strongly facilitate the task of planning and constructing the trap.

Insect succession on cadavers can be better studied using this trap and with this contribution we believe that building one will be simpler.

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Chapter 3

CARRION FLIES OF FORENSIC INTEREST: A STUDY OF SEASONAL COMMUNITY COMPOSITION AND SUCCESSION IN LISBON, PORTUGAL

Prado e Castro, C., Serrano, A., Martins da Silva, P. & García, M.D. (submitted) Carrion flies of forensic interest: a study of seasonal community composition and succession in Lisbon, Portugal. *Medical and Veterinary Entomology*.

Carrion flies of forensic interest: a study of seasonal community composition and succession in Lisbon, Portugal

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Short title: Carrion flies in Portugal

Abstract

Information on Diptera community, seasonality and successional patterns in every geographic region is fundamental for the use of flies as forensic indicators of time of death. In order to obtain these data from Lisbon area (Portugal), experiments were conducted during the four seasons of the year, using piglet carcasses as animal models.

Five stages were recognized during the decomposition process. The stages, besides visually defined, could be separated taking into account the occurrence and abundance of the specific groups of Diptera collected. Bloated stage in general recorded higher abundance and species richness values.

71 species were identified, belonging to 39 families, in a total of 20144 adult Diptera collected. Autumn yielded the highest values of species richness, while winter had the lowest. In all seasons of the year, Calliphoridae was the dominant family;

Muscidae and Fanniidae were very abundant as well. *Calliphora vicina*, *C. vomitoria*, *Chrysomya albiceps*, *Lucilia ampullacea*, *L. caesar, L. sericata* (Calliphoridae); *Hydrotaea ignava*, *Muscina prolapsa, Synthesiomyia nudiseta* (Muscidae), *Piophila megastigmata, Stearibia nigriceps* (Piophilidae) and *Nemopoda nitidula* (Sepsidae) revealed to be very important members of the Diptera community collected. The necrophagous behaviour, demonstrated by their immatures, using carrion as food source makes them useful forensic indicator species. It is also relevant the presence of *Chrysomya megacephala, S. nudiseta* and *P. megastigmata*, foreign species established in the local carrion communities. This paper also marks the first record for *S. nudiseta* in Portugal.

Keywords: Diptera, carrion decomposition, forensic entomology, succession, Portugal

Introduction

Forensic entomology is nowadays considered an important branch of forensic science, in which the biology and ecology of insects are studied, particularly to determine the postmortem interval (PMI) (Amendt *et al.*, 2007). The difficulties that forensic medical specialists have concerning the determination of time since death during investigations (Prieto *et al.*, 2004) and the accurate estimations regarding this question that can be made through the study of carrion fauna, even in advanced stages of decomposition, is leading to an increasing acceptation and use of this discipline (Greenberg & Kunich, 2002).

Postmortem interval can be calculated using developmental data of certain species and also through analysis of the arthropod community composition and its succession on the carcass (Amendt *et al.*, 2007). The first method tends to be used in initial decomposition stages, with Diptera species from Calliphoridae family as the main indicators, while the second can be used in long postmortem intervals (Catts & Goff, 1992), after weeks, months or even years after death (Anderson, 1995). The succession-based method requires knowledge of insect fauna in the geographic region where the corpse is found, since species and their times of colonization vary among bio-geographic regions (Anderson, 1995, 2010; Anderson & VanLaerhoven, 1996). Hence, composition of local carrion fauna, its seasonal variations and patterns of succession, as well as the periods of time each life stage spends on a cadaver, comprise crucial information to be used in forensic cases of a particular region (Anderson, 1995, 2010; Matuszewski *et al.*, 2010a). This sort of information is generated from field experiments on carrion decomposition, usually made with animal models, particularly focusing on insect succession (Wells & LaMotte, 2010). However, in Europe just few studies have been conducted on this subject (Matuszewski *et al.*, 2008). Particularly in southern Europe, the scarcity of entomo-forensic data has obligated researchers to use information from other bio-geographical areas, with different fauna and environmental conditions (Vanin *et al.*, 2008). Such use of foreign data may not provide a sufficient degree of accuracy in the interpretation of forensic cases (Arnaldos *et al.*, 2004). Entomological evidence should, thus, be evaluated on a regional scale (Grassberger & Frank, 2004; Vanin *et al.*, 2008). In southern Europe, particularly in the Iberian Peninsula, a region with remarkable climatic, environmental and faunistic differences from Central and Northern Europe, hardly any work has been focused on seasonal patterns of arthropod succession in cadavers, with the exception of Arnaldos *et al.* (2001, 2004) and Castillo Miralbes (2002), in Spain. While the first authors characterized carrion communities within an arid region in the southeastern part of the Iberian Peninsula, the second author’s study focused on a northern continental region. However, climatic conditions diverge remarkably along the Iberian Peninsula. For instance, the predominant climate types along the Portuguese country, i.e. mediterranean and atlantic, may have an influence on dipteran communities of forensic interest, yet, the only data available from the Portuguese area (Prado e Castro *et al.*, 2011a, 2011b), from an atlantic region, just focused on Diptera activity in late spring/summer. As stated by Matuszewski *et al.* (2008), no models of succession useful in the process of PMI estimation exist for most of the bio-geographic areas of Europe and its typical habitats. Actually, this lack of suitable reference data is even more pronounced in southern European regions.

The objective of this study was to investigate the dipteran community composition and successional patterns on cadavers decomposing in the four different seasons of the year in Lisbon, Portugal. The entomological data obtained may serve as reference to be applied in other regions with similar bio-geoclimatic characteristics, in southern Europe.

Material and methods

Site description. The experiment was performed in Lisbon, the Portuguese capital and biggest urban centre, with a population of ≈ 2.8 million inhabitants in its metropolitan area. Lisbon has a mediterranean climate. According to the National Weather Institute, summers are hot and dry, with average daily temperatures of 20–23°C and winters are cool and rainy, with daily means around 12°C, while spring and autumn are generally mild, or even warm during daytime. Annual rainfall is around 700–750 mm/year, spread over 100 rainy days, mostly from October to May.

The study site was located in Instituto Superior de Agronomia, Tapada da Ajuda (38º42'41''N 09º11'28''W), a small patchy woodland park inside urban perimeter, mainly composed of *Ailanthus altissima* (Mill.) Swingle, *Fraxinus angustifolia* Vahl and *Ulmus minor* Miller. The chosen site was located in a shaded area, undisturbed by human activity.

Carcasses and experimental procedures. Four different experiments, of 8 to 10 weeks each, were performed, in order to cover all seasons of the year. The dates were the following: Autumn- 18.10.2006 to 2.01.2007; Winter- 17.01 to 3.04.2007; Spring- 16.04 to 16.06.2007; Summer- 27.06 to 27.08.2007. In each experiment, a domestic piglet, *Sus scrofa domesticus* L., of 7.5 to 8 Kg weight was used. The animals were stunned first and then killed trough incision in the jugular vein.

A version of Schoenly trap (Prado e Castro *et al.*, 2009) was used, with the freshly killed piglets as bait. The trap is a dodecahedral structure constructed in plywood, where the carcasses stay in direct contact with soil and that allows good aeration and odour dissipation. The traps designed by Schoenly (Schoenly 1981; Schoenly *et al.* 1991; Prado e Castro *et al.*, 2009) collect the entomofauna from carcasses with a dual-functioning system, capturing arriving as well as emerging populations while successional processes and cadaver decomposition are left undisturbed. The trap has been confirmed to be a superior methodology for collecting adult dipterans in a decaying corpse, and recommended as the most effective device for characterizing the sarcosaprophagous succession (Ordóñez *et al.* 2008). The bottles that capture the arthropods contained a killing and temporary preservative solution of 40% ethylene glycol with formalin and detergent. Collection of samples from the trap consisted in removing the bottles with arthropods and replacing them with others filled with clean solution. The visits were made always at the same hour, daily in the first 23 days, and thereafter every 2, 3 or 5 days until the end of each experiment. Besides the continuous collections made by the trap, in each visit, manual sampling was also performed to collect Diptera eggs and larvae. The immature stages collected were reared in small containers and fed with a piece of pork meat. This was done in a "field laboratory", located 500 m distance from the study site, also in a shaded area, with similar ambient temperatures, except for Piophilidae and Sepsidae that were reared in the laboratory at the Faculty of Sciences, University of Lisbon, at ambient temperature.

Ambient temperatures were continuously registered with a HOBO Data logger (24 records/day) placed into the trap.

The date of death and placement of the pig carcass in the trap was designated as day 0. The collections started 24 hours after, on day 1. Specimens were all kept in 80% ethanol.

Data analysis. Average numbers of Diptera abundance (N) and richness (S - number of species) found in the carcass were recorded in each sampling season and decomposition stage. Number of species refers only to those identified (some families, not identified to the species level, count as one species). To evaluate the effect of season, decomposition stage and their interaction, a two-way multivariate analysis of variance (MANOVA), followed by pair-wised Bonferroni *post hoc* tests, was performed to N and S values of Diptera, and with a particular focus on the Calliphoridae family. If assumptions of homogeneity of variances and normality (verified previously using Bartlett and Kolmogorov–Smirnov tests, respectively) were not met, data were log (x+1) transformed. The above statistical analyses were performed in the SigmaStat software (SPSS 17.0 for Windows).

In the estimation of seasonal relative abundances (within families and species), only the first 15, 40, 15 and 12 days of experiment were considered (for autumn, winter, spring and summer, respectively), in order to exclude emerging flies and in all cases to include the same decomposition stages. All families with less than 10 individuals for the studied period were grouped in "Other families".

Indicator Diptera species of each sampling season were identified by using the Indicator Value (IndVal) method (Dufrêne & Legendre, 1997). The analysis was performed to species/families that bred on the cadaver and those that even breeding was not confirmed, are considered of potential forensic importance (Matuszewski *et al.*, 2010b; Velásquez *et al.*, 2010). According to Dufrêne & Legendre (1997) IndVal values vary from 0 to 100 and indicator values of 25 are considered the minimum significant level for the index. In this analysis, species with the highest IndVal values were considered the indicators of each respective sampling season.

Diptera community composition related to the different decomposition stages, within each season was analyzed by ordination methods. A previous detrended correspondence analysis (DCA) based on Diptera species composition was performed to identify the gradient length, in order to select the correct constrained ordination method (Lepš & Smilauer, 2003). Subsequently, a Redundance analysis (RDA) was performed using log transformed data and based on the ‘‘sampling days *vs.* Diptera *taxa*’’ data matrices. Statistical significance of the ordination axes was evaluated by a Monte Carlo permutation test. RDAs were performed in CANOCO 4.5 for Windows.

Taxa with at least 10 adult specimens collected during the study period and all species that bred on the cadaver were included in the succession tables. The presence of eggs, larvae, pupae, gravid or teneral adults is based on what was captured by the trap and also on species rearing. The fact that some stages are not mentioned does not mean they are not present, but they were not observed.

Results

Climatic data. The evolution of temperature throughout the four seasonal sampling periods is given in Fig. 1. As could be expected, winter was the season in which the temperatures were considerably lower compared to the other seasons. Regarding the first week of carcass exposure, the mean temperatures were 18.3ºC in autumn, 10.9ºC in winter, 18.9ºC in spring and 20.2ºC in summer.

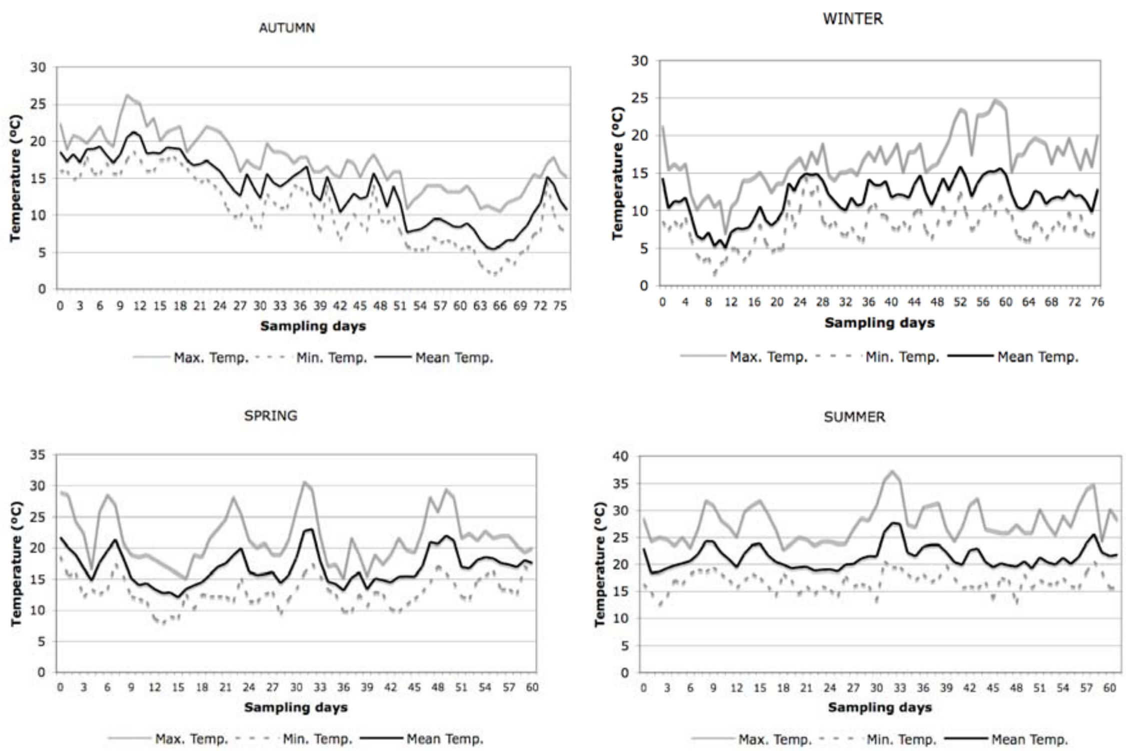


Fig. 1. Temperature data for the studied periods.

Decomposition process. Five decomposition stages were identified, as described in Anderson & VanLaerhoven (1996): fresh (F), bloated (B), decay (D), advanced decay (AD), and dry/remains (DR). The characteristics that define each stage of decomposition are summarized in Table 1. All stages were observed, with different durations, in all seasons of the year, except in autumn, where D was not clear, as deflation started simultaneously with larval migration (Table 1). Differently to the other seasons, also in autumn, humidity and mold was usually present in the DR stage. Head decomposed faster than trunk in all seasons. The aspect of carcasses in each decomposition stage, is shown in Appendix 1.

Table 1. Decomposition stages.

Decay stage	Defining characteristics of decomposition stages	Days postmortem Autumn	Winter	Spring	Summer
Fresh	Fresh appearance; no odor	0-3	0-17	0-3	0-2
Bloated	Bloating, initiating as slight inflation of the abdomen; odor of putrefaction	4-7	18-28	4-6	3-5
Decay	Carcass starts to deflate; larval masses feeding on soft tissues; strong odor of decay	---	29-34	7	6
Advanced decay	Intense migration of larvae; decrease in odor; at the end of the stage, most of the flesh has been removed	8-26	35-54	8-19	7-12
Dry	Carcass consists of bones, skin and hair; little to no odor	27-76+	55-76+	20-60+	13-60+

Diptera community. A total of 20144 adult Diptera, belonging to 39 families and 71 species were collected along all sampling seasons (Table 2).

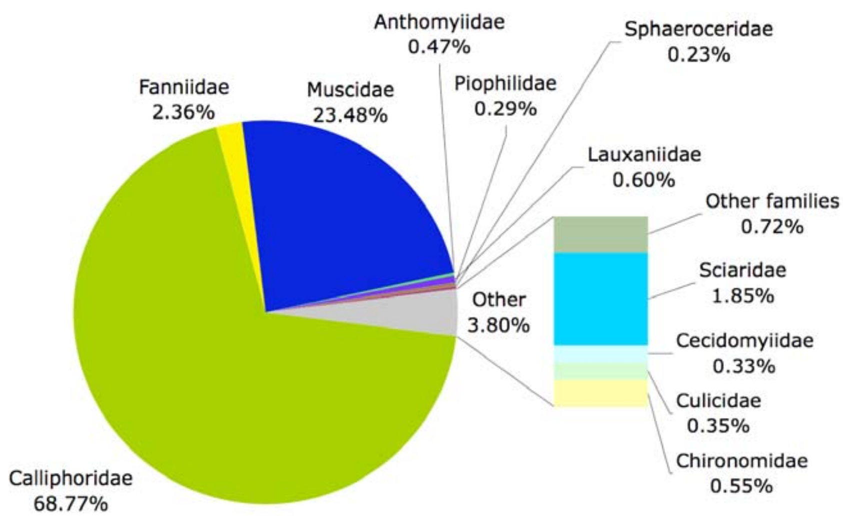
Table 2. Absolute seasonal abundance of adult Diptera collected on pig carcasses in the four seasons of the year.

FAMILY	SPECIES	Autumn	Winter	Spring	Summer	Total
Anthomyiidae		24	4	22	221	271
Calliphoridae	*Calliphora vicina*	249	580	315	27	1171
	Calliphora vomitoria	19	150	218	16	403
	Chrysomya albiceps	2896	1	1	1399	4297
	Chrysomya megacephala	23	0	1	0	24
	Lucilia ampullacea	39	0	363	14	416
	Lucilia caesar	181	5	1158	648	1992
	Lucilia illustris	1	0	2	0	3
	Lucilia sericata	292	1	147	103	543
	Melinda viridicyanea	0	0	3	1	4
	Pollenia sp.	2	0	171	21	194
	Stomorhina lunata	1	0	0	1	2
Carnidae		1	0	3	54	58
Chloropidae		0	0	1	3	4
Dolichopodidae		0	0	1	0	1
Drosophilidae		4	2	1	2	9
Ephydridae		0	0	1	0	1
Fanniidae	*Fannia* sp.	139	96	1054	425	1714
Heleomyzidae		85	21	31	11	148
Hybotidae		2	2	0	0	4
Lauxaniidae		31	0	19	242	292
Muscidae	*Atherigona varia*	1	0	0	4	5
	Graphomya maculata	1	0	2	2	5
	Hydrotaea aenescens	2	0	0	0	2
	Hydrotaea armipes	7	28	159	16	210
	Hydrotaea capensis	3	0	13	62	78

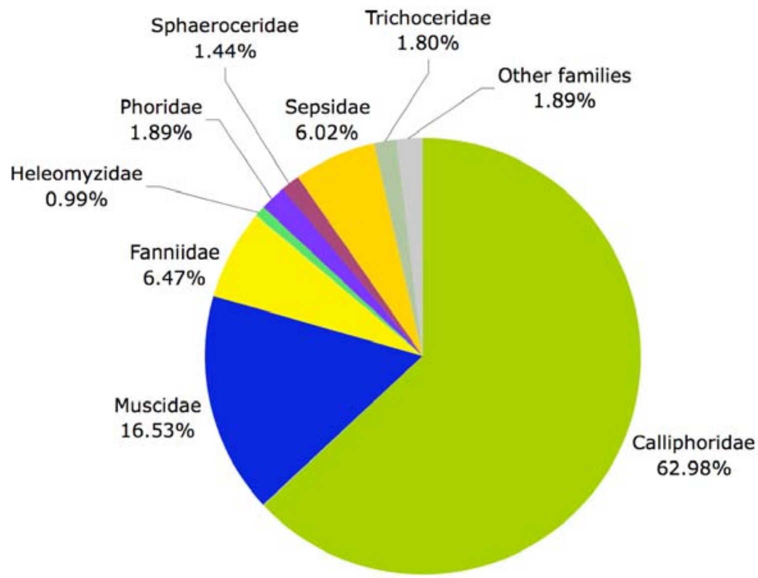
FAMILY	SPECIES	Autumn	Winter	Spring	Summer	Total
	Hydrotaea dentipes	9	3	20	4	36
	Hydrotaea ignava	727	6	1971	1719	4423
	Hebecnema sp.	37	20	51	7	115
	Musca domestica	187	0	0	118	305
	Muscina levida	39	1	14	78	132
	Muscina pascuorum	1	0	0	0	1
	Muscina prolapsa	58	1	120	145	324
	Muscina stabulans	33	4	50	15	102
	Phaonia subventa	137	141	252	17	547
	Phaonia sp.	6	0	10	0	16
	Synthesiomyia nudiseta	24	2	0	17	43
	Other Muscidae species	1	0	9	17	27
Phoridae		35	40	41	9	125
Piophilidae	*Piophila casei*	0	0	0	7	7
	Piophila megastigmata	4	0	1	38	43
	Prochyliza nigrimana	6	0	25	4	35
	Protopiophila latipes	2	0	9	9	20
	Stearibia nigriceps	8	0	16	112	136
Platystomatidae	*Platystoma* sp.	0	0	0	5	5
Rhinophoridae		0	0	8	24	32
Sarcophagidae		7	0	17	316	340
Scathophagidae		2	2	0	0	4
Sepsidae	*Nemopoda nitidula*	23	139	236	0	398
	Sepsis lateralis	7	1	2	1	11
	Sepsis sp.	0	0	0	1	1
Sphaeroceridae		31	36	60	152	279
Stratiomyidae		1	0	0	0	1
Syrphidae		0	0	0	1	1
Tachinidae		0	0	1	0	1
Tephritidae		0	0	4	2	6
Trixoscelididae	*Trixoscelis* sp.	0	0	3	32	35
Ulidiidae		0	0	0	6	6
Xylomyidae		7	0	3	1	11
NEMATOCERA						
Bibionidae		0	1	0	0	1
Cecidomyiidae		26	5	13	84	128
Ceratopogonidae		2	0	0	1	3
Chironomidae		57	8	1	0	66
Culicidae		19	0	0	1	20
Keroplatidae		0	1	0	0	1
Limoniidae		3	0	0	0	3
Mycetophilidae		4	0	7	1	12
Psychodidae		18	9	47	8	82
Scatopsidae	*Scatopse notata*	10	6	2	0	18
Sciaridae		203	91	7	21	322
Trichoceridae	*Trichocera* sp.	41	24	0	0	65
Diptera abundance (N)		5778	1432	6689	6245	20144
Species richness (S)		56	32	51	53	

Higher values of Diptera abundance and species richness were found, respectively, in spring and autumn (Table 2). Conversely, winter was the season showing significantly the lowest values of abundance (F = 23.1, p = 0) and richness (F = 30.6, p = 0). A similar result was obtained for the most important forensic family (Calliphoridae), recording significantly lower abundance (F = 5.56, p = 0.001) and species richness (F = 21.3, p = 0) in winter.

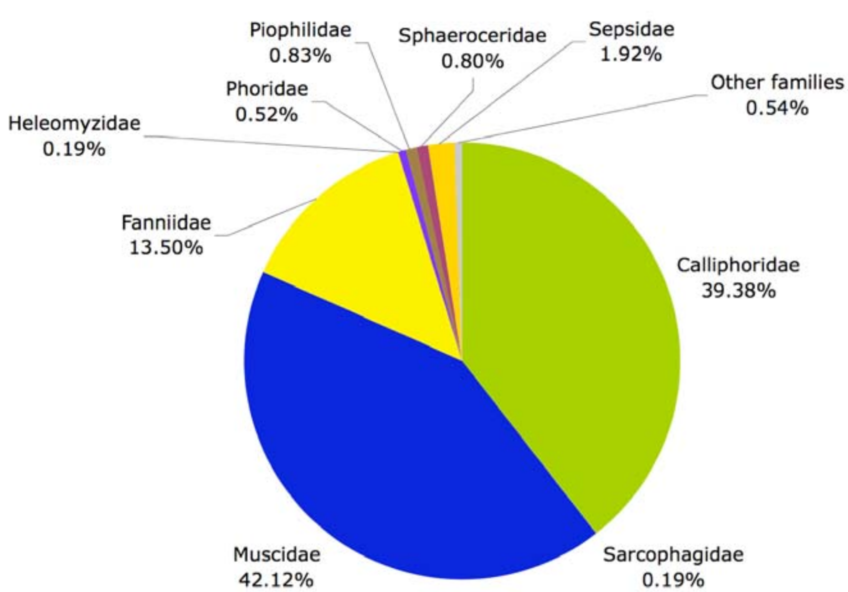
In what concerns the abundance of Diptera families, in autumn and winter Calliphoridae was clearly dominant, followed by Muscidae and Fanniidae (Fig. 2). In spring and summer, in turn, Muscidae was the most abundant family, followed by Calliphoridae and also Fanniidae. Sepsidae were quite abundant in winter and spring while Sarcophagidae only in summer had a marked presence. Some families did not occur in all seasons or their relative abundance was considerably different among them (e.g. Carnidae, Lauxaniidae, Piophilidae, Sarcophagidae, Sepsidae) (Table 2, Fig. 2).



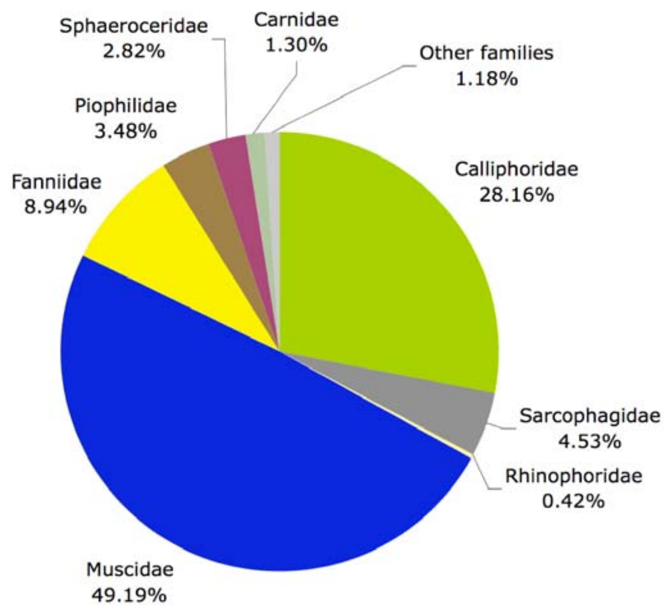
A


Sphaeroceridae
1.44%
Trichoceridae
1.80%
Other families
1.89%
Phoridae
1.89%
Sepsidae
6.02%
Heleomyzidae
0.99%
Fanniidae
6.47%
Muscidae
16.53%
Calliphoridae
62.98%

B


Piophilidae
0.83%
Sphaeroceridae
0.80%
Sepsidae
1.92%
Phoridae
0.52%
Other families
0.54%
Heleomyzidae
0.19%
Fanniidae
13.50%
Calliphoridae
39.38%
Muscidae
42.12%
Sarcophagidae
0.19%

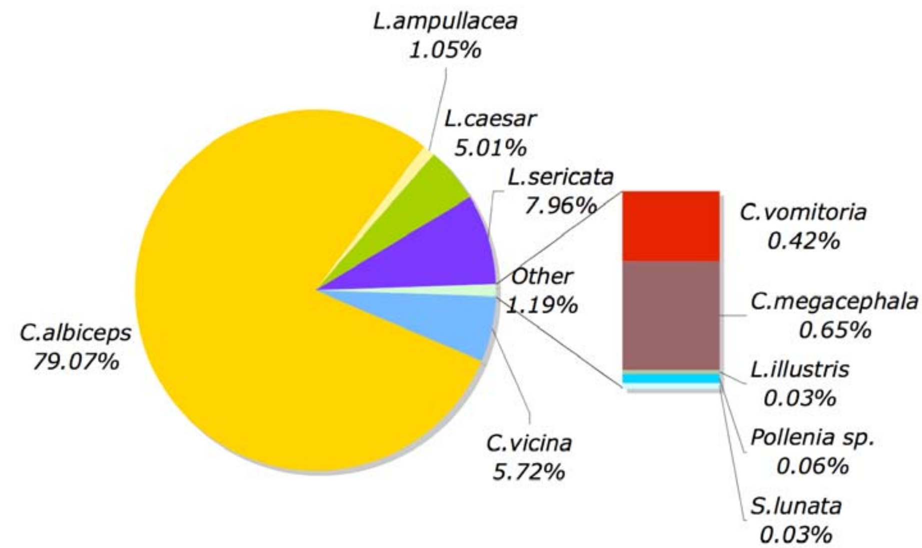
C



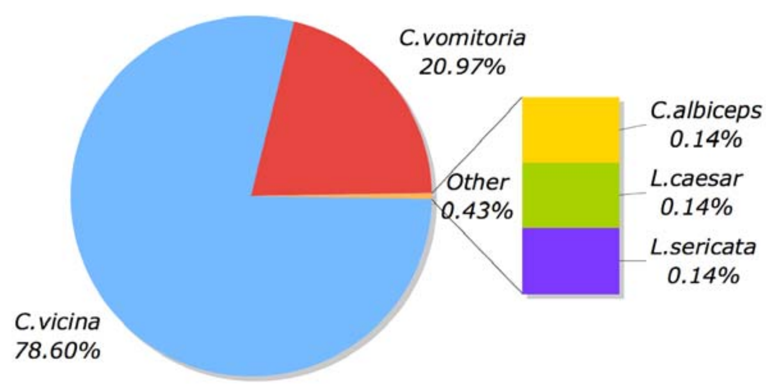
D

Fig. 2. Relative abundance of Diptera families present in each season of the year. A) autumn (15 days); B) winter (40 days); C) spring (15 days); D) summer (12 days)

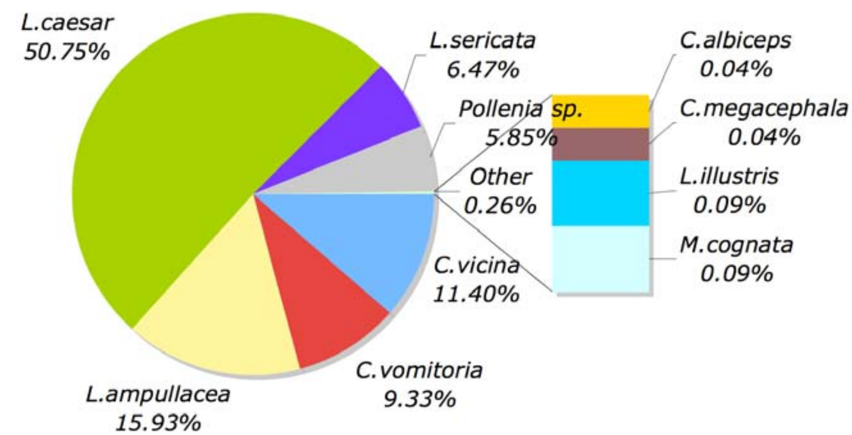
The species most attracted to the carcass during autumn were *Chrysomya albiceps* (Wiedemann, 1819) and *Hydrotaea ignava* (Harris, 1758); in winter *Calliphora vicina* Robineau-Desvoidy, 1830 and *Phaonia subventa* (Harris, 1780) were the most represented; in spring and summer *Lucilia caesar* (Linnaeus, 1758) and *H. ignava* were the most abundant species (Figs. 3 and 4). There was a clear seasonal shift in the Calliphoridae species present on the carcasses and in their abundances. While in autumn, *C. albiceps*, *Lucilia sericata* (Meigen, 1826), *C. vicina* and *L. caesar* were the most represented species, in winter, *C. vicina* and *Calliphora vomitoria* (Linnaeus, 1758) dominated. *Lucilia caesar* was very abundant in spring and summer, yet in spring it was accompanied mainly by *Lucilia ampullacea* Villeneuve, 1922, *C. vicina* and *C. vomitoria* and in summer mostly by *C. albiceps* and *L. sericata* (Figure 3). *Hydrotaea ignava* was the dominant muscid in all seasons, except in winter, where it was practically absent and was replaced by *Phaonia subventa* (Fig. 4).


L.ampullacea
1.05%
L.caesar
5.01%
L.sericata
7.96%
Other
1.19%
C.albiceps
79.07%
C.vicina
5.72%
C.vomitoria
0.42%
C.megacephala
0.65%
L.illustris
0.03%
Pollenia sp.
0.06%
S.lunata
0.03%

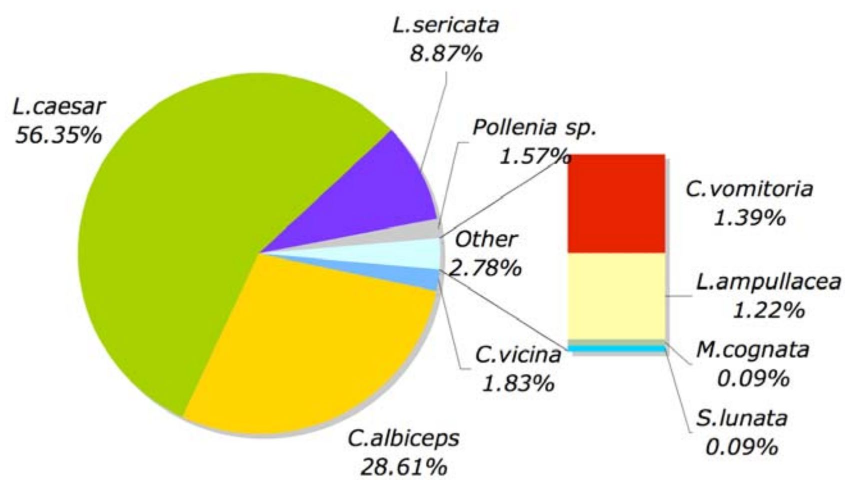
A


C.vomitoria
20.97%
Other
0.43%
C.vicina
78.60%
C.albiceps
0.14%
L.caesar
0.14%
L.sericata
0.14%

B

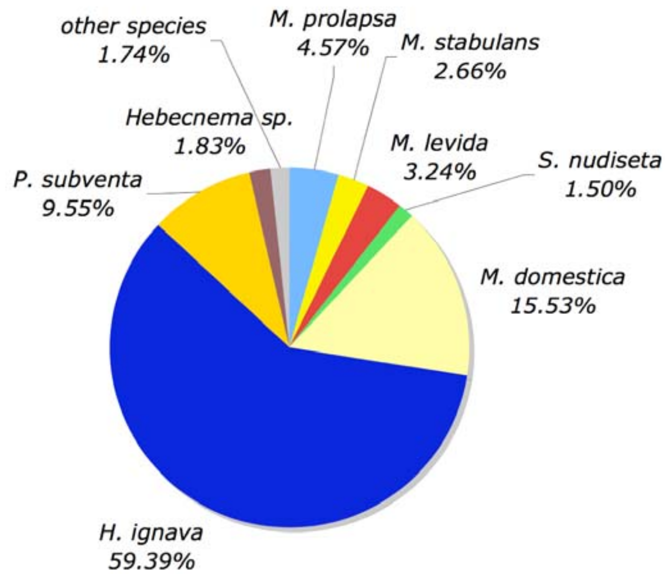


C

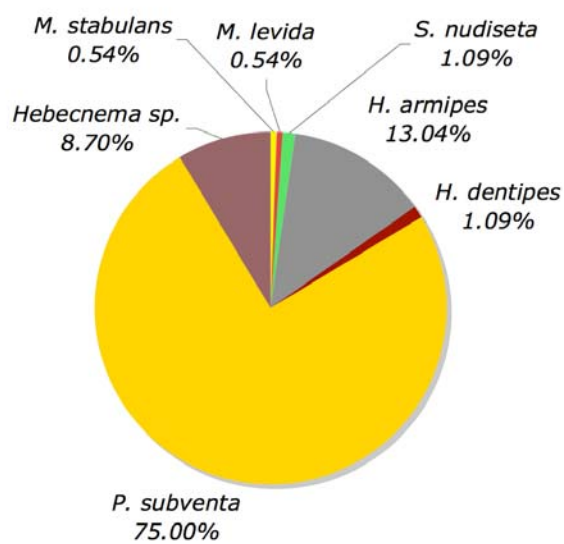


D

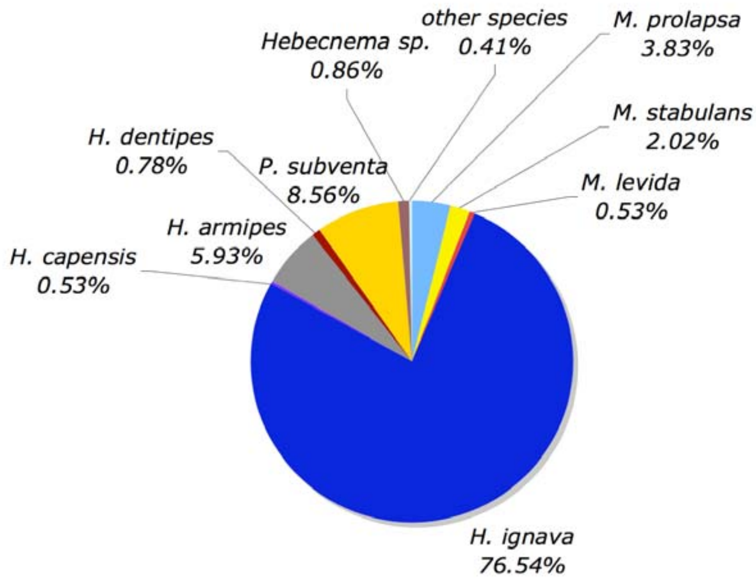
Fig. 3. Relative abundance of Calliphoridae species present in each season of the year. A) autumn (15 days); B) winter (40 days); C) spring (15 days); D) summer (12 days)


other species
1.74%
M. prolapsa
4.57%
M. stabulans
2.66%
Hebecnema sp.
1.83%
M. levida
3.24%
S. nudiseta
1.50%
P. subventa
9.55%
M. domestica
15.53%
H. ignava
59.39%

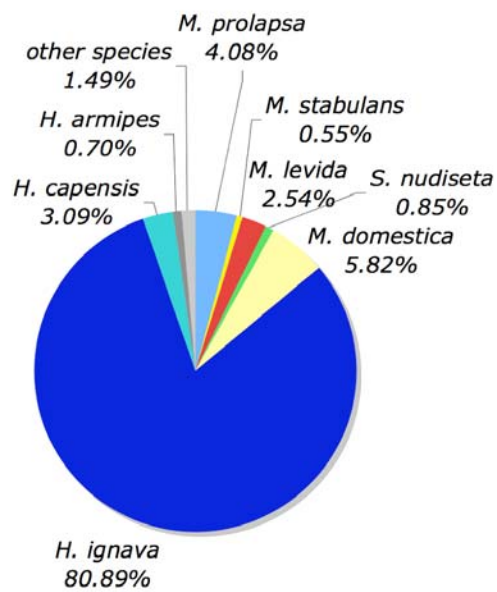
A


M. stabulans
0.54%
M. levida
0.54%
S. nudiseta
1.09%
Hebecnema sp.
8.70%
H. armipes
13.04%
H. dentipes
1.09%
P. subventa
75.00%

B



C



D

Fig. 4. Relative abundance of Muscidae species present in each season of the year. A) autumn (15 days); B) winter (40 days); C) spring (15 days); D) summer (12 days)

According to the Indicator Value method, no strong indicators were in general found for the different seasons (Appendix 2). Still, 10 Diptera species were indicators of spring, 9 were indicators of summer and 4 were indicators of autumn, whereas no indicators were found in winter. Average indicator values among all indicators recorded in spring and summer were very similar (45,3 in spring and 46,3 in summer) while in autumn indicator values were in average slightly lower (36,7) (Appendix 2). The muscid *Hydrotaea armipes* (Fallén, 1825) was the species with the highest indicator value in spring (IndVal = 74,2) followed by the sepsid *Nemopoda nitidula* (Fallén, 1820) (IndVal = 61,8). On the other hand, the Sarcophagidae family appeared as a strong indicator of summer (IndVal = 77,3), as well as the piophilid species *Piophila megastigmata* McAlpine, 1978 (IndVal = 62,7). In autumn, only few calliphorid and muscid species were found as indicators of this season, particularly *C. albiceps* and *Musca domestica* (Linnaeus 1758) (with IndVal values of 52,3 and 37,4, respectively).

Regarding the stages of decomposition within each season, the B stage recorded generally higher abundance values in relation to the other stages, both taking into account all Diptera families and only species from the Calliphoridae family. Significantly lower abundance values were generally recorded in the DR stage in relation to the B stage, particularly when accounting for all Diptera families (Fig. 5). In autumn, winter and summer the D stage also recorded higher abundance values in relation to F and DR stages, while in spring the F stage presented higher abundance of Diptera and, particularly, of Calliphoridae species (Fig. 5). In terms of species richness, B and D stages presented again in general higher species numbers (Fig. 6). Yet, values from the F stage were not significantly different from B and D in autumn and spring, inclusively recording a slightly higher average number of species than the D stage in spring (Fig. 6).

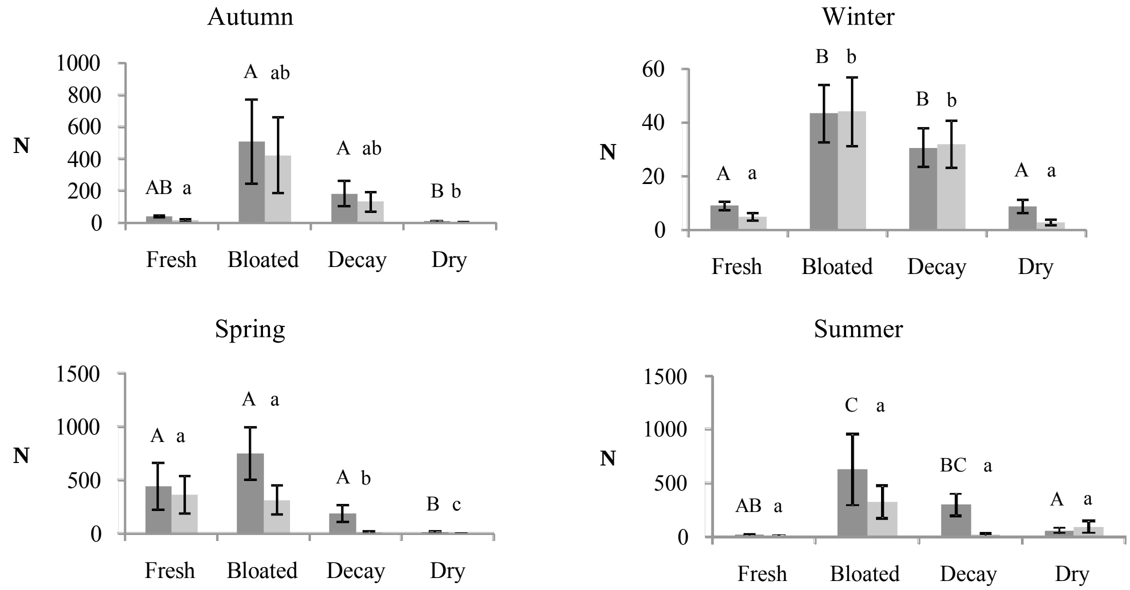


Fig. 5. Mean values of Diptera (dark grey bars) and Calliphoridae (light grey bars) abundance (N) along carcass' decomposition stages in each sampling season (error bars represent the standard error of the mean). Decay and advanced decay stages are treated as one stage (Decay). A, B and C (a, b and c) represent different groups after pair-wised Bonferroni tests between decomposition stages.

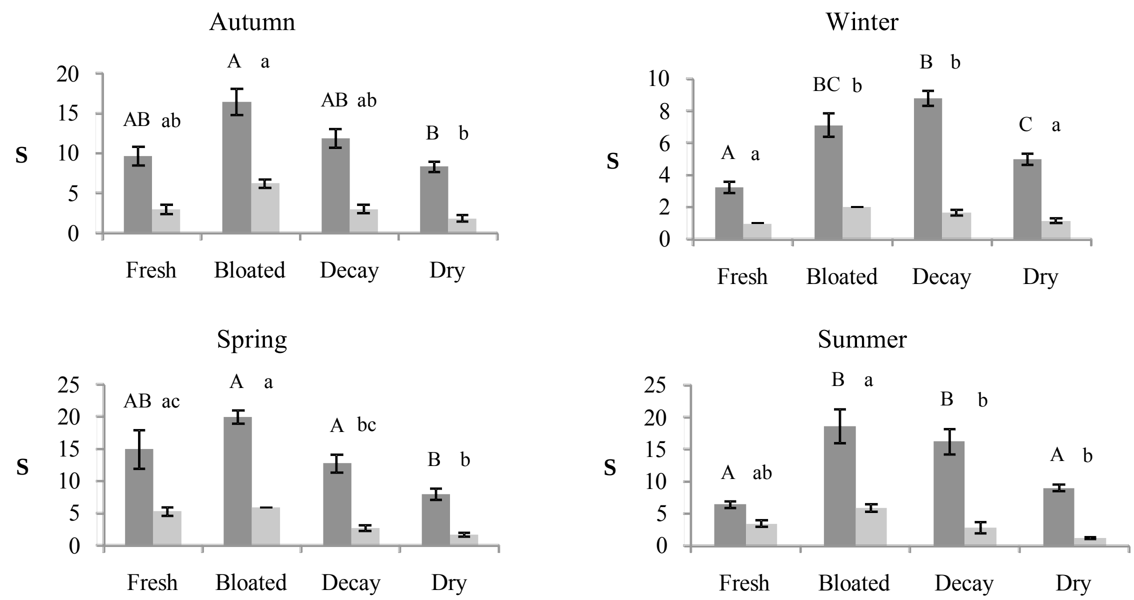
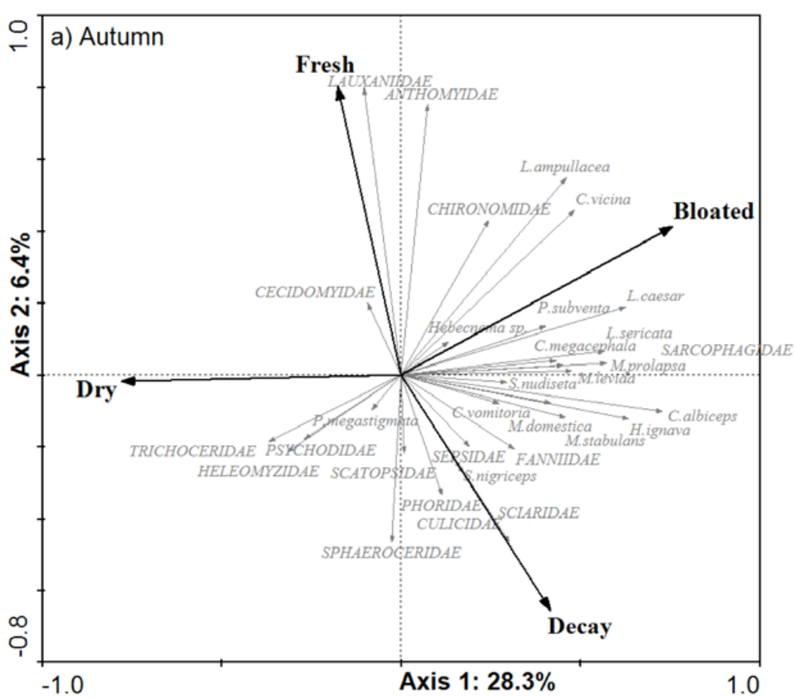
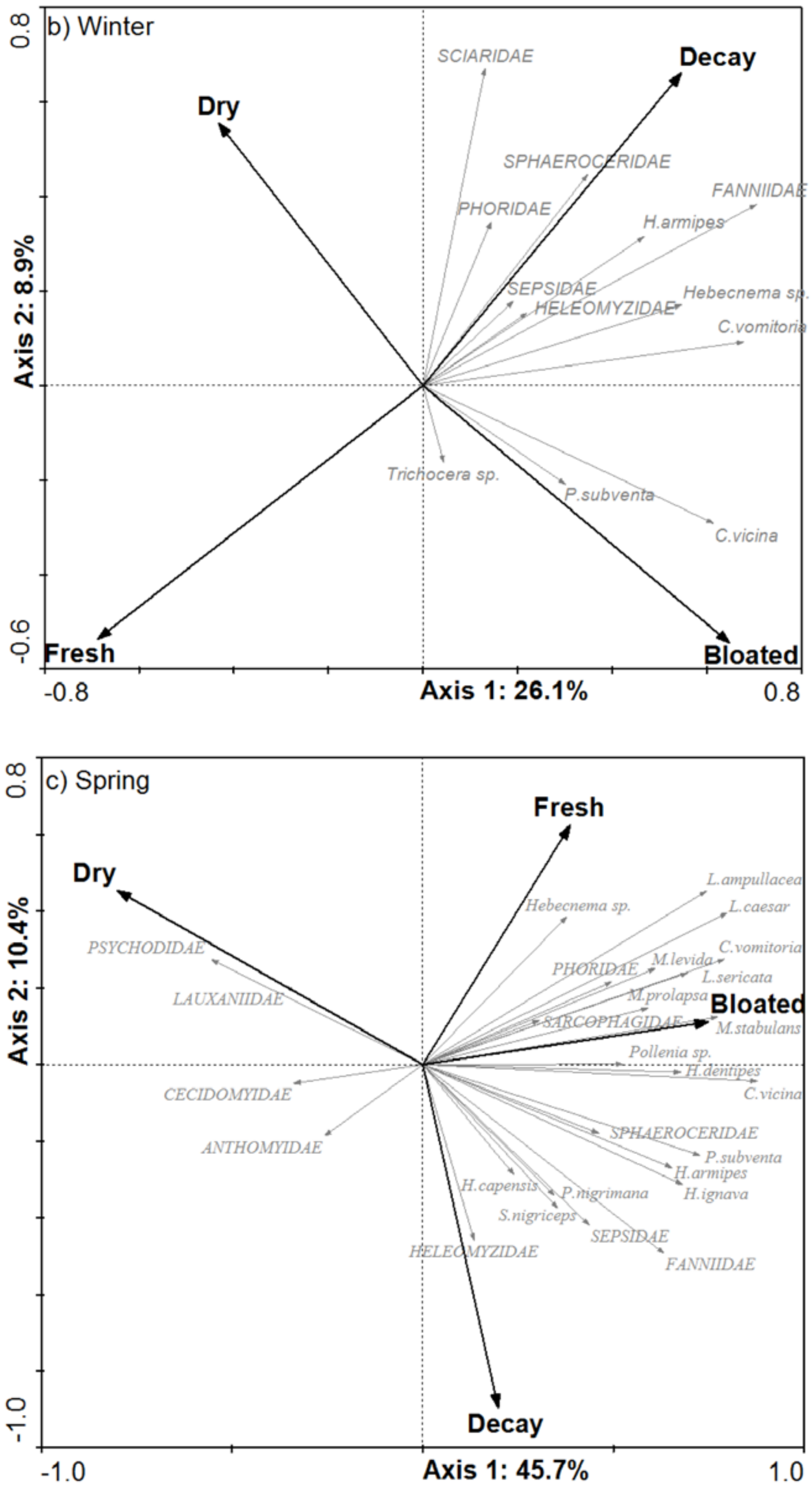


Fig. 6. Mean values of Diptera (dark grey bars) and Calliphoridae (light grey bars) species richness (S) of along carcass' decomposition stages in each sampling season (error bars represent the standard error of the mean). Decay and advanced decay stages are treated as one stage (Decay). A, B and C (a, b and c) represent different groups after pair-wised Bonferroni tests between decomposition stages.

In all sampling seasons Diptera community composition was clearly different among decomposition stages. The first two axes of the RDA, significantly explaining 34.7%, 35%, 56.1% and 49.9% of community variance in autumn, winter, spring and summer, respectively, segregated DR stage from B and D (Figs. 7a, b, c and d). These decomposition stages were clearly detached in the first RDA axis. Their separation was mainly due to the poorest occurrence and abundance of typical forensic fauna in the DR stage besides the highest relative abundance of some Diptera families not so frequent in the other stages, particularly Psychodidae (in autumn and spring), as well as Cecidomyidae, Lauxaniidae and Anthomyidae (in spring and summer). The second RDA axis also separated the stages F from D along all seasons of the year, mainly due to the higher association of Lauxaniidae family to the former, particularly in autumn and summer (this family did not occur in winter) than to the latter (Fig. 7). The second RDA axis has also showed a finer separation between stages B and D, due to the higher relative abundance of the species *C. vicina*, *L. caesar* and *L. ampullacea* in the former, and *Stearibia nigriceps* (Meigen, 1826) in the latter (Figs. 7a, c and d), although almost all the above Diptera species were absent in winter (Fig. 7b). In winter, separation between B and D was explained mainly by *C. vicina.*




b) Winter
0.8
-0.6
-0.8
0.8
Axis 2: 8.9%
Axis 1: 26.1%
Dry
Decay
Fresh
Bloated
SCIARIDAE
SPHAEROCERIDAE
FANNIIDAE
PHORIDAE
H.armipes
SEPSIDAE
HELEOMYZIDAE
Hebecnema sp.
C.vomitoria
Trichocera sp.
P.subventa
C.vicina
c) Spring
0.8
-1.0
-1.0
1.0
Axis 2: 10.4%
Axis 1: 45.7%
Fresh
Dry
Bloated
Decay
L.ampullacea
Hebecnema sp.
L.caesar
C.vomitoria
PSYCHODIDAE
PHORIDAE
M.levida
L.sericata
LAUXANIIDAE
M.prolapsa
SARCOPHAGIDAE
M.stabulans
Pollenia sp.
H.dentipes
CECIDOMYIDAE
C.vicina
SPHAEROCERIDAE
ANTHOMYIDAE
P.subventa
H.armipes
H.capensis
P.nigrimana
H.ignava
S.nigriceps
SEPSIDAE
HELEOMYZIDAE
FANNIIDAE

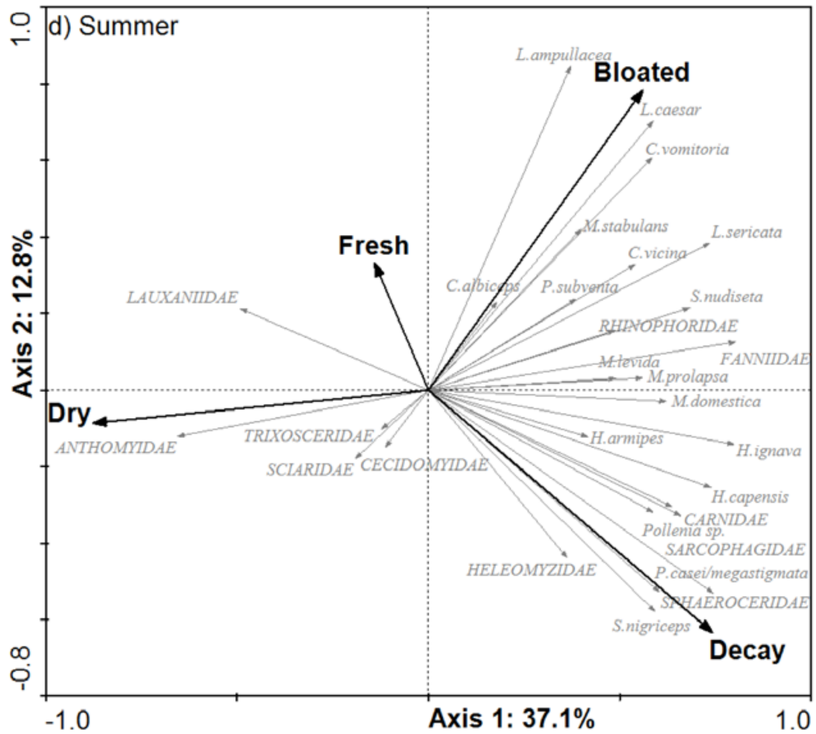


Fig. 7. RDA based on Diptera community composition from carcass decomposition stages in each sampling season: a) autumn, b) winter, c) spring and d) summer. Taxa names are abbreviated (names in caps correspond to Diptera families instead of species). Significance of RDA axes: a) Axis 1: F = 14.6 (p = 0.002), all axes: F = 7.17 (p = 0.002); b) Axis 1: F = 13.1 (p = 0.004), all axes: F = 7.55 (p = 0.002); c) F = 28.6 (p = 0.002), all axes: F = 15.3 (p = 0.002); d) F = 20 (p = 0.002), all axes: F = 12 (p = 0.002).

Diptera succession. The presence of adult and immature stages of the different species in each day of the sampling period is presented on Tables 3 to 6 .

Calliphora vicina was present all over the year. It was the dominant species in winter and scarce in summer. In autumn and spring it was present, laying eggs since the first day. Probably due to the low temperature in winter, eggs were only laid by this species on day 8. Emerging adults were caught from day 25 (autumn), 58 (winter) and 31 (spring) onwards. *Calliphora vomitoria* was also present throughout the year. It was numerous in winter, where it was the second most abundant species in the cadaver. In every case, the species arrived to the cadaver always after *C. vicina* and bred on it in all seasons of the year except in summer (Tables 3 to 6).

Table 3. Diptera occurrence matrix for autumn. Numbers refer to adults collected. From day 25 onwards, numbers are average number of specimens per day. Abbreviations: g = adult gravid females; E= eggs; L= larvae; P= pupae; t= teneral adults.

	Autumn	F			B				AD															
	Day of decomposition	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
	ANTHOMYIIDAE	7	3	6	7											1								
CALLIPHORIDAE	*C.vicina*	1	10	29	11	10	28	43	31	34	4		1				4	1	2	3			1	
		E	EL	EL	L	L	L	L	L	L	L	LP	LP	LP	P	P	P	P	P	P	P	P	P	P
	C.vomitoria						2	1	3	5	4													
									L															
	C.albiceps				21	40	951	394	487	333	454	107	4				4	4	2	7	2	11	36	13
					E		E	g	L	L	gL	L	L	L	L	LP	LPt	LPt	LPt	Pt	t	t	t	t
	C.megacephala					1	1	6	9	6														
									g															
	L.ampullacea	1	5	3	10	1	3	3	4	7									1					
			E	gL	gL	L	gL	L	L	L	L	L				P	P	P	P	P	P	P	P	P
	L.caesar		1		14	10	26	23	47	54	2						1							
			E	L	EL	L	L	gL	gL	gL	L	L				P	P	P	P	P	P	P	P	P
	L.sericata		2	1	2	4	47	48	81	44	37	15					2	2	3	1				
			E	L	L	gL	gL	L	gL	gL	gL	L				P	P	gP	gP	gP	P	P	P	P
	FANNIIDAE			1	2	1		7	43	15	33	13	3	1	2		1		1	1				
	Fannia sp.				E						g				L	L			g	g				
	HELEOMYZIDAE		2						1							2		1		1	1	7	2	10
																								g
	LAUXANIIDAE	20	6	4	1																			
MUSCIDAE	*H.ignava*				5	5	101	67	125	137	157	86	26	4	1	1	2		1			1	4	
					g		g	g	g	g	g	g				g	g					g		
	Hebecnema sp.	1		1	3			2	6	7	2											2		
					g						g													
	M.domestica			2		1	4	20	52	46	27	27	6	2										
				g				g	g		g			g										
	M.levida			1	2		3	4	7	10	8	1		3										
	M.prolapsa			1	3	1	10	3	17	10	8	2						1	1	1				
		E	L	L	L	gL	gL	L	L	L	gL	L	L	L	L	L	L	L	L	L		P	P	P
	M.stabulans					2	1	3	5	5	11	4	1				1							
						g																		
	P.subventa	1		5	7		5	25	20	32	15	1	1	1	1	1	1							
	S.nudiseta						4	2		3	7	1	1											
			E		E																			
	PHORIDAE							1	2	1					2					2	5	1	5	1
PIO	*P. megastigmata*											2												
	S.nigriceps												1	3		2	1							
	SARCOPHAGIDAE						2	1	1	1		2												
		E																						
	SEPSIDAE				1			1	1						1		1		1	2			3	
	N.nitidula																g							
	SPHAEROCERIDAE									1	1	1	3	1	4	1	1			1			2	1
													g											
	CECIDOMYIIDAE	6	3										1	2	4	1		1	1	1	1			
	CHIRONOMIDAE	1		3	7	4	1	5		4	1	1		1										
	CULICIDAE									1		6	8	3									1	
	PSYCHODIDAE																		2			1	1	1
																			g				g	g
	SCATOPSIDAE														1			1	1					
	S.notata																							
	SCIARIDAE	4			3	1		15	8	5	15	12	8	6	9	9	3	6	7	2	3	4	2	3
	TRICHOCERIDAE										1	1								1	2			
	Trichocera sp.																							

	Autumn	AD	Dry																
	Day of decomposition	25	27	29	31	33	35	37	40	43	46	49	52	55	58	61	66	71	76
	ANTHOMYIIDAE																		
CALLIPHORIDAE	*C.vicina*		1					2	2	3	1	1	3	1					1
		Pt	Pt			t	t												
	C.vomitoria								1	1									
							t												
	C.albiceps	8	1		2		1		1	1		1							
		Pt	Pt		t		t	t	tg	t									
	C.megacephala																		
	L.ampullacea			1															
		P		g			t					t							
	L.caesar								1	1									
		P										t	t	t					
	L.sericata								1	1		1							
		P	P			t	t	t	t	t		t							
	FANNIIDAE	2		1	2			1	1	1			1	1		1			
	Fannia sp.	L	L	L	gL		LP		LP	LP		t	g	t					
	HELEOMYZIDAE	2	1	5	2	3	1	1	1	1		2	1	1		1	1	1	2
		g							g										
	LAUXANIIDAE																		
MUSCIDAE	*H.ignava*	2						1											
		g																	
	Hebecnema sp.			1			2	1		1	1	1				1			
								g											
	M.domestica																		
	M.levida																		
	M.prolapsa																		
		LP	LP	P	P	P	Pt	Pt	Pt										
	M.stabulans																		
	P.subventa							2	1	3			2	1	1				
	S.nudiseta									1	1	1	1						
			L				L			tg	t	t							
	PHORIDAE	3	2				1				1			1				1	1
PIO	*P. megastigmata*		1		1														
		L	L	L	L	L	LP	L	LPt	LPt	LPt	LPt	L	LPt	Lt	LP	L	Lt	LPt
	S.nigriceps	1														A	A		
		L	L	L	L	L	LP	L	LPt	LPt	LPt	LPt	L	LPt	Lt	LPt	L	Lt	LPt
	SARCOPHAGIDAE																		
							L			t									
	SEPSIDAE	2		1				1	1							A	A	1	1
	N.nitidula	gL	L	LP	L	L	LP	LP	LPt	LPt	LPt	LP	L	P	L	t	t	t	t
	SPHAEROCERIDAE	2		1	1		1	1						1	1	1		1	1
															t				
	CECIDOMYIIDAE			1			1	1					1						1
	CHIRONOMIDAE	1	1	1	3	1	1	1	1				1	1	2	1		1	1
	CULICIDAE																		
	PSYCHODIDAE				1			1			2		1	1	1		1	1	
									L	L	L	L	L	L	g	L	L		
	SCATOPSIDAE	1															1		1
	S.notata	g										L		P		t		t	t
	SCIARIDAE	2	5	4	6	4	5	2	1	1	2	1	1	1	1	1	1	1	1
	TRICHOCERIDAE	1		1		1		2	4	1	1	1	1	1	1				1
	Trichocera sp.									g									

Table 4. Diptera occurrence matrix for winter. Numbers refer to adults collected. From day 25 onwards, numbers are average number of specimens per day. Abbreviations: g = adult gravid females; E= eggs; L= larvae; P= pupae; t= teneral adults.

	Winter	**F**																	**B**								**D**			**AD**							**Dry**					
	Day of decomposition	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**25**	**27**	**29**	**31**	**33**	**35**	**37**	**40**	**43**	**46**	**49**	**52**	**55**	**58**	**61**	**66**	**71**	**76**
CAL	*C.vicina*		1		1	1	2	1	4	2	3	4		4	9	12	6	19	26	26	24	2	17	70	23	48	19	15	18	24	6	6	2	2	1		1	1	1		1	2
CAL									E	E	E	E	E	EL	EL	EL	EL	EL	EL	EL	EL		L	g	gL	EL	L	L	P	P	P	P	P	P	P	P	P	t	t	t	t	
CAL	*C.vomitoria*																		3	2	1	1	1	13	7	8	11	4	16	11	8	1								1	1	
CAL																							EL	EL	gL	EL	g	L	L		gLP	P	P	P	P	P	P	P	t	t	t	
	FANNIIDAE																	1	1	1			1	1	2	2	6	2	6	11	5	1	4	3	1	1						
	Fannia sp.																										g		g	g				g	g					t	t	
	HELEOMYZIDAE									1				2										2		2	1	1						1	1	1		1				1
																																				g						
MUSC	*H.armipes*																	1			1			2			5		2	3	1		1	1	1							
MUSC																											g			g												
MUSC	*Hebecnema sp.*						1													1				2	1	2	1	1	2	1				1	1	1			1			
MUSC	*P.subventa*		4	6	10	7	6		3	1					1	1	1	1	1	7	1		1	5	3	7	11	5	8	8	1		1	1								
MUSC				g	g		g																	g	g		g		g	g	g											
	PHORIDAE			2										1		2		3	1			1		1	1		1		1	1	3		1	1	1	1	1	1	1	1		1
																																				g						
	SEPSIDAE				3	1	3	3	1				1		3		1			3	1		2	4	5	2	3	2	3	4	1	1	1		1	1		11	10	1	1	
	N.nitidula														g											g		g	g	g	g	g	gt	L	g	L	LP	Pt	Pt	t	t	t
	SPHAEROCERIDAE	1													1			1			1	1	1		1	1		1	2		1	1	1	2	1	1	1	1	1		1	1
																							g											g	g			g				Pt
	SCIARIDAE		1	1																	1				1		1		1	1	1		1	9	4	2	2	2	3	1	1	1
																					g														g			g		g		
	Trichocera sp.				3		1	1	1	1		1		1	1								1	1		1			2	1		1	1									

Table 5. Diptera occurrence matrix for spring. Numbers refer to adults collected. From day 25 onwards, numbers are average number of specimens per day. Abbreviations: g = adult gravid females; E= eggs; L= larvae; P= pupae; t= teneral adults.

	Spring	F			B			D	AD												Dry																	
	Day of decomposition	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	25	27	29	31	33	35	37	40	43	46	49	52	55	58	60
	ANTHOMYIIDAE													1	5	1		2	1		1		1	1					1			1	1		1	1	1		1
CALLIPHORIDAE	*C.vicina*	10	37	35	58	28	35	20	4	9	6	7	4	1	2	3	10	11	13	2	4	1						1		3	1	1	1	1			1		
		E	gL	L	L	L	L	L	L	L	L	L	L	LP	LP	LP	LP	LP	LP	LP	LP	LP	P	P	P	P	P	t											
	C.vomitoria		20	43	62	20	56	9	2										4		1												1						
			E	E		E		L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	LP			LP	t		t	t		t						
	L.ampullacea	1	173	123	39	14	9	1		1					1			1																					
		E	EL	EL	EL	L	L	L	L	L	L	L	L	LP	LP	LP	LP	LP	LP	LP	LP	LP	P	P	P	P	P	t	t	t	t		t	t					
	L.caesar	5	222	357	382	61	91	30	2	1		2																1		1		1	1						
		L	EL	EL	EL	gL	gL	gL	L	L	L	L	L	L	L	LP	LP	LP	LP	LP	LP	LP	P	P	P	P	P	t	t	t	t		t	t	t				
	L.sericata		25	27	29	2	32	26	6																														
			g				g																																
	Pollenia sp.	1	12	7	15	4	19	41	20	1	1	5	4	2	1				1	5	6	3	4	11	1	1		1	1				1	1	1				
	FANNIIDAE	1	14	40	104	12	170	158	86	20	19	26	60	33	25	11	26	25	75	35	20	4	7	11	1	1	1			3	3	7	8	1	1			1	4
	Fannia sp.			g	g		g		g	g	g	g	g	g	g	g	g	g	g				g	L		L	L	LP		t	t		t		t			t	
	HELEOMYZIDAE			1		1		1				1	1	2	4		4	1	4	2	1										1		1	1					1
												g	g	g	g		g		g	g	g																		
	LAUXANIIDAE																								1				3			1			1	1	1	1	
MUSCIDAE	*H.armipes*		1	2	26	7	34	47	15	4	2	1	2	1	2		1	2	3		3	1		1								1	1	1					
					g	g	g	g							g			g			g	g																	
	H.capensis						2	7	3	1																													
							g	g																															
	H.dentipes		1		4	6	2	3	2			1																				1							
								g																															
	H.ignava		35	54	125	58	474	534	351	88	46	38	31	19	7		4	1	20	12	6	1	2	1								1	3		10	3	2	1	1
			g		g	g	g	g	g	L		L						g													t		t		t	t	t		
	Hebecnema sp.	1	3	3	2	4	4	2			1	1						1		1	1	5	1	6	1	2						1	2	1					1
																				g																			
	M.levida		3	2	3		2	1	1	1																						1							
	M.prolapsa		11	18	21	2	15	12	5	1	5	1		1		1			2		3			6	1					1	1	1	2						1
				g	g	g	g																																
	M.stabulans	1	5	2	12	2	14	9	2				2						1																				
	P.subventa		1	21	42	26	39	48	19		2	4	2	1		3	3	10	14	2	1										1	2	3						
					g				g					g					g																				
	PHORIDAE		4	5	3		12	2	3	1									2		2	1		2					1			1							
PIO	*P.nigrimana*					2	2	12	6	1	1			1																									
	S.nigriceps					1	2	6	1	2			3					1																					
												E										L	L	L		L	L	LP		LP			LPt	LPt	LPt	t	t	t	t
	SARCOPHAGIDAE		3		2		1	4	1																			1	2							1			
	SEPSIDAE	1		3	8	12	30	6	11	6	8	7	7	5	1	5	1	10	20	19	31	9	9	19	2	1			1						1	1			
	N.nitidula	g			g	g	g	g	g	g	g	g	g	g	g	g			g	g	g	L	L	LP		LP	LP	P		P			P		g				
	SPHAEROCERIDAE	1	1	3	4	2	3	8	5	2	1	8	5	2		1		1			1	1	1	6				1			1		1		1				
				g					g			g																											
	CECIDOMYIIDAE								1	1			1					1					1		1			1	1	1	1		1	1		1			
	PSYCHODIDAE																		1		2		3	4	1	1		1	1		1	1	1	3	2	2		1	
																					g		g	g	g	g			g			g	g	g	g	g		g	

Table 6. Diptera occurrence matrix for summer. Numbers refer to adults collected. From day 25 onwards, numbers are average number of specimens per day. Abbreviations: g = adult gravid females; E= eggs; L= larvae; P= pupae; t= teneral adults.

	Summer	**F**		**B**			**D**	**AD**						**Dry**																								
	Day of decomposition	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**25**	**27**	**29**	**31**	**33**	**35**	**37**	**40**	**43**	**46**	**49**	**52**	**55**	**58**	**61**
	ANTHOMYIIDAE		1						1		2			2	1	5	6	3	1	1		2	2	2	3	4	3	1	3	3	5	2	2	7	3	4	12	12	9
CALLIPHORID	*C.vicina*		1	4	6	1	3	5				1			1				2							1			1				1						
	C.vomitoria			1	6	7	2																																
	C.albiceps	1 g			47 g	242 E	27 L	12 L	L	LP	L	LP		LP	40 t	268 t	601 t	153 t	8 t	t																			
	L.ampullacea	3 EL	2 L	3 L	4 L	2 L	L	L	L	L				LP	LP	LP		P	P	P	P	P	t			t	t		t	t									
	L.caesar	9 EL	15 EL	59 gL	224 gL	307 gL	29 L	5 L	L	L		LP	LP	LP	LP	LP		P	P	Pt	P	Pt	t	Pt	t	t	t												
	L.sericata		1	5	31 g	33 g	19	8	4		1										1																		
	Pollenia sp.					2	3	3	6	1		3											1			1									1				
	CARNIDAE					5	11	20	10	6	1																					1							
	FANNIIDAE *Fannia sp.*		2	24 g	42 g	89 g	90 g	66 g	33 g	10	2	4	3	2	2			4	1		3					2	7	1	1		1	1	1		1	3	1	2	2
	HELEOMYZIDAE						2	1	1			1	1		1	1										1													1
	LAUXANIIDAE	1	3	3	1									2	8	3	1				3	1	1	1		2	2	1	2	1	2	3	3	4	3	5	10	22 g	17
MUSCIDAE	*H.armipes*				2		1	10 g	1						2																								
	H.capensis				1 g	7	15	9 g	23 g	4	1 g	2																											
	H.ignava			2	135 g	433 g	364 g	363 g	165 g	59	29 g	62	13	20	12	10		4	3	20	2			1	1	7	1		1		1					1 t			
	M.domestica				3	21 g	59 g	29 g	4 g	1																								1 g					
	M.levida				5	15	10	16	3			1	1	2	2			2 g	1	1				3	1	3								1				1	2
	M.prolapsa	1		1	6	35 g	15 g	11	4	2	3	2	2	3	2	3 g		4		5	4	2	1	1	1	2	2	1	2		2	1	1	2		1		1	3
	M.stabulans				2	7	2																			1	1												1
	P.subventa			1	1	2		3				1						1								3										1			1
	S.nudiseta			1	2	4 g	2 g	7 g	1																														
PIO	*P. megastigmata*					1	1	4	6	5	13 E	5 E	3 E	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	LP	LP	LP	LP	LPt	LPt		t	t		
	S.nigriceps					1	4	18	18	28	8 E	10 E	4 E	4 EL	4 L	4 L	2 L	1 L	1 L	L	1 L	L	L	L	1 L	1 L	L	L	LP	LP	LP	1 LP	LP	LPt		t	t		
	RHINOPHORIDAE	1		1	2	2		1	7	3						1										1		1	1			1						1	
	SARCOPHAGIDAE			1 L	12 L	25	34	31	38	17 L	7	11 L	9 L	7 L	9	8 L	6	4 L	3	4 LP	1	4 LP	2	3 LP		4 LP	5 LP	3	6 Pt	2 Pt	5 t	2 t	1 t	2	1	1	2	2	1
	SPHAEROCERIDAE					5	13	9	37	19	14	10	8	4 g	7 g	2	7	3 g		1		1	1			1 g	1	1	3 g	1									
	TRIXOSCELIDIDAE *Trixoscelis sp.*			2					1	1	1		1	2	1		1 g	3 g	2	1	1	1		1	1	1	1	1 g	1	1	1		1	1					
	CECIDOMYIIDAE					1	1	1	2	1						1		1							1				3		3	1	4	4	4	2	2	3	1
	SCIARIDAE								4						1									1		1 g		1	1	1	1	1	1	1	1		1 g	1	

Species of *Lucilia* were present in all seasons of the year, except in winter. *Lucilia ampullacea* arrived to the cadaver always during the 1st day. In spring, it was the second most abundant species in the carcass. In the other seasons, although not very abundant, it always bred on the cadaver. *Lucilia caesar* was the dominant species in spring and summer. In spring it appeared in less than 5 minutes and laid eggs 1 hour after; in summer it arrived immediately and laid eggs in less than 1 hour; in autumn, specimens arrived on the 2nd day and in a lower number, but also bred on the corpse. *Lucilia sericata* was the second most abundant species in autumn, season in which apparently it was more successful in breeding. It arrived always in the 2nd day (Tables 3, 5 and 6).

Chrysomya albiceps was present in autumn, where it was extremely abundant, and in summer. It arrived in large numbers to the cadaver on the 4th day in both seasons and successfully bred on it. New adults started to emerge on day 16 (autumn) and 14 (summer) (Tables 3 and 6). This species is a later colonizer, in comparison with *Calliphora* spp. and *Lucilia* spp., yet, due to its shorter lifecycle, new adults emerge before every other species. *Chrysomya megacephala* (Fabricius, 1794), a recently introduced species in Portugal (Prado e Castro & García, 2009), also shows a tendency to be abundant in autumn. The species was present between days 5 and 9, with gravid females present.

Calliphoridae larval migration occurred from day 7 to 10 (mostly on day 8) in autumn, day 31 to 43 in winter (the largest number migrating from days 35 to 40), day 8 and 9 in spring (more intensely on day 8) and day 7 to 10 in summer (mainly on days 7 and 8).

Fannia sp. was present all over the year, being particularly abundant in spring, where it arrived in the 1st day. Breeding occurred in all seasons, only in summer that could not be confirmed.

Heleomyzidae was always present, with a preference for AD stages. Although gravid females were present in autumn, winter and spring, no immature stages or teneral adults were observed.

Lauxaniidae, similarly to Anthomyiidae were quite abundant in summer, especially in DR stage. However in autumn they appeared in the first days of decomposition, only adults being captured.

From the *Hydrotaea* Robineau-Desvoidy 1830 (Muscidae) group, *Hydrotaea ignava* was the dominant. It was the most abundant captured Diptera species in spring and summer, being the dominant muscid in all seasons except winter. It arrived between the 2nd and the 4th day, always after the first calliphorids, and successfully bred on the cadaver.

Musca domestica was present in autumn and summer, in both seasons being the second most abundant muscid. Bloated and decay stages were the most attractive to this species. Gravid females were captured but no immature stages or teneral adults (Tables 3 and 6).

Muscina prolapsa (Harris 1780) was the most abundant from the *Muscina* group, and the one that, with no doubts bred on the carcass. In autumn, eggs of this species were collected on the 1st day. In spring and summer it was probably also successful breeder, since gravid females were caught and in late decomposition stages adults were also captured by the trap, which probably represents a second generation (Tables 3, 5 and 6).

Phaonia subventa was captured throughout the year. It was the dominant muscid in winter and had important abundances in autumn and spring. It was present in the carcasses generally since the F stage, but more abundantly in B and decay stages. Many gravid females were present, mainly in winter, but we are uncertain if breeding was successful.

Synthesiomyia nudiseta (van der Wulp, 1883), an introduced species previously unrecorded in Portugal, was present in low numbers in autumn and summer. Eggs of this species were collected from the 2nd day in autumn and a second generation emerged from day 27 to 52 (Table 3); in summer, adults were present from the 3rd day, with gravid females, but no teneral adults were captured (Table 6).

Piophila megastigmata, another novel record for Portugal, as well as a very recent finding in Europe (Martín-Vega *et al.*, 2011) and *Stearibia nigriceps* (Piophilidae) arrived to the carcass in autumn on days 11 and 12, respectively. Larvae of these species began to be most visible (skipping) on the 25th day and new emerged flies emerged from days 61 to 66 in the field, while in the laboratory flies emerged from puparia from day 40 onwards. In spring, *S. nigriceps* appeared on day 5, immature stages were also observed, with teneral adults emerging only in the lab, from day 43. The two species arrived to the carcass on the 5th day in summer, and eggs were collected from day 10 however, in this season, no larval activity of these species could be observed in the carcass, only in the laboratory. Teneral adults started to emerge on day 43 (*P. megastigmata*) and 46 (*S. nigriceps*) (Tables 3, 5 and 6).

Sarcophagidae was especially abundant in summer, breeding successfully; in autumn, although present in low numbers, they could also breed on the cadaver (Tables 3 and 6).

Nemopoda nitidula was present in all seasons, except in summer. Even though adults appeared quite early, larvae were only present in later decomposition stages, as in the case of Piophilidae (Tables 3 to 5).

Discussion

The process of decomposition was clearly affected by the season of the year, not only in length of stages but also in some faunal composition characteristics.

The duration of the decomposition stages differed in the four seasons of the year, especially in winter, due to the lower temperatures that retarded decomposition and insect activity. In this season, flies only oviposited on day 8 (máx. temp. = 12.16ºC), even though similar and even higher temperatures occurred in the days before. In autumn and spring, the decomposition pattern was similar, due to similar temperatures, especially in the first week. However, in autumn temperatures were decreasing with time, which lead to a longer AD stage. Summer was the season in which decomposition was faster, because of higher temperatures. The carcass was dry in less than 2 weeks.

Winter, as expected, was the season with the lowest values of abundance and richness, showing that cold limits Diptera activity. Other European studies on carrion communities characterization (Arnaldos *et al.*, 2004; Matuszewski *et al.*, 2010b) demonstrated that spring was the richest season of the year. In our case, spring yielded the highest values of Diptera abundance, but autumn was the richest season. Among decomposition stages, in general, B stage presented the highest abundance and richness regarding Diptera communities.

Stages of decomposition are usually recognized and defined visually in most forensic entomological works, for a convenient description of a process known as continuous (Boulton & Lake 1988). The composition of Diptera community certainly changes with carcass decomposition along the days of exposure. In this study, through multivariate analysis it was showed that Diptera communities were clearly different among decomposition stages. The occurrence and abundance of species/ families formed specific groups that separated the stages of decomposition. These results, therefore, support the underlying assumption that different Diptera communities may segregate the *a priori* visually defined stages of decomposition.

The most abundant flies were Calliphoridae (autumn and winter) and Muscidae (spring and summer). The high numbers of adult Muscidae did not represent, however, dominance. Calliphorids were the most successful breeding on the cadaver, monopolizing soft tissues removal, which is a general conclusion of most studies in various geographical areas (Anderson & VanLaerhoven, 1996; Grassberger & Frank, 2004; Matuszewski *et al.*, 2008, 2010a; Battán Horenstein *et al.*, 2010). In winter, *C. vicina* and *C. vomitoria* larvae were the only calliphorids present in the cadaver. In the other seasons is outstanding the presence of a very high diversity of Calliphoridae species breeding on the carcasses (Tables 3 to 6, e.g. 6 species in autumn). While *Lucilia* spp. were not present in winter, *Calliphora* spp. were not able to breed in the carcass in summer. *Chrysomya albiceps*, when present (autumn and summer) colonized the carcass later than other calliphorids and was always very abundant, their adults emerging before those of other species.

The taxonomic spectrum of summer Calliphoridae was the most similar to the one reported from end of spring in Prado e Castro *et al.* (2011b) from another region in Portugal (Coimbra). In the spring experiment of the present study, *C. albiceps* does not occur, while in the summer one it does, as one of the dominant species, similarly to Prado e Castro *et al.* (*op. cit.*), in which experiments started at the end of May. This is related to the period of activity of the species, which seems to start around May. *Lucilia caesar*, the dominant species in the present study, was the second most abundant in Prado e Castro *et al.* (*op. cit.*), where it was surpassed by *C. vomitoria*. Even though some particular differences may exist between these two studies, there is a common pattern, in which *L. caesar* and *L. ampullacea* have a marked constant presence (except in winter), while *L. sericata* has a much more discrete occurrence. This agrees with the information reported in Cainé *et al.* (2009) for human cadavers in open conditions in the north of Portugal and is different from most of the studied regions of the Iberian Peninsula (Martínez-Sánchez *et al.* 2000, 2005; Arnaldos *et al.* 2001; Castillo Miralbes 2002; García-Rojo 2004), where *L. sericata* is the dominant fly in all types of habitat, regardless the degree of anthropization. The group of Calliphoridae species collected in this study is very similar however, to the one presented from the north of Spain (Moneo Pellitero & Saloña Bordas, 2007).

Regarding the dominant muscid, *H. ignava*, abundant from April to November, and *M. prolapsa*, present in the same period, they are recorded for the first time breeding in cadavers in the Iberian Peninsula.

Piophila megastigmata and *S. nigriceps* (Piophilidae) were found laying eggs as soon as 10 days after death in summer and teneral adults could emerge about 30 days later. *Nemopoda nitidula* (Sepsidae) is another tiny fly and successful breeder with periods of activity in the carcass similar to Piophilidae. However, Sepsidae species were not present in summer, while Piophilidae species did not appear in winter. There are very few records of species from these families in carrion succession studies in the Iberian Peninsula (Castillo Miralbes, 2002; Martín-Vega *et al.*, 2011), which demonstrates the present lack of knowledge about these groups in this geographical area.

Two species recently introduced in Portugal were found in this study. *Chrysomya megacephala* (Calliphoridae) present mainly in autumn, in low numbers and apparently not yet found developing in the carcasses (Prado e Castro & García, 2009) and the muscid *S. nudiseta*, present in autumn as well as summer and breeding on carcasses. These two species have a limited distribution in Europe, being still restricted to Portugal, Spain (Carles-Tolrá, 2002) and Malta (Ebejer & Gatt, 1999; Ebejer, 2007). The presence of *P. megastigmata* is also a novel finding. This species is abundant and well established in Portugal, as it is in Spain, raising the possibility of having been introduced in Europe some time ago and maybe confused with *Piophila casei* (Linnaeus, 1758) (Martín-Vega *et al.*, 2011).

It is interesting to note that some species, despite being present in low numbers, bred on the cadaver [e.g. *M. prolapsa*, *S. nudiseta*, *P. megastigmata* (in autumn), *L. ampullacea* (in summer)]. On the other hand, some species do not seem to be able to breed on the cadaver, despite its abundance and the presence of gravid females (e.g. *M. domestica* and *P. subventa*). This is probably explained by their biology and the fact that the latter species are not strictly necrophagous. The same occurred with Heleomyzidae and Lauxaniidae, of which only adults were caught.

IndVal method designates species that are reliable indicators of each season of the year, based on the analysis of adult specimens. This method had successfully found true forensic indicators (e.g., *C. megacephala* in autumn and Sarcophagidae spp. in summer), although caution is needed while interpreting the results recorded for some species, particularly those that were collected in the samples but did not breed on the carcasses (e.g. *H. armipes*). Furthermore, some species were considered indicators of a certain season but have not exclusively occurred there (e.g. *C. vicina*, *C. vomitoria*, *C. albiceps*, *L. casesar*). The numeric results contrast with the field observations, highlighting the importance of the collector in the interpretation of data in this kind of experiments.

The information presented in this work is the first reporting seasonality and succession of sarcosaprophagous Diptera in Portugal. Data on the phenology of species is crucial in estimating the period (season, months) of death in cases of long PMI if empty puparia of these flies are found (Vanin *et al.*, 2008). Moreover, species patterns of succession and the periods of time each life stage spends on the cadaver in a particular region and climatic situation (season) is fundamental information to be used as reference in the analysis of forensic cases in this geographical area.

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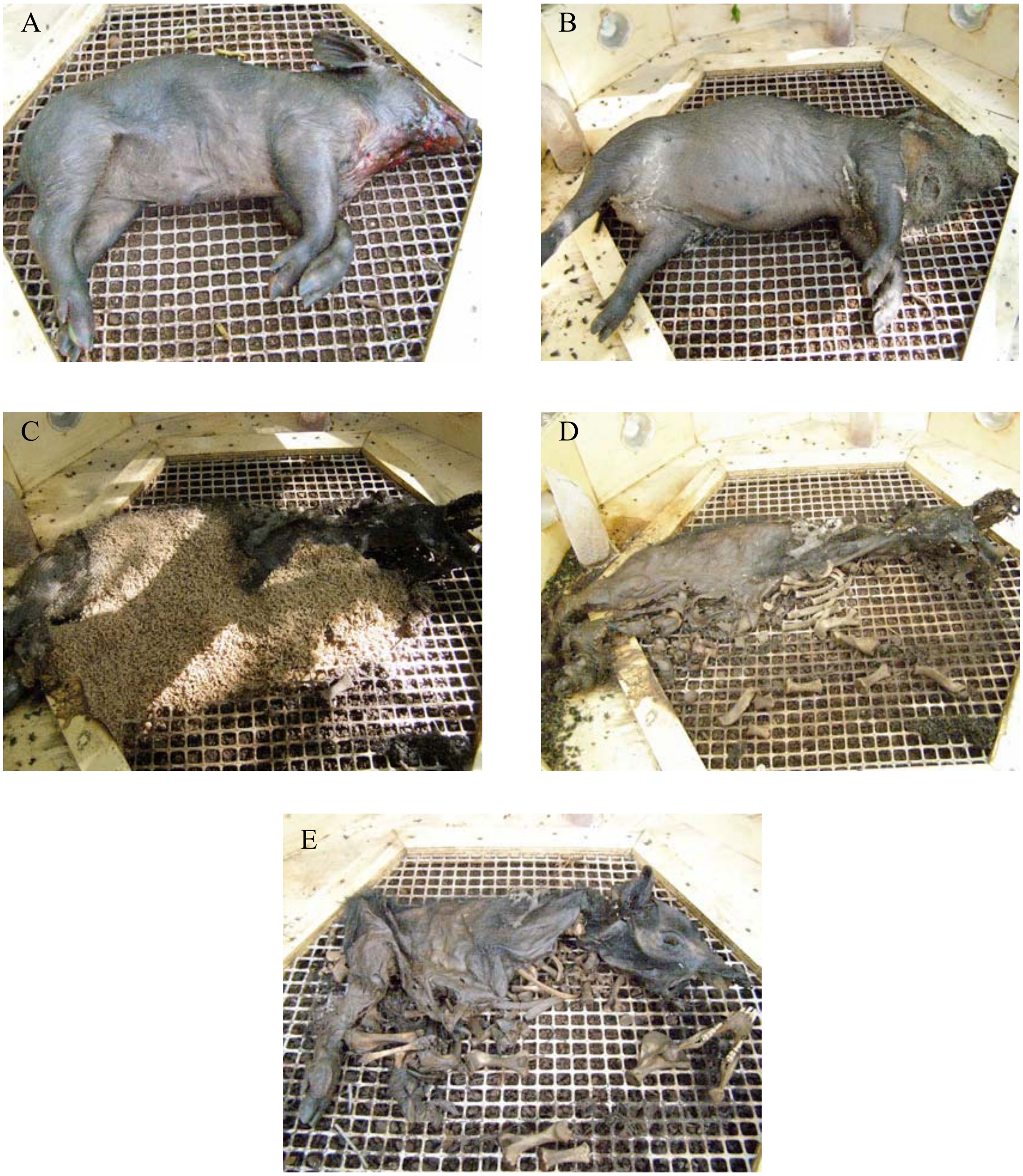
Appendix 1. Decomposition stages throughout spring experiment: A- Fresh stage (day 1); B- Bloated stage (day 5); C- Decay stage (day 7); D- Advanced decay stage, (day 8); E- Dry stage (day 15).



Appendix 2. Indicator species (indicator values according to Dufrêne & Legendre, 1997) obtained for the different sampling seasons (Aut – autumn, Win – winter, Spr – spring, Sum summer) regarding the most abundant Diptera species collected in the pig carcasses. Only significant IndVal values (i.e. superior to 25) were included. The maximum indicator value of each species is indicated in bold. Nij – Total abundance of individuals of each species collected among all samples divided by the total number of samples; B – Frequency of each species among all samples divided by the total number of samples; IndVal – Nij (of each season) / SUM (Nij of all seasons) * B * 100.

	Nij				SUM	Autumn			Winter			Spring			Summer		
	Aut	Win	Spr	Sum		Nij/SUM	B	IndVal	Nij/SUM	B	IndVal	Nij/SUM	B	IndVal	Nij/SUM	B	IndVal
C. vicina	13,5	10,1	17,3	1,8	42,5	0,3	0,7		0,2	0,9		0,4	1	**40,6**	0	0,6	
C. vomitoria	1	2,2	14,1	1,3	18,7	0,1	0,3		0,1	0,4		0,8	0,5	**35,3**	0,1	0,3	
C. albiceps	186,1	0	0	27,4	213,5	0,9	0,6	**52,3**	0	0		0	0		0,1	0,4	
C. megacephala	1,5	0	0	0	1,5	1	0,3	**33,3**	0	0		0	0		0	0	
L. ampullacea	2,5	0	24,1	1,2	27,8	0,1	0,6		0	0		0,9	0,6	**52,1**	0	0,4	
L. caesar	11,8	0	76,9	54	142,7	0,1	0,5		0	0		0,5	0,7	**35,9**	0,4	0,6	
L. sericata	18,7	0	9,8	8,5	37	0,5	0,7	**33,7**	0	0		0,3	0,5		0,2	0,7	
H. ignava	47,7	0	124	135,4	307,1	0,2	0,8		0	0		0,4	0,9	35	0,4	0,8	**36,7**
M. domestica	12,5	0	0	9,8	22,2	0,6	0,7	**37,4**	0	0		0	0		0,4	0,5	
M. levida	2,6	0	0,9	4,3	7,7	0,3	0,6		0	0		0,1	0,5		0,6	0,6	**32,1**
M. prolapsa	3,7	0	6,2	6,8	16,7	0,2	0,6		0	0		0,4	0,8	29,7	0,4	0,9	**37,5**
M. stabulans	2,1	0	3,3	0,9	6,3	0,3	0,5		0	0		0,5	0,6	31	0,1	0,3	
P. subventa	7,7	2,5	13,9	0,7	24,7	0,3	0,9	26,9	0,1	0,6		0,6	0,8	**44,9**	0	0,4	
S. nudiseta	1,2	0	0	1,4	2,6	0,5	0,4		0	0		0	0		0,5	0,5	**27,1**
H. armipes	0	0,5	9,6	1,2	11,2	0	0		0	0,3		0,9	0,9	**74,2**	0,1	0,3	
H. capensis	0	0	0,9	5,2	6	0	0		0	0		0,1	0,3		0,9	0,7	**57,1**
H. dentipes	0	0	1,3	0	1,3	0	0		0	0		1	0,5	**46,7**	0	0	
Fannia sp.	8,1	1,2	51,9	30,4	91,6	0,1	0,7		0	0,4		0,6	1	**56,7**	0,3	0,9	30,4
P. casei	0	0	0	0,6	0,6	0	0		0	0		0	0		1	0,2	
P. megastigmata	0,1	0	0,1	3,2	3,4	0	0,1		0	0		0	0,1		0,9	0,7	**62,7**
S. nigriceps	0,4	0	1	7,6	9	0	0,2		0	0		0,1	0,4		0,8	0,7	**56,3**
Sarcophagidae	0,5	0	0,7	15,4	16,6	0	0,3		0	0		0	0,3		0,9	0,8	**77,3**
N. nitidula	0,3	3,5	7,3	0	11,1	0	0,3		0,3	0,7		0,7	0,9	**61,8**	0	0	
Sphaeroceridae	0,8	0,6	3,1	9,6	14	0,1	0,5		0	0,5		0,2	0,9		0,7	0,7	**45,6**
Psychodidae	0	0	0	0	0	0	0		0	0		0	1,0		0	0	
S. notata	0,1	0	0	0	0,1	1	0,1		0	0		0	0		0	0	

Chapter 4

ADDITIONS TO THE CALLIPHORIDAE (DIPTERA) FAUNA FROM PORTUGAL, WITH DESCRIPTION OF NEW RECORDS

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Additions to the Calliphoridae (Diptera) fauna from Portugal, with description of new records

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ABSTRACT

Faunistic information is presented for 10 species of Calliphoridae flies from Portugal: *Calliphora vicina* Robineau-Desvoidy, 1830; *Calliphora vomitoria* (Linnaeus, 1758); *Chrysomya albiceps* (Wiedemann, 1819); *Lucilia ampullacea* Villeneuve, 1922; *Lucilia caesar* (Linnaeus, 1758); *Lucilia illustris* (Meigen, 1826); *Lucilia sericata* (Meigen, 1826); *Lucilia silvarum* (Meigen, 1826); *Melinda viridicyanea* (Robineau-Desvoidy, 1830) and *Protophormia terraenovae* (Robineau-Desvoidy, 1830). Six of these species are reported for the first time for Portugal and it is the first record of the 10 species for Portuguese mainland. The material studied was collected from rabbit and piglet cadavers, in experiments related to forensic entomology, in Coimbra and Lisbon.

Key words: Diptera, Calliphoridae, *Calliphora*, *Chrysomya*, *Lucilia*, *Melinda*, *Protophormia*, faunistic, new records, Portugal.

Contribución a la fauna de Calliphoridae (Diptera) de Portugal, con nuevas citas

RESUMEN

Se presenta información faunística de diez especies de dípteros de la familia Calliphoridae, de Portugal: *Calliphora vicina* Robineau-Desvoidy, 1830; *Calliphora vomitoria* (Linnaeus, 1758); *Chrysomya albiceps* (Wiedemann, 1819); *Lucilia ampullacea* Villeneuve, 1922; *Lucilia caesar* (Linnaeus, 1758); *Lucilia illustris* (Meigen, 1826); *Lucilia sericata* (Meigen, 1826); *Lucilia silvarum* (Meigen, 1826); *Melinda viridicyanea* (Robineau-Desvoidy, 1830)

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and *Protophormia terraenovae* (Robineau-Desvoidy, 1830). Seis de las especies se citan por primera vez para Portugal y es el primer registro de las diez para Portugal peninsular. El material estudiado fue recogido de cadáveres de conejo y cerdo en experimentos de entomología forense, en las localidades de Coimbra y Lisboa.

Palabras clave: Diptera, Calliphoridae, *Calliphora*, *Chrysomya*, *Lucilia*, *Melinda*, *Protophormia*, faunística, primeras citas, Portugal.

INTRODUCTION

Calliphoridae is a large family of Diptera that contains over 1000 described species (SMITH, 1986), of which about 115 are present in Europe (OOSTERBROEK, 2006).

Calliphorids have very diverse habits, especially the larvae. While the adults generally visit flowers, faeces and carrion, there are species whose larvae feed on dead animals, faeces or other decaying organic matter, some can feed on bird's blood, others are associated with living snails, earthworms and insect larvae, acting as parasitoids or predators (ROGNES, 1998). Larvae can also infest living vertebrate animals, including humans, feeding on dead or living tissues, a process called myiasis, which can lead to death of the host (ZUMPT, 1965; ROGNES, 1998).

These flies have a high hygienic importance, as they are vectors of bacteria, viruses, protozoans and helminths, causing various diseases (ROGNES, 1998). They are also very important in the process of organic matter decomposition since they are the initial and main consumers of carrion. In fact, Calliphoridae are considered the most important group of flies used as indicators of the postmortem interval in forensic entomology (LANE, 1975; SMITH, 1986; GREENBERG, 1991). In this respect, the knowledge of species composition of this particular fauna is fundamental for each region because, although many families of carrion Diptera are relatively ubiquitous, the individual species involved in decomposition vary from region to region, as well as their times of arrival (ANDERSON, 2001).

In the Iberian Peninsula, 46 Calliphoridae species are recorded for Spanish mainland (MARTÍNEZ-SÁNCHEZ *et al*., 2002, 2005), but in continental Portugal only 12 are registered to be present (MARTÍNEZ-SÁNCHEZ *et al*., 2002; PRADO E CASTRO & GARCÍA, 2009), although 19 species have been referred for the country (mainland Portugal and islands) (MARTÍNEZ-SÁNCHEZ *et al*., 2002). Even though the presence of some species should be obvious in Portugal, no bibliographic sources could give reliable information about it (MARTÍNEZ-SÁNCHEZ *et al*., 2002).

Table I. Calliphoridae species records from Portuguese mainland and islands: A- MARTÍNEZ-SÁNCHEZ *et al*. (2002); B- PRADO E CASTRO (2005); C- CAINÉ *et al*. (2009); D- PRADO E CASTRO & GARCÍA (2009).
Tabla I. Especies de Calliphoridae presentes en Portugal continental e insular: A- MARTÍNEZ-SÁNCHEZ *et al*. (2002); B- PRADO E CASTRO (2005); C- CAINÉ *et al*. (2009); D- PRADO E CASTRO & GARCÍA (2009).

	PORTUGAL		
Species	**Mainland**	**Azores**	**Madeira**
Calliphora vicina	B,C	A	A
Calliphora vomitoria	B,C	A	A
Chrysomya albiceps	B,C	A	A
Chrysomya megacephala	D		A
Lucilia ampullacea	B,C		
Lucilia caesar	B,C		
Lucilia illustris	B,C		
Lucilia sericata	B,C	A	A
Lucilia silvarum	B		
Protophormia terranovae	B		
Pollenia angustigena	A		
Pollenia bicolor	A		
Pollenia contempta	A		
Pollenia intermedia		A	
Pollenia pediculata	A		
Pollenia ponti	A		
Pollenia rudis	A	A	A
Pollenia verneri	A		
Rhinia apicalis		A	
Rhyncomyia columbina	A		
Rhyncomyia cuprea	A		
Rhyncomyia impavida		A	
Rhyncomyia felina	A		
Stomorhina lunata	A	A	A

Recently in Portugal, PRADO E CASTRO (2005, and unpublished data) has performed several experiments concerning the study of insect composition and faunal succession on cadavers and its use in forensic entomology. These studies were the first in the country dealing with sarcosaprophagous fauna, and therefore are bringing some novelties, namely, 9 Calliphoridae species were reported for the first time for continental Portugal (PRADO E CASTRO, 2005).

In the same country, CAINÉ et al. (2006, 2009) have made genetic identification of some Calliphoridae species, mainly from immature stages collected from human bodies. Seven Calliphoridae species were identified from the north of Portugal; however, faunistic information supplied is very poor.

Table I summarizes the records of Calliphoridae in Portugal (mainland, Azores and Madeira islands).

Due to the character of PRADO E CASTRO (2005) work, to the fact that CAINÉ *et al*. (2006) do not present faunistic data and CAINÉ *et al*. (2009) present very insufficient information regarding basic data on species, we did not consider them as valid records of species. Consequently, even though several species have been mentioned in those works, they are treated here as first records.

The aim of this paper is to provide previously unavailable faunistic information about 10 Calliphoridae species collected in Portugal mainland, and thus contributing to a better knowledge of this Diptera family in the country. New records are provided as well as some data about distribution and biology.

MATERIAL AND METHODS

Fieldwork was conducted in the city of Coimbra (UTM coordinate 29TNE45), at Escola Superior Agrária de Coimbra (May 2003) and Jardim Botânico da Universidade de Coimbra (from May to September 2004) and in Lisbon (UTM coordinate 29SMC88), at Instituto Superior de Agronomia (from October 2006 to August 2007). Localities are indicated in Figure 1. All the sites are forested areas inside the city.

The experiments consisted on leaving a dead animal in the field (a rabbit in the first experiment and piglets in the others) and during periodical visits, collecting the insects that colonized the body, both adult and immature forms. This was made daily in the first three to four weeks and afterwards in alternate days.

In the 2003 experiment, the collection of insects from the rabbit carcass was made by manual sampling. In the other experiments, a version of Schoenly trap designed to collect sarcosaprophagous arthropods (PRADO E CASTRO *et al*., 2009) was used, with piglets as bait. In the 2004 experiment, two traps, each one baited with a 5 kg piglet, were used during 4 months. In 2006-2007, four different experiments of 10 weeks were performed, with four different piglets, in order to cover all seasons of the year. LEECH (1966) solution was used to kill and preserve the arthropods in the trap, after which they were moved to 80% ethanol. The adult Calliphoridae collected, which are focused in the present work, have been identified based on GONZÁLEZ-MORA & PERIS (1988); GONZÁLEZ-MORA (1989) and PERIS & GONZÁLEZ-MORA (1991).

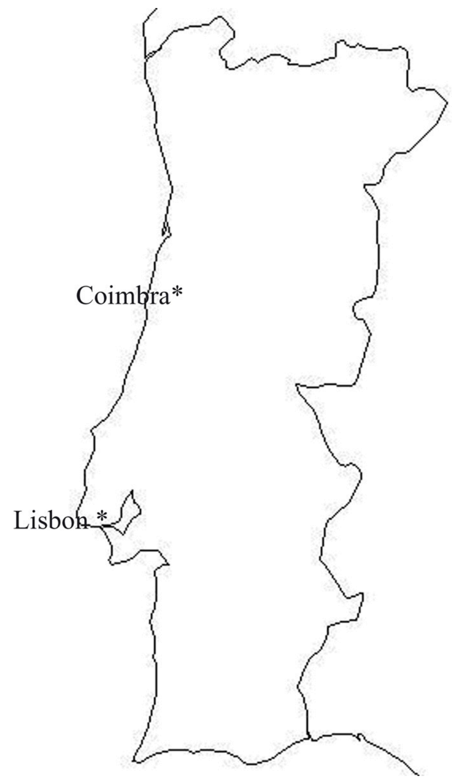

Figure 1. Portugal map, with study sites indicated.
Figura 1. Mapa de Portugal con la situación de las localidades de estudio.

RESULTS AND DISCUSSION

Subfamily Calliphorinae
Genus *Calliphora* Robineau-Desvoidy, 1830

***Calliphora vicina* Robineau-Desvoidy, 1830**

Cosmopolitan species widely distributed all over the world and closely related with human activity (ZUMPT, 1965; GREENBERG, 1971; GONZÁLEZ-MORA, 1989; MARTÍNEZ-SÁNCHEZ *et al*., 2002).

Adults are attracted to fruit, decaying meat and faeces; larvae are mainly necrophagous, usually developing in carrion (ZUMPT, 1965; GREENBERG, 1971). In Portugal this species is present all over the year and is one of the most important carrion breeders (PRADO E CASTRO, 2005).

Material examined: Coimbra: 31.5.2003, 1♀; from 28.5.2004 to 25.9.2004, 1357♀, 936♂; Lisbon: from 19.10.2006 to 2.1.2007, 207♀, 34♂; from 19.1.2007 to 3.4.2007, 495♀, 85♂; from 17.4.2007 to 10.6.2007, 209♀, 106♂; from 29.6.2007 to 30.7.2007, 17♀, 8♂; 9.8.2007, 1♂

***Calliphora vomitoria* (Linnaeus, 1758)**

Species distributed trough the Holartic (ZUMPT, 1965; GREENBERG, 1971; GONZÁLEZ-MORA, 1989; MARTÍNEZ-SÁNCHEZ *et al*., 2002) and Australian regions (ROGNES, 2004). It is more rural in distribution than *C. vicina* (SMITH, 1986). Like *C. vicina*, it is a saprophagous species (GREENBERG, 1971) and a successful breeder in cadavers (PRADO E CASTRO, 2005) that seems to be present practically all year.

Material examined: Coimbra: 31.5.2003, 1♀, 1♂; from 28.5.2004 to 4.7.2004, 1415♀, 952♂; 14.7.2004, 1♀; 10.9.2004, 1♀, 1♂; Lisbon: from 24.10.2006 to 28.10.2006, 14♀, 3♂; from 27.11.2006 to 30.11.2006, 4♀; from 4.2.2007 to 26.2.2007, 142♀, 5♂; from 24.3.2007 to 29.3.2007, 1♀, 2♂; from 18.4.2007 to 29.5.2007, 159♀, 59♂; from 30.6.2007 to 3.7.2007, 10♀, 6♂.

Subfamily Chrysomyinae

Genus *Chrysomya* Robineau-Desvoidy, 1830

***Chrysomya albiceps* (Wiedemann, 1819)**

Chrysomya albiceps can be found from the southern Palaearctic region (southern Europe, Arabia, India), throughout Africa (ZUMPT, 1965) and America where, since its introduction (GUIMARÃES *et al*., 1978), its range is rapidly expanding to northern areas (BAUMGARTNER & GREENBERG, 1984). Within Europe, the species is very abundant in Spain (MARTÍNEZ-SÁNCHEZ *et al*., 2002) and is expanding north, having reached countries as France, Switzerland, Austria (GRASSBERGER *et al*., 2003), Ukraine (VERVES, 2004) and Poland, its actual northern extreme (SZPILA *et al*., 2008).

Chrysomya albiceps normally breeds in carrion, hatched larvae feed on exudations of the decomposing flesh, but the second and third larval stages are predaceous, feeding on other blowfly larvae (ZUMPT, 1965). This behavior makes them good competitors and may eventually lead to a decline in numbers of the native species population (GUIMARÃES *et al*., 1978; MARILUIS & SCHNACK, 1986; GRASSBERGER *et al*., 2003; BATTÁN HORENSTEIN *et al*., 2007)

In Portugal it was observed that they act as secondary flies in sarcosaprophagous succession, arriving after *Calliphora* and *Lucilia* spp., they are very abundant in the end of spring, summer and autumn, being strong carrion breeders (PRADO E CASTRO, 2005). *Chrysomya albiceps* was captured from April to December.

Material examined: Coimbra: 31.5.2003, 4♀, 6♂; from 30.5.2004 to 29.8.2004, 2271♀, 922♂; Lisbon: from 22.10.2006 to 6.12.2006, 2611♀, 271♂; 22.4.2007, 1♂; from 28.6.2007 to 15.7.2007, 713♀, 685♂.

Genus *Protophormia* Towsend, 1908

***Protophormia terraenovae* (Robineau-Desvoidy, 1830)**

New species record for Portugal. *Protophormia terraenovae* is a Holartic species but, although very common in the northern parts, in the southern parts it is more or less restricted to higher altitudes (ZUMPT, 1965). There is only one previous published reference to this species in Spain, from Viella (Lérida province), where it is considered rare (GONZÁLEZ-MORA & PERIS, 1988). *Protophormia terraenovae* was also collected recently, in Madrid city, by MARTÍNEZ-SÁNCHEZ *et al*. (2007), which collected an empty pupa in a human corpse.

The presence of *Protophormia terraenovae* in Coimbra confirms its presence in the occidental slope of the Iberian Peninsula.

Protophormia terraenovae is a saprophagous species (ZUMPT, 1965; GREENBERG, 1971), its development is favored by cool weather and is the most cold tolerant of all calliphorid species (BYRD & CASTNER, 2001). It is a forensically important blowfly in many regions (ANDERSON, 1995; BYRD & CASTNER, 2001), including European cool regions (SMITH, 1986; LECLERCQ & VERSTRAETEN, 1993; BENECKE, 1998), but also has records from more southern locations at low altitude, as Brindisi (Italy) (INTRONA *et al*., 1998).

In Portugal, 12 specimens were collected, from May to July, in Coimbra, a low altitude (55 m) location.

Material examined: Coimbra: 31.5.2004, 1♂; 2.6.2004, 2♀; 3.6.2004, 1♂; 4.6.2004, 1♀, 3♂; 6.6.2004, 1♂; 17.6.2004, 1♂; 23.6.2004, 1♂; 4.7.2004, 1♂.

Subfamily Luciliinae

Genus *Lucilia* Robineau-Desvoidy, 1830

***Lucilia ampullacea* Villeneuve, 1922**

New species record for Portugal. *Lucilia ampullacea* is distributed through the Palaearctic, Oriental and Australian regions (ZUMPT, 1965;

SMITH, 1986; PERIS & GONZÁLEZ-MORA, 1991). It is a common species in the Far East, but is rarer and has a patchy distribution in Europe. In the Iberian Peninsula, the species appears only referred in the works of PERIS & GONZÁLEZ-MORA (1991) with records from Huesca (near Pyrenees), Asturias, Granada (Nevada mountain range), Pontevedra and Santander, in Spain and MONEO PELLITERO & SALOÑA-BRODAS (2007), who recorded it from Vizcaya. So, this reference greatly enlarges the known distribution of the species in the Iberian Peninsula.

Lucilia ampullacea is another carrion breeding species (ZUMPT, 1965; SMITH, 1986). In Portugal, in spring, after *Lucilia caesar*, is the most abundant *Lucilia* species present on cadavers and breeding on them (PRADO E CASTRO, 2005). It was captured from April to November.

Material examined: Coimbra: from 29.5.2004 to 25.9.2004, 498♀, 112♂; Lisbon: from 19.10.2006 to 16.11.2006, 38♀; from 17.4.2007 to 3.5.2007, 337♀, 26♂; from 28.6.2007 to 2.7.2007, 14♀.

***Lucilia caesar* (Linnaeus, 1758)**

New species record for Portugal. *Lucilia caesar* is restricted to the Palaearctic region, being more common in the western parts than in the east (ZUMPT, 1965), with several records from the Iberian Peninsula (PERIS & GONZÁLEZ-MORA, 1991; MARTÍNEZ-SÁNCHEZ *et al*., 1998).

Lucilia caesar is a carrion breeder (ZUMPT, 1965); in Portugal it is the most abundant *Lucilia* species collected from pig cadavers (PRADO E CASTRO, 2005). It was collected from April to November.

Material examined: Coimbra: 31.5.2003, 33♀, 1♂; from 29.5.2004 to 23.7.2004, 1633♀, 123♂; from 24.8.2004 to 10.9.2004, 19♀, 6♂; Lisbon: from 20.10.2006 to 30.11.2006, 160♀, 11♂; from 17.4.2007 to 29.5.2007, 1038♀, 119♂; from 28.6.2007 to 4.7.2007, 539♀, 107♂.

***Lucilia illustris* (Meigen, 1826)**

New species record for Portugal. *Lucilia illustris* has a Holartic distribution, also occurring in the Oriental and Australian regions (ZUMPT, 1965; GREENBERG, 1971; PERIS & GONZÁLEZ-MORA, 1991). In North America, Japan and Finland is a common species, while in other parts of Europe is evidently rarer (ZUMPT, 1965). It was recorded for the first time to be present in Spain in Asturias, Huesca, Pontevedra and Santander by PERIS & GONZÁLEZ-MORA (1991). The present records in Portugal amplify the distribution of the species and localize it in much more southern latitudes than it was previously known.

Lucilia illustris is a carrion breeder (ZUMPT, 1965), primarily attracted to fresh carrion, and less often found on human faeces (GREENBERG, 1971). This species, that appears to be rare in Portugal, is forensically important is other regions (ANDERSON, 2000; BYRD & CASTNER, 2001). Eleven specimens were captured in Coimbra and Lisbon, from April to July and October.

Material examined: Coimbra: 30.5.2004, 5♀; 15.6.2004, 1♀; 18.6.2004, 1♀; 22.7.2004, 1♀; Lisbon: 26.10.2006, 1♀; 19.4.2007, 1♀; 22.4.2007, 1♀.

***Lucilia sericata* (Meigen, 1826)**

Very common fly in temperate areas of the Holartic (ZUMPT, 1965) and widespread throughout the major zoogeographical regions (SMITH, 1986), being practically cosmopolitan, directly connected to human activity (MARTÍNEZ-SÁNCHEZ *et al*., 2002). This is the commonest *Lucilia* species in the Iberian Peninsula (PERIS & GONZÁLEZ-MORA, 1991).

The adults are attracted by carrion, open wounds and, also, to faeces, in which the larvae can also complete their development (ZUMPT, 1965). In Portugal, although *L. sericata* is able to breed on cadavers, is not the most abundant necrophagous species (PRADO E CASTRO, 2005) differently to what is observed in some parts of Spain (MARTÍNEZ-SÁNCHEZ *et al*., 2000; ARNALDOS *et al*., 2001, 2004; CASTILLO MIRALBES, 2002). The species was captured from April to December.

Material examined: Coimbra: from 28.5.2004 to 10.9.2004, 399♀, 35♂; Lisbon: from 20.10.2006 to 6.12.2006, 277♀, 15♂; from 18.4.2007 to 24.4.2007, 136♀, 11♂; from 29.6.2007 to 17.7.2007, 91♀, 11♂.

***Lucilia silvarum* (Meigen, 1826)**

New species record for Portugal. *Lucilia silvarum* is present in the Palaearctic, Neartic and Neotropical regions (MARTÍNEZ-SÁNCHEZ *et al*., 1998). It is known to be present in the Iberian Peninsula, in several locations in Spain (PERIS & GONZÁLEZ-MORA, 1991; MARTÍNEZ-SÁNCHEZ *et al*., 1998, CASTILLO MIRALBES, 2002), with wide distribution.

Larvae of this species parasite toads (SMITH, 1986). Although *L. silvarum* appears on cadavers (CASTILLO MIRALBES, 2002; PRADO E CASTRO, 2005) it seems to be very scarce in this particular environment.

Material examined: Coimbra: 2.6.2004, 1♀; 10.6.2004, 1♀.

Subfamily Melanomyinae

Genus *Melinda* Robineau-Desvoidy, 1830

***Melinda viridicyanea* (Robineau-Desvoidy, 1830)**

New species record for Portugal. *Melinda viridicyanea* is present in Europe, Kazakhstan, middle Asia from the ex Soviet Union and north of Africa (GONZÁLEZ-MORA, 1989). The species is widely distributed in Spain (GONZÁLEZ-MORA, 1989) and it was also found in cadavers in Huesca province (CASTILLO MIRALBES, 2002).

Melinda viridicyanea larvae parasite snails (GONZÁLEZ-MORA, 1989) and their presence on cadavers appear to be casual.

Material examined: Lisbon: 22.4.2007, 1♀; 8.5.2007, 1♀.

The information presented in this work enlarges the knowledge of the distribution of ten Calliphoridae species in Portugal. It is particularly worth of mention the case of *P. terraenovae*, with only one previous published record in Spain, so, the present record of the species in Coimbra confirms it in the Iberian Peninsula, considerably extending its European distribution. *Lucilia ampullacea* and *L. illustris,* that have been cited few times in Spain, also have much extended their known distribution.

Besides the faunistic information, the data presented in this work has practical interest for forensic purposes. It provides information on the most common Calliphoridae species in Portuguese territory, which certainly will be of major relevance in forensic cases.

ACKNOWLEDGEMENTS

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Chapter 5

FIRST RECORD OF *CHRYSOMYA MEGACEPHALA* (FABRICIUS, 1794) (DIPTERA: CALLIPHORIDAE) FROM PORTUGAL

Prado e Castro, C. & García, M.D. (2009) First record of *Chrysomya megacephala* (Fabricius, 1794) (Diptera, Calliphoridae) from Portugal. *Graellsia*, **65**, 75-77.

First record of *Chrysomya megacephala* (Fabricius, 1794) (Diptera, Calliphoridae) from Portugal

C. Prado e Castro[1, 2]* & M. D. García[3]

The blowfly *Chrysomya megacephala* (Fabricius, 1794), also known as the oriental latrine fly (Zumpt, 1965), is considered one of the most dangerous dipteran vectors of pathogens (Wells, 1991) showing a heavy potential sanitary risk (Maldonado & Centeno, 2003). It is normally a faeces and carrion breeder and larvae can be found as facultative parasites in traumatic myiasis (Zumpt, 1965). *Chrysomya megacephala* is commonly found in cadavers in many parts of the world (Gruner *et al*., 2007; Sukontason *et al*., 2007; Wang *et al*., 2008) and is used in forensic entomology cases, for postmortem interval determination (Goff & Odum, 1987; Goff *et al*., 1988; Goff, 1992).

From its original distribution over the Oriental and Australian regions, *Ch. megacephala* has tremendously expanded its range in the last decades since its introduction in Brazil (Guimarães *et al*., 1978), probably from southern Africa (Baumgartner & Greenberg, 1984) and is a recent invader of the continental United States, where it is expanding (Greenberg, 1988; Wells, 1991; Baumgartner, 1993; De Jong, 1995; Wells, 2000; Tomberlin, 2001). It also occurs in the Palaearctic region (Zumpt, 1965; Smith, 1986), presently covering the siberian sub-region, Iran, Afghanistan, China, Japan, Egypt and in the Macaronesian region, the Canary Islands and Madeira (Martínez Sánchez *et al*., 2001). It was recently collected in Malta (Ebejer, 2007). In continental Europe it was cited for the first time, in southeastern Spain (Martínez Sánchez *et al*., 2001).

During October 2006 and April 2007, in Lisbon, Portugal (UTM coordinate 29SMC88), *Ch. megacephala* was collected using a modified Schoenly trap (Prado e Castro *et al*., 2009) for the collection of fauna associated with pig carrion. Specimens were identified based on González-Mora & Peris (1988). The material is stored in 80% alcohol solution and kept in the Animal Biology Department of the University of Lisbon Faculty of Sciences.

Twenty-three specimens of *Ch. megacephala* were captured in Lisbon, mainly during autumn. The species was present in low numbers compared with other blowflies that were simultaneously on the carcasses and only adults of the species were found. This is the first record of this species for Portugal, broadly enlarging the known distribution in the Iberian Peninsula, and the second record for mainland Europe.

Chrysomya megacephala is easily distinguished from its congener species, *Chrysomya albiceps* (Wiedemann, 1819), already established in Portugal (Prado e Castro, 2005) and other European countries (Rognes, 2004) recently having reached Poland (Szpila *et al*., 2008) in its northward expansion. The adults of both species present several differences, namely in eye size, face, the anterior thoracic spiracle and in the thoracic squamae (González-Mora & Peris, 1988). In larval stage, *Ch. albiceps* in known as the "hairy maggot", due to the distinct, stout and elongated tubercles encircling the body segments, structures that are not present in *Ch. megacephala* (Queiroz *et al*., 1997; Sukontason *et al*., 2003, 2008).

The collection of the reported specimens in Lisbon, in autumn, and the ones previously collected in Alicante, southeastern Spain, in the same season of the year (Martínez Sánchez *et al*., 2001) suggests that the species may be established in the Iberian Peninsula, and a broader distribution throughout it can be presumed.

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Chrysomya megacephala is one of the primary flies associated with decomposing corpses in areas in which the species is present (Sukontason *et al*., 2003). In the Iberian Peninsula it probably hasn't reached the population levels found in other geographical areas, but it is clearly in expansion, for which it should not be discarded as a possible forensic indicator in this geographical area.

MATERIAL EXAMINED: 29SMC88 – Lisbon, Estremadura; altitude: 140 m; 24.X.2006, 1♀; 25.X.2006, 6♀♀; 26.X.2006, 6♀♀, 3♂♂; 27.X.2006, 1♀, 5♂♂; 23.IV.2007, 1♂.

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Chapter 6

ADDITIONS TO THE PIOPHILIDAE (DIPTERA) FAUNA FROM PORTUGAL, WITH NEW RECORDS

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Additions to the Piophilidae (Diptera) fauna from Portugal, with new records

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The Piophilidae are small to medium-sized flies (3-8 mm), brown to black in colour (Ozerov, 2000). The adults visit decaying carcasses, bones, garbage, filth, excrements, sewage, and other proteinaceous animal and vegetable matter (McAlpine, 1977). Larvae of Piophilidae breed mainly on decomposing fungi and plant matter or carrion of vertebrate animals and high-protein resources. At least one species, *Neottiophilum praeustum* (Meigen, 1826), lives in birds nests, where they act as ectoparasites, feeding on nestlings' blood (McAlpine, 1977; Ozerov, 2000). Several species prefer a human environment, where food supply and living conditions are favourable (McAlpine, 1977). Piophilidae larvae are often recovered from carcasses, including human corpses in advanced stages of decay, as the body begins to dry, even though adults may be found in corpses only 3 to 4 days after death (Smith, 1986).

The habit of jumping and skipping is well documented for *Piophila casei* (Linnaeus, 1758), but it is probable that the larvae of many other species (possibly all) in the family possess the same capacities (McAlpine, 1977; Ozerov, 2000). The skipping ability is most pronounced during migration, even if undisturbed, but it is also a useful escape mechanism in times of danger (Simmons, 1927).

Piophilidae are found in all continents, but the family is richest in the temperate portion of the Northern Hemisphere, many species having Holarctic distributions. A few species developed synanthropic tendencies —e.g. *Piophila casei*, *Stearibia nigriceps* (Meigen, 1826) and *Prochyliza nigrimana* (Meigen, 1826)— and have accompanied man to many parts of the world (McAlpine, 1977).

Presently, more than 70 species of 24 genera are included in Piophilidae (Ozerov, 2000), of which some 16 genera and about 30 species are present in Europe (Oosterbroek, 2006). In the Iberian Peninsula, 3 Piophilidae species are recorded for Portugal (1 in portuguese mainland, 2 in Azores and Madeira) and 9 for Spain (Carles-Tolrá & Báez, 2002; Gómez-Gómez *et al*., 2008).

The aim of this paper is to report 4 Piophilidae species new for continental Portugal, within a total of 5 that were collected from animal carcasses. The biology and distribution of the species is commented and data on seasonality of species is presented, in order to contribute to a better knowledge of this family, in a country where Diptera are still poorly known.

Fieldwork was conducted in the city of Coimbra, at Jardim Botânico da Universidade de Coimbra (UTM coordinate 29TNE45 – Coimbra, Beira Litoral; altitude: 55 m), from May to September 2004 and in Lisbon, at Instituto Superior de Agronomia (UTM coordinate 29SMC88 – Lisbon, Estremadura; altitude: 80 m), from October 2006 to August 2007. Both sites are small patchy woodland parks inside the urban perimeter. The study site, in Coimbra, had vegetation mainly composed of *Ailanthus altissima* (Mill.) Swingle*, Laurus nobilis* L.*, Celtis australis* L.*, Olea europaea* L. *and Eucalyptus* L'Hér. The site was in a clearing with direct sunlight almost all day. In Lisbon, the habitat had *Ailanthus altissima* (Mill.) Swingle*, Fraxinus*

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angustifolia Vahl and *Ulmus minor* Miller as the predominant species. The site had less solar exposure.

The experiments consisted of leaving a dead animal (piglet) in the field and collecting the insects that colonized the body, both adult and immature forms, during periodical visits. This was made daily for the first three to four weeks and afterwards on alternate days. A version of a Schoenly trap designed to collect sarcosaprophagous arthropods (Prado e Castro *et al*., 2009) was used, with piglets as bait. In the 2004 experiment, a 5 kg piglet was used for a period of 4 months (Prado e Castro, 2005). In 2006-2007, four different experiments of 10 weeks were performed, with four different piglets, in order to cover all seasons of the year. A 40% ethylene glycol solution with formalin and detergent was used in the trap as killing and temporary preservative agent for the arthropods, after which they were placed in 80% ethanol. The adult Piophilidae collected have been identified based on McAlpine (1977).

The classification used is according to McAlpine (1977). All species belong to Piophilini tribe and Piophilina subtribe.

Liopiophila varipes (Meigen, 1830)

The genus *Liopiophila* Duda, 1924 has a single, common species, *L. varipes* that occurs in the Holarctic region (McAlpine, 1977; Zuska, 1984). It is the first record of the species for Portugal. According to Ozerov (2004), *Liopiophila* Duda is a synonym of *Prochyliza* Walker.

Immature stages live on carcasses and bones of a variety of dead animals (McAlpine, 1977). Smith (1986) also refers to cases in which this species was collected in animal carcasses (chicken, rabbit, fox), Kentner & Streit (1990) collected it in rats, Bonduriansky & Brooks (1999) in fish, rodents, moose carcasses and Carles-Tolrá (2001), mainly in dead chicken and animal fat. Only adults were collected in our experiment, in Coimbra.

MATERIAL EXAMINED: Coimbra - from 31.V.2004 to 3.VI.2004, 9♀♀, 3♂♂; 23.VI.2004, 1♂; 24.VI.2004, 2♂♂.

Piophila casei (Linnaeus, 1758)

Piophila casei and *Piophila megastigmata* McAlpine, 1978 are the two species belonging to genus *Piophila* Fallén, 1810. *Piophila casei* is cosmopolitan in distribution (McAlpine, 1977; Zuska, 1984). This is the first record of the species for mainland Portugal.

Larvae, also known as cheese skippers, infest meat, hides and bones of various animals, including fish. They often cause serious damage in the meat, leather and cheese industries (McAlpine, 1977). Many publications report the presence of this synanthropic species in animal carcasses (e.g. Wolff *et al.*, 2001; Castillo-Miralbes, 2002; Watson & Carlton, 2003; Sharanowski *et al*., 2008; Gomes *et al*., 2009) as well as in human corpses (e.g. Smith, 1986; Goff & Flynn, 1991; Carvalho *et al*., 2000; Sukontason *et al*., 2001b; Wyss & Cherix, 2006; Lefebvre & Gaudry, 2009; Prado e Castro, unpublished data). We collected adults and larvae of this species.

The anterior spiracles of *P. casei* third-instar larvae present 10 papillae, arranged in two groups of 5, as described by Sukontason *et al*. (2001a). This clear bilobed arrangement that we also could observe with regular stereomicroscope, is not illustrated in Smith's (1986) textbook, which can lead to erroneous identification of larval stages of this species.

MATERIAL EXAMINED: Lisbon - 29.X.2006, 2♂♂; 14.XI.2006, 1♀; 18.XI.2006, 1♀; 27.IV.2007, 1♀; from 2.VII.2007 to 9.VII.2007, 25♀♀, 20♂♂.

Prochyliza nigrimana (Meigen, 1826)

Prochyliza nigrimana is one of the 8 species of the genus *Prochyliza* Walker, 1849 and seems to have accompanied man from the Holarctic to the Neotropical Region. The species is present in Eurasia, North and South America, Madeira, Azores and Canary islands (McAlpine, 1977; Zuska, 1984). New species record for continental Portugal.

Larvae have been reported to damage stored bones, having bred from decaying leaves (Zuska & Lastovka, 1965). Few references associate this species with carcasses, as far as we are aware, only Carles-Tolrá (2001) reported it mainly from dead hedgehogs and chickens and Castillo-Miralbes (2002) from piglets. In our experiments, they also had a regular appearance, although only adults were collected. However one female was gravid in spring 2007, season in which this was the most abundant piophilid collected.

MATERIAL EXAMINED: Coimbra - from 1.VI.2004 to 4.VI.2004, 3♀♀, 2♂♂; 23.VI.2004, 1♀; Lisbon - from

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25.X.2006 to 1.XI.2006, 2♀♀, 4♂♂; from 21.IV.2007 to 29.IV.2007, 6♀♀, 18♂♂; from 2.VII.2007 to 17.VII.2007, 2♀♀, 2♂♂.

Protopiophila latipes (Meigen, 1838)

Protopiophila Duda, 1924 contains 10 species (Bonduriansky, 1995), primarily distributed in the Southern Hemisphere. *Protopiophila latipes*, the most widespread species, is present in the Holarctic Region and also in the Oriental and Australasian Regions (McAlpine, 1977; Zuska, 1984). New species record for Portugal.

Protopiophila latipes reproduces on corpses in advanced stages of decay, where females go to mate and oviposit (Bonduriansky, 1995). This species has been collected in carcasses of rats (Kentner & Streit, 1990), rodents, turtles, fish, moose (Bonduriansky, 1995; Bonduriansky & Brooks, 1999), chicken and hedgehogs, as well as in animal fat (Carles-Tolrá, 2001). In our case, only adults were collected, with 4 females gravid in spring 2004, season when it was the most abundant piophilid species.

MATERIAL EXAMINED: Coimbra - from 29.V.2004 to 27.VI.2004, 17♀♀, 22♂♂; 7.VII.2004, 1♂; 23.VIII.2004, 1♀; from 9.IX.2004 to 16.IX.2004, 5♀♀, 3♂♂; Lisbon - 30.X.2006, 1♀; 3.XI.2006, 1♀; from 27.IV.2007 to 13.V.2007, 7♂♂; from 6.VII.2007 to 21.VIII.2007, 1♀, 8♂♂.

Stearibia nigriceps (Meigen, 1826)

Stearibia nigriceps is the only species of the genus *Stearibia* Lioy, 1864. It occurs in Eurasia, North and South America (McAlpine, 1977; Zuska, 1984).

McAlpine (1977) stated that immature stages probably lived on cadavers of various animals. This has been confirmed by several investigators that found this species in animal (e.g. Anderson & VanLaerhoven, 1996; Bonduriansky & Brooks, 1999; Carles-Tolrá, 2001; Castillo-Miralbes, 2002; Watson & Carlton, 2003; Tabor *et al*., 2005; Matuszewski *et al*., 2008) and human cadavers (e.g. Smith, 1986; Wyss & Cherix, 2006; Lefebvre & Gaudry, 2009). We collected both adults and larvae. The species was particularly abundant in summer.

MATERIAL EXAMINED: Coimbra - from 1.VI.2004 to 13.VII.2004, 13♀♀, 13♂♂; Lisbon - from 30.X.2006 to 12.XI.2006, 8♂♂; from 21.IV.2007 to 3.V.2007, 7♀♀, 9♂♂; from 2.VII.2007 to 6.VIII.2007, 55♀♀, 57♂♂.

The seasonal distribution of the different species collected is shown in Table 1.

In the spring 2004 experiment, in Coimbra, *L. varipes* was present, whereas the species was never collected in Lisbon. *Piophila casei* did not appear in Coimbra, but it was frequent in Lisbon. Since the type of bait and collecting technique was the same, these differences may be due to possible differences between habitats, even though both places are forested "islands" inside urbanized areas.

No species was active during winter, and summer was the season where a higher number of specimens were present in the carcass. *Protopiophila latipes* and *P. nigrimana* were the most abundant species in spring 2004 (Coimbra) and spring 2007 (Lisbon), respectively. *Stearibia nigriceps* was dominant, especially in the summer.

Piophilidae is clearly a saprophagous family, with a great proportion of species collected in

Table 1.– Total Piophilidae specimens (adults) collected in pig carcasses in Spring 2004 (Coimbra) and 2006-07 (Lisbon).

Table 1.– Número total de ejemplares adultos de Piophilidae colectados en cadáveres de cerdo en la primavera de 2004 (Coimbra) y en 2006-07 (Lisboa).

	L. varipes	*P. casei*	*P. nigrimana*	*P. latipes*	*S. nigriceps*	Total
Spring 2004	15		6	49	26	96
Autumn 2006		4	6	2	8	20
Winter 2007						0
Spring 2007		1	25	9	16	51
Summer 2007		45	4	9	112	170
Total	**15**	**50**	**41**	**69**	**162**	**337**

decomposing organic matter (Carles-Tolrá, 2001). The majority of works dealing with this Diptera family report *P. casei* and *S. nigriceps* as practically the only species associated with cadavers. It is interesting that for *P. nigrimana* and *P. latipes*, species with considerable abundance in our experiments, there are similar records in the Iberian Peninsula (Carles-Tolrá, 2001; Castillo-Miralbes, 2002). However we cannot be certain if the two species have bred on the cadavers.

All five species treated in this contribution have known and widespread distributions in Europe, therefore their presence in Portugal is not surprising. Despite this fact, the information presented greatly enlarges the knowledge on Piophilidae in Portugal, since the only contribution up to date was due to Hennig (1943). Besides the faunistic information, the data presented in this work has practical interest for forensic purposes. It provides information on the most common Piophilidae species in Portuguese territory, which certainly will be of major relevance in forensic cases.

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Chapter 7

PIOPHILA MEGASTIGMATA, A NEW FORENSIC IMPORTANT SPECIES, ASSOCIATED WITH PIG CARRION AND HUMAN CORPSES IN PORTUGAL

Prado e Castro, C., Cunha, E., Serrano, A. & García, M.D. (submitted) *Piophila megastigmata*, a new forensic important species, associated with pig carrion and human corpses in Portugal. *Forensic Science International*.

Piophila megastigmata, a new forensic important species, associated with pig carrion and human corpses in Portugal

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Short title: *P. megastigmata*, a new forensic important species

Abstract

Piophila megastigmata McAlpine, 1978, during many years only known from South Africa, was recently recorded in Spain. The present work reports for the first time the occurrence of *P. megastigmata* in human corpses, in Portugal. The species was also collected in succession studies carried with piglet carcasses, where it coexisted with *Stearibia nigriceps* (Meigen, 1826), showing similar periods of presence in the carcass. It was observed though that *P. megastigmata* was more abundant than *S. nigriceps* in autumn, and the contrary in summer. In winter period none of the species was able to colonize carrion and in spring, only *S. nigriceps*. The first record of *P. megastigmata* in human corpses confirms it as a forensic important species in Europe. Moreover, *P.*

megastigmata was the only piophilid present in human cadavers. The species, well established in the Iberian Peninsula, has probably a wider distribution but has been unnoticed and maybe confused with *Piophila casei* (Linnaeus, 1758) in many studies.

Keywords: *Piophila megastigmata*, *Stearibia nigriceps*, Piophilidae, forensic entomology, Portugal

Introduction

Piophila casei (Linnaeus, 1758) and *Piophila megastigmata* McAlpine, 1978 are the only two species belonging to the genus *Piophila* Fallén, 1810 [1,2]. *Piophila casei* is a cosmopolitan, synanthropic and well-known species [3] due to its economic, medical and forensic interest. This species is attracted to proteinaceous substrates, infesting meat, fish, ham, cheese, etc. They often cause serious damage in industries connected with these kinds of foods [3,4]. Curiously, larvae of this species are used in the production of valuable cheeses in Italy and Croatia [5]. Larvae can occasionally produce intestinal myiasis in man after the ingestion of cheeses or other infested food; they can live for a long time in the intestine, causing colic in the patients [6]. *Piophila casei* is frequently found in human cadavers in advanced stages of decay [e.g. [6-13]], being a species of forensic interest.

On the other hand, *P. megastigmata* is practically an unknown species, during many years only recorded from South Africa [1,14], until it was very recently reported for Spain [15]. This species has been collected from impala carcasses in the Kruger National Park, South Africa [1,14] and from carrion-baited traps as well as pig carcasses in Madrid area, Spain [15].

Several characters were described by McAlpine [1] and summarized by Martín-Vega *et al*. [15] to distinguish *P. megastigmata* from *P. casei*. Among them, and characteristic of the former are the enlarged abdomen spiracles, the strong ocellar bristles and all the femora yellow.

At the light of these recent findings [15] in Spain, we revised the *Piophila* speciments collected in Portugal, proceeding from both human cadavers and carrion succession studies carried out with piglet carcasses. As a result, the first report of *P. megastigmata* breeding on human cadavers is presented, as well as further data on Piophilidae related to animal carcasses in Portugal.

Material and Methods

Carrion succession studies

From October 2006 to August 2007, in Lisbon (Portugal), Piophilidae specimens were collected using a modified Schoenly trap [16] in order to sample the fauna associated with pig carrion. Four piglets (≈ 8 Kg weight) were used in four different experiments of 10 weeks each, in order to cover all seasons of the year. The experiments consisted on leaving the dead animal as bait inside the trap and during periodical visits collecting the insects that colonized the carcass and bred on it. The visits were made always at the same hour, daily in the first 23 days, and thereafter every 2, 3 or 5 days until the end of each experiment. Besides the continuous collections made by the trap, in each visit, manual sampling was also performed to collect Diptera eggs and larvae, when present. The immature stages of Piophilidae were collected with the substrate in which they were feeding on and reared in the laboratory at ambient temperature, in that same substrate, inside plastic boxes (size: 20x12x12 cm). A thin layer of sand covered the bottom of the boxes and ventilation was provided trough fine holes in the plastic cover of the container.

The field experiments were performed in Instituto Superior de Agronomia, Tapada da Ajuda, Lisbon (38º42’41’’N 09º11’28’’W). The location is a small patchy woodland park inside urban perimeter, mainly composed of *Ailanthus altissima* (Mill.) Swingle, *Fraxinus angustifolia* Vahl and *Ulmus minor* Mill., in an altitude of 80 m a.s.l.. Ambient temperatures and relative humidity were continuously registered with an HOBO Data logger (24 records/day) placed into the trap. Figure 1 shows the temperatures recorded during the four different experiments.

The specimens collected are deposited in the collection of the Department of Animal Biology, University of Lisbon.

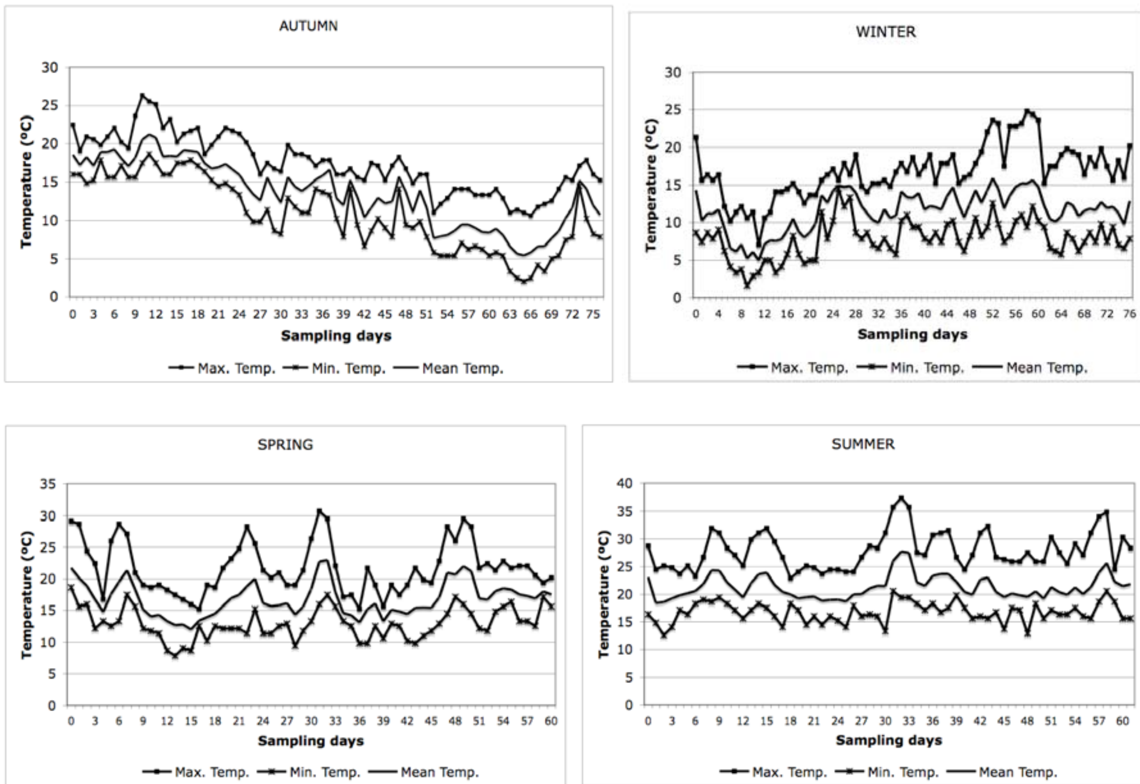

Figure 1- Temperature data during the studied periods.

<u>Forensic cases</u>

Specimens were collected during autopsy, at the National Institute of Legal Medicine, in Lisbon. In the first case, the corpse was found inside a car, in a place of difficult access in Arrábida Mountain (located close to Lisbon), in March 2007. Twenty larvae, dozens of pupae and empty puparia of Piophilidae were collected mainly from the abdomen area. The second case concerns a body found in his own room, in a house that was crowded with garbage and abandoned, in Portimão, Algarve (south of Portugal) by the end of July 2007. Dozens of larvae and pupae of Piophilidae were collected.

Results

Carrion succession studies

After the revision of the piophilids previously identified as *P. casei* [17], we found that, among the 50 *Piophila* adult specimens collected, 43 are in fact *P. megastigmata* and only 7 are *P. casei*. This revision modifies the information presented in Prado e Castro & García [17].

Thus, five Piophilidae species were attracted to the carcasses: *Piophila casei, Piophila megastigmata, Prochyliza nigrimana* (Meigen, 1826), *Protopiophila latipes* (Meigen, 1838) and *Stearibia nigriceps* (Meigen, 1826) (Table 1).

Table 1- Total Piophilidae specimens (adults) collected in pig carcasses from Autumn 2006 to Summer 2007.

	Autumn	Winter	Spring	Summer	Total
Piophila casei				7	7
Piophila megastigmata	4		1	38	43
Prochyliza nigrimana	6		25	4	35
Protopiophila latipes	2		9	9	20
Stearibia nigriceps	8		16	112	136

Stearibia nigriceps and *P. megastigmata* were the most abundant and the only found breeding on the cadavers. Immature stages of *Piophila megastigmata* were found in autumn and summer, while *S. nigriceps* was present in the same seasons and also in spring. Table 2 shows, for the different seasons of the year, the presence of both adults and immature stages of *P. megastigmata* and *S. nigriceps* in the carcasses. In autumn, only a few adults of *P. megastigmata* were collected despite the species successfully bred on the cadaver (Table 2). Larvae were detected on day 25, being clearly visible (and presenting skipping behaviour) until day 35. The trap collected migrating larvae from day 25 until the end of the experiment. Days 29 and 37 presented the highest number of larvae collected (123 and 175 respectively), thus being the period of most intense migration. Teneral adults emerged from days 61 to 66 in the field, while in the laboratory flies emerged from puparia from day 40 onwards (Table 2), which can be attributed to the different temperature regimes. In spring, only *S. nigriceps* was able to breed in the cadaver. Eggs of this species could be collected from the carcass on the 11th day and larvae were observed, although very small and not skipping, between days 21 and 23. In the laboratory, skipping was observed for the first time on day 28 and teneral adults started to emerge on day 43 (Table 2), however, this was not observed in the carcass. In summer, eggs of both *P. megastigmata* and *S. nigriceps* were collected from the carcasses between days 10 and 13. These were taken to the laboratory and were successfully bred until adult stage. Larvae started to skip on day 21 and adult stage was reached on day 43 (Table 2). However, in this season, no larval activity of these species could be observed in the carcass.

Regarding the abundance in which the two species were collected as immatures in the cadavers, *P. megastigmata* was more abundant than *S. nigriceps* in autumn and the converse happened in summer.

Table 2- *Piophila megastigmata* and *Stearibia nigriceps* occurrence matrix (Autumn- 18.10.2006 to 2.01.2007; Spring- 16.04 to 16.06.2007; Summer- 27.06 to 27.08.2007).

Numbers refer to adults collected. Abbreviations: E= eggs; L= larvae; P= pupae; T= teneral adults. Grey shading refers to rearing in the laboratory (ambient temperature).

Autumn	**Fresh**			**Bloated**				**Decay**																	**Dry**																
Day of decomposition	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**25**	**27**	**29**	**31**	**33**	**35**	**37**	**40**	**43**	**46**	**49**	**52**	**55**	**58**	**61**	**66**	**71**	**76**
P. megastigmata											2														1		1														
																								L	L	L	L	L	L			L						T	T		
																								L	L	L	L	L	L P	L P	LPT	LPT	LPT	LPT	LPT	LPT	LPT	LPT	LPT	LPT	P T
S. nigriceps												1	3		2	1								1																	
																								L	L		L	L	L									T	T		
																								L	L	L	L	L	L P	L P	LPT	LPT	LPT	LPT	LPT	LPT	LPT	LPT	LPT	LPT	P T

Spring	**Fresh**			**Bloated**			**Decay**													**Dry**																		
Day of decomposition	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**25**	**27**	**29**	**31**	**33**	**35**	**37**	**40**	**43**	**46**	**49**	**52**	**55**	**58**	**60**
S. nigriceps					1	2	6	1	2			3					1																					
											E										L	L	L															
											E		L	L	L	L	L	L	L	L	L	L	L	L	L	L	L P	L P	L P	L P	L P	LPT	LPT	LPT	LPT	LPT	LPT	LPT

Summer	**Fresh**		**Bloated**			**Decay**							**Dry**																									
Day of decomposition	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**25**	**27**	**29**	**31**	**33**	**35**	**37**	**40**	**43**	**46**	**49**	**52**	**55**	**58**	**61**
P. megastigmata					1	1	4	6	5	13	5	3																										
										E	E	E	E																									
										E	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L P	L P	L P	L P	LPT	LPT	P T	T	T		
S. nigriceps					1	4	18	18	28	8	10	4	4	4	4	2	1	1		1				1	1						2							
										E	E	E	E																									
										E	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L P	L P	L P	L P	L P	LPT	P T	T	T		

Forensic cases

Among the entomological evidence collected (Table 3), *P. megastigmata* was the only piophilid found in both cases. In the two situations, the corpses were in advanced stage of decay. In the first case, while the skull was completely skeletonized, the remaining body, fully dressed, was mainly mummified, in particular the limbs, with the ribs skeletonized. The body was found seated inside the vehicle, still with the seatbelt on and with several pieces of clothes. Soil, grass and insects were found allover the body, mainly on the axial skeleton. The body belonged to a middle age male, caucasian individual who was positively identified by genetic analysis. A period of 5 months was known to have passed since the person was missing (October 2006) and the discovery of the body (March 2007). The date of disappearance was most likely the time of death.

Table 3- Entomological evidence of the two forensic cases studied.

Case number	**Entomological evidence**	
	Diptera	Coleoptera
1	*Piophila megastigmata* (LIII, P, empty puparia) Muscidae sp. (empty puparia) *Chrysomya albiceps* (empty puparia)	*Necrobia* sp. (elytra)
2	*Piophila megastigmata* (LIII, P) Phoridae sp. (P)	*Dermestes* sp. (exuviae)

In the second case, the individual was only half dressed, since it retained only boxers and a shirt. The body was found lying down on the individuals ‘bed and was almost completely skeletonized. Yet, the bones were not fully dry since they retained dried soft tissues still attached. Some hair was still preserved on the skull. The anthropological analysis attributed the body to a caucasian male individual near his sixties. This biological profile matched the one of the missing person who lived absolutely alone and had no relatives, which preclude a genetic identification. The last time he was seen was one and a half year before. By that time he stopped paying the house rents. It can be therefore assumed he died one to one and half year before his body was found.

Discussion

Piophila megastigmata was able to successfully breed on piglet carcasses in autumn. In summer, the species laid eggs, but maybe due to high temperatures and the rapid desiccation of the tissues that serve as breeding substrate, larvae did not develop. Hegazi *et al*. [18] observed that *P. casei* eggs laid on dry places never hatched because of lack of humidity and Russo *et al*. [5] recorded highest mortality of eggs maintained at high temperatures. The scarcity of Piophilidae during summer months was also noted by Martín-Vega *et al*. [15]. Interestingly, in human cadavers, immature stages of this species were found to be present both in spring and summer. Probably due to body size and availability of resources, the activity of these larvae in human cadavers can occur until very late in the decomposition process, e.g. 5 months in case 1 (in this case, *P. megastigmata* most likely colonized the corpse in late autumn and larvae remained present until spring) and more than a year in case 2. Similarly, *P. casei* has been reported in late stage corpses, from 2 to 17 months postmortem [7,9,11,12,19] but also as early as 26 days postmortem [9] or ≈ 35 days [8], which is congruent to what we found in the piglet carcasses.

In winter experiment (17.01.2007 to 3.04.2007), no piophilid species was attracted to the carcass, despite dry stage having been reached (in other seasons adults arrived in bloated or decay stage (Table 2)), so colonization didn't occur. Even though most species of Piophilidae occur in the cooler regions of the north temperate zone [3] and e.g. *P. casei* being a well adapted species to a wide range of thermic regimens [5], the mean temperatures generally below 15ºC during the winter study period (Figure 1) were probably too low for the activity of these flies. The fact that *P. megastigmata* didn't colonize the spring carcass (only *S. nigriceps* did) might be due to the own phenology of the species, not still being active in spring. The absence of *Piophila* in spring has been noted also in a different experiment conducted in Portugal [17].

Piophila megastigmata has been reported coexisting on carrion with *P. casei* [1,15,20]. In our experiment that was not the case, being *S. nigriceps* the coexisting species. According to the literature, *Stearibia nigriceps* is another species frequently found in cadavers [6,13,21]. The two species showed very similar periods of appearance and presence on the carcasses. *Piophila megastigmata* immatures were collected in higher number than *S. nigriceps* in autumn and the contrary in summer. Similar observations were made by Martín-Vega *et al*. [15], where *P. megastigmata* was more abundant than *P. casei* in autumn and the opposite in spring. It may be a usual pattern of coexistence between *P. megastigmata* and the other dominant piophilid in the area. In human cadavers, nevertheless, *P. megastigmata* was the only piophilid present.

The first instars of piophilid larvae are very small, being difficult to locate. In autumn experiment they were only detected as mature third instar, already exhibiting the very characteristic habit of skipping or jumping. This ability, best documented for *P. casei* but probably characteristic of all the family [3,4] is most pronounced in the mature larvae, during migration, even if undisturbed but it is also a useful escape mechanism in times of danger [22].

In the Iberian Peninsula, only Martín-Vega *et al*. [15] reported the presence of immature stages of Piophilidae (*P. casei* and *P. megastigmata*) in carrion succession studies. In the present work we also mention, even though temperature regimes were not ideally controlled, periods of time spent in each developmental stage, for *P. megastigmata* and *S. nigriceps* (Table 2).

Piophila megastigmata was collected in three different localities in Portugal (Lisboa, Setúbal (Arrábida Mountain) and Portimão), being an abundant species on carrion. This supports that it must be a synanthropic species, as suggested by Martín-Vega *et al*. [15] and contrarily to what was previously supposed [1].

The fact that *P. megastigmata* is so well established in the Iberian Peninsula raises the strong possibility that the species has a much wider distribution and probably has been unnoticed during an unknown period of time (given that it was possibly an introduced species in Europe). Its general appearance strongly resembling *P. casei*, the belief that it was an endemic species from South Africa [1] and its absence from McAlpine [3] key probably contributed to its non-recognition as a different species.

Even though Piophilidae are commonly referred both in succession studies and forensic cases, it is still a poorly known group. Immature stages of some species are described [3,4,19,23,24] but no complete key currently exists. Developmental data, at different temperatures, are presently available for *S. nigriceps* [25] and *P. casei* [5]. *Piophila megastigmata* is now reported for the first time associated to human corpses, which confirms it as a "new" forensic important species to be taken into account in forensic practice. It is very probable that the species has been confused with *P. casei* in many studies. Piophilidae are valuable in determining time of death in later stage corpses [9] and *Piophila megastigmata* definitely deserves attention to clarify its distribution and to gather data about time of colonization and development.

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Chapter 8

CARRION BEETLES OF FORENSIC INTEREST: A STUDY OF SEASONAL COMMUNITY COMPOSITION AND SUCCESSION IN LISBON, PORTUGAL

Prado e Castro, C., García, M.D., Martins da Silva, P., Faria e Silva, I. & Serrano, A. (in preparation) Carrion beetles of forensic interest: a study of seasonal community composition and succession in Lisbon, Portugal.

Carrion beetles of forensic interest: a study of seasonal community composition and succession in Lisbon, Portugal

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Abstract

Beetles are an important component of the fauna that colonizes a cadaver. Some of these insects feed on carrion, many are predators and other can consume both carcass and associated arthropods. Coleoptera are recognised as being forensic important as PMI indicators, especially in the later stages of cadaver decomposition. It is known that insect species and their timings of appearance in the cadaver vary according to geographic location. For this reason it is important to know the patterns of succession of the different coleopteran species occurring in cadavers, as well as its seasonality at a regional level. In order to obtain these data from Lisbon area (Portugal), experiments were conducted during the four seasons of the year, using piglet carcasses as animal models.

Five stages were recognized during the decomposition process. The stages, besides visually defined, could be separated taking into account the occurrence and abundance of the specific groups of Coleoptera collected. Decay stages in general recorded higher abundance and species richness values.

82 species were identified, belonging to 30 families, in a total of 1968 adult Coleoptera collected. Autumn yielded the highest values of species abundance and

richness, while winter had the lowest. Staphylinidae was the most abundant family in all seasons of the year; in spring and summer, Dermestidae was quite dominant.

In general, most species were related to the decay and advanced decay stage, particularly *Margarinotus brunneus* and *Creophilus maxillosus*, but also *Saprinus detersus* and *Thanatophilus sinuatus*, while only few were related to the dry stage, namely *Oligota pusillima* and Dermestidae spp. larvae. On the other hand, *Anotylus complanatus* and *Atheta pertyi* were apparently more associated to the stages fresh and bloated, respectively.

The presence of some species was markedly seasonal, performing a season characterization on the basis of certain taxa, which can be useful for forensic purposes.

Keywords: Coleoptera, carrion decomposition, forensic entomology, succession, Portugal

Introduction

Beetles (Insecta, Coleoptera) are an important component of the fauna that colonizes a cadaver. Some of these insects feed on carrion, many are predators and other can consume both carcass and associated arthropods. Although beetle fauna has received more attention in the literature than any other carrion insect group, the assessment of its value as forensic indicators has not been fully investigated (Smith, 1986; Midgley *et al*., 2010). Most research has focused on flies – their greater mobility and faster rate of larval development enables them to reach a cadaver before the beetles and complete their development before the food source is exhausted. Many carrion beetles avoid competition with blowflies by occurring on carcasses at a later, drier stage of decomposition (Smith, 1986). In advanced decomposition stage, Coleoptera comprise the main entomological evidence present in a corpse and the analysis of their communities can be used to estimate postmortem interval (PMI) (Smith, 1986; Kulshrestha & Satpathy, 2001).

A comparison of the fauna collected on a corpse at the time of discovery with the fauna collected in controlled studies (with animal models) in similar environments is the base of the succession method of PMI estimation and can often provide information when little or no other evidence is available (Schoenly *et al.*, 1996). The community and patterns of arthropod succession can provide valuable clues to the investigation, particularly when used in conjunction with development of the earliest colonizers of the corpse (Kreitlow, 2010). Regarding development under controlled conditions, very little research has been conducted on Coleoptera. However, as several coleopteran species that inhabit cadavers are common and widespread, often, stored product pests, scattered information exist on their development (Midgley *et al*., 2010).

Many families of carrion insects are relatively ubiquitous; nevertheless the species vary from region to region, which highlights the importance of performing local studies, to obtain information about the species present, as well as their seasonal availability (Anderson, 2010). In southern Europe, only the works of Castillo Miralbes (2002) and Arnaldos *et al.* (2004) list coleopteran species collected in animal carcasses during an annual period, in Spain. In Portugal, Prado e Castro *et al.* (2010) recorded Staphylinidae species collected in piglet carcasses and Grosso-Silva & Soares-Vieira (2009) compiled scattered information on Coleoptera associated with different animal carcasses.

The objective of this study was to investigate the coleopteran community composition and successional patterns on cadavers decomposing in the four seasons of the year in Lisbon, Portugal. The entomological data obtained may serve as reference to be applied in other regions with similar bio-geoclimatic characteristics, in southern Europe.

Material and methods

Site description. Lisbon is the Portuguese capital and biggest urban centre, with a population of ≈ 2.8 million inhabitants in its metropolitan area. Lisbon has a Mediterranean climate and according to the National Weather Institute, summers are hot and dry with average daily temperatures of 20–23°C, winters are cool and rainy with daily means around 12°C, while spring and autumn are generally mild or even warm during daytime. Annual rainfall is around 700–750 mm/year, spread over 100 rainy days, mostly from October to May.

The experiment was performed at Instituto Superior de Agronomia, Tapada da Ajuda (38º42’41’’N, 09º11’28’’W), a small patchy woodland park inside urban perimeter, mainly composed of *Ailanthus altissima* (Mill.) Swingle, *Fraxinus angustifolia* Vahl and *Ulmus minor* Miller. The chosen site was located in a shaded area, undisturbed by human activity.

Carcasses and experimental procedures. Domestic piglets, *Sus scrofa* L., of 7.5 to 8 kg weight, were used as animal models. They were stunned first and then killed trough incision in the jugular vein. Field experiments were performed from October 2006 to August 2007. Four different trials, of 8 to 10 weeks, were carried out, in order to cover all seasons of the year. In every experiment, a piglet carcass was used.

Each piglet carcass was placed inside a modified Schoenly trap (Prado e Castro *et al.*, 2009). The trap is a dodecahedral structure constructed in plywood, where the carcasses stay in direct contact with soil and that allows good aeration and odour dissipation. The traps designed by Schoenly (Schoenly 1981; Schoenly *et al.* 1991; Prado e Castro *et al.*, 2009) collect arthropods that are attracted to a carcass, continuously and automatically, reducing the disturbance in the colonization and collector's bias is not introduced. The trap has been confirmed and recommended as the most effective device for characterizing the sarcosaprophagous succession (Ordóñez *et al.* 2008). The bottles that capture the arthropods contained a killing and temporary preservative solution of 40% ethylene glycol with formalin and detergent. Collection of samples from the trap consisted in removing the bottles with arthropods and replacing them with others filled with clean solution. The visits were made always at the same hour, daily in the first 23 days, and thereafter every 2, 3 or 5 days until the end of each experiment. Besides the continuous collections made by the trap, in each visit, manual sampling was also performed.

Ambient temperatures and relative humidity were registered with a HOBO Data logger (24 records/day) placed into the trap.

The date of death and placement of the pig carcasses in the traps was designated as day 0. The collections started 24 hours after, on day 1. Specimens were all kept in 80% ethanol.

Data analysis. Average numbers of Coleoptera abundance (N) and richness (S - number of species) found in the carcass were recorded in each sampling season and decomposition stage. Number of species refers only to the ones identified (some families, not identified to species level, count as one species). To evaluate the effect of season, decomposition stage and their interaction, a two-way multivariate analysis of variance (MANOVA), followed by pair-wised Bonferroni *post hoc* tests, was performed to N and S values of Coleoptera. If assumptions of homogeneity of variances and normality (verified previously using Bartlett and Kolmogorov–Smirnov tests, respectively) were not met, data were log transformed. The above statistical analyses were performed in the SigmaStat software (SPSS 17.0 for Windows).

Coleoptera community composition related to the different decomposition stages, within each season was analyzed by ordination methods. A previous detrended correspondence analysis (DCA) based on Coleoptera species composition was performed to identify the gradient length, in order to select the correct constrained ordination method (Lepš & Smilauer, 2003). Subsequently, a Canoninical Correspondence Analysis (CCA) was performed using log transformed data and based on the ‘‘sampling days *vs*. Coleoptera *taxa*’’ data matrices. Statistical significance of the ordination axes was evaluated by a Monte Carlo permutation test. CCAs were performed in CANOCO 4.5 for Windows.

Results

Climatic data. Temperatures throughout the four seasonal sampling trials are given in Fig. 1. As expected, winter was the season in which the temperatures were considerably lower compared to the other seasons. Autumn and spring had similar mean temperature during the first week of carcass exposure.

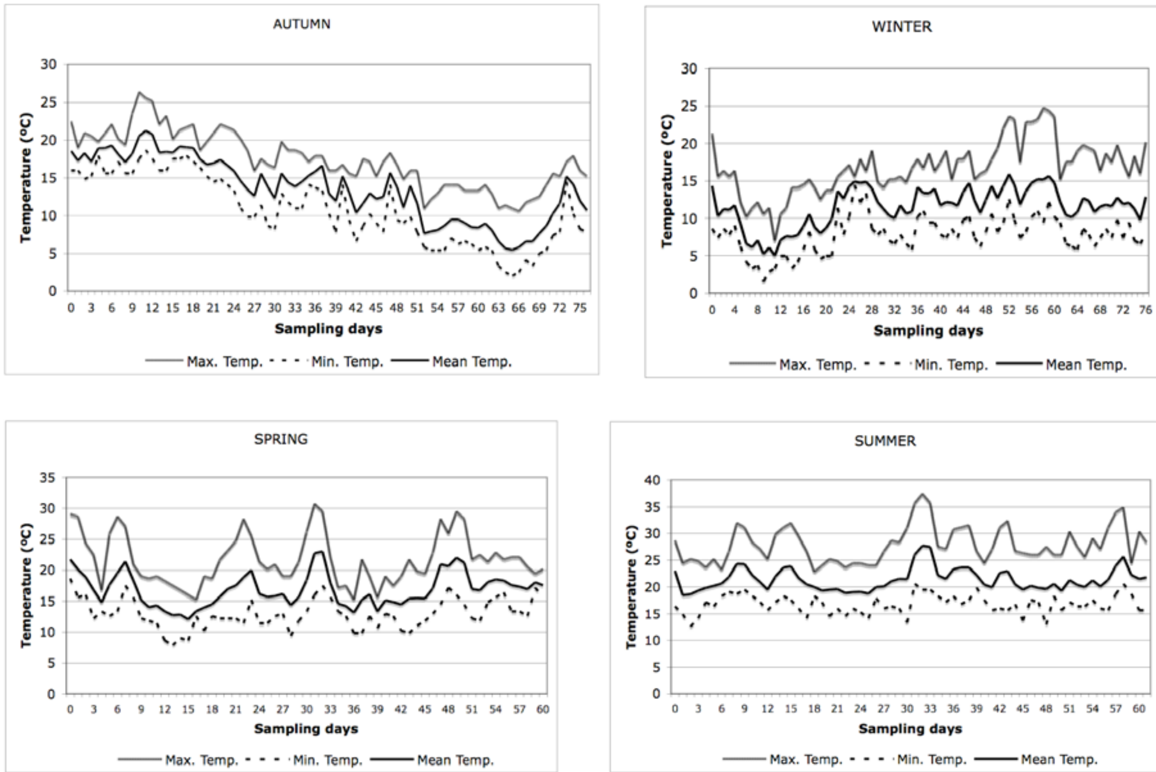


Fig. 1. Temperature data for the studied periods.

Decomposition process. Five decomposition stages were recognized, as described in Anderson & VanLaerhoven (1996): fresh (F), bloated (B), decay (D), advanced decay (AD), and dry/remains (DR). The description of each stage of decomposition is summarized in Table 1. All stages were observed, with different durations, in all seasons of the year, except in autumn, where D was not clear, as deflation started simultaneously with dipteran larval migration (Table 1). Differently to the other seasons, also in autumn, humidity and mold was usually present in the dry stage. Head decomposed faster than trunk in all seasons.

Table 1. Decomposition stages.

Decay stage	Defining characteristics of decomposition stages	Days postmortem Autumn	Winter	Spring	Summer
Fresh	Fresh appearance; no odor	0-3	0-17	0-3	0-2
Bloated	Bloating, initiating as slight inflation of the abdomen; odor of putrefaction	4-7	18-28	4-6	3-5
Decay	Carcass starts to deflate; dipteran larval masses feeding on soft tissues; strong odor of decay	---	29-34	7	6
Advanced decay	Intense migration of Diptera larvae; decrease in odor; at the end of the stage, most of the flesh has been removed	8-26	35-54	8-19	7-12
Dry	Carcass consists of bones, skin and hair; little or no odor	27-76+	55-76+	20-60+	13-60+

Coleoptera community. A total of 1977 adult Coleoptera and 1118 larvae were collected along all sampling seasons (Fig. 2).

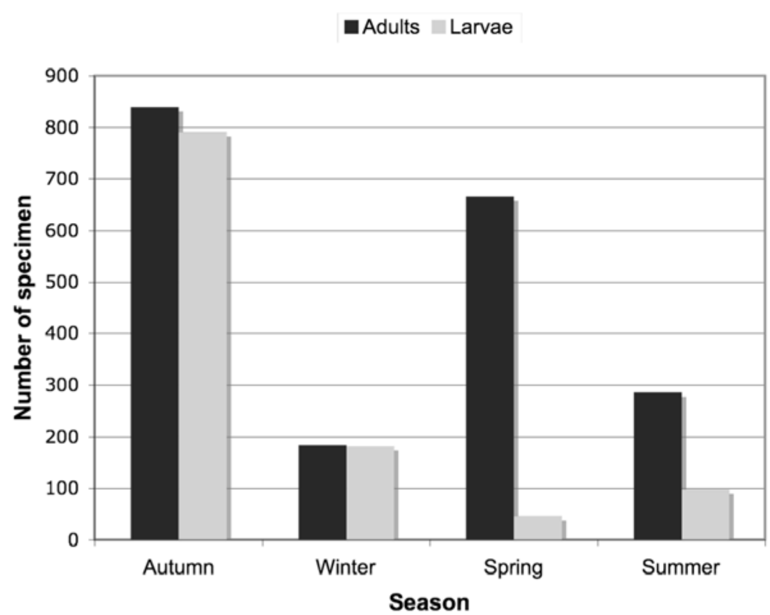


Fig. 2. Total number of Coleoptera (adult and larvae) captured during the four sampling seasons.

The larvae were predominantly Staphylinidae in autumn and winter and Dermestidae in spring and summer.

The adults belong to 30 families and 82 species (Table 2).

Table 2. Seasonal abundance (N) of adult Coleoptera sampled on pig carcasses during the study period of the four seasons of the year. The families, subfamilies (Staphylinidae), genera and species were ordered alphabetically.

FAMILY (Subfamily)	SPECIES	Autumn	Winter	Spring	Summer
Anthicidae		0	0	0	1
Cantharidae		0	0	61	2
Carabidae		4	1	5	0
Chrysomelidae	*Psylliodes* sp.	0	1	0	0
Cleridae	*Necrobia ruficollis*	7	2	16	17
	Necrobia rufipes	28	0	1	4
	Necrobia violacea	8	2	15	13
Coccinellidae		0	0	0	3
Corylophidae	*Orthoperus aequalis*	0	0	0	1
	Orthoperus anxius	0	0	0	1
	Sericoderus lateralis	1	0	3	6
Cryptophagidae		2	0	0	0
Curculionidae		0	0	3	1
Dermestidae	*Dermestes frischii*	34	0	12	24
	Dermestes maculatus	0	0	10	6
	Dermestes undulatus	2	0	78	54
	Anthrenus sp.	0	0	2	0
Histeridae	*Margarinotus brunneus*	11	15	47	8
	Margarinotus ignobilis	1	4	2	0
	Margarinotus uncostriatus	0	2	0	0
	Saprinus caerulescens	0	0	1	0
	Saprinus cruciatus	0	0	1	0
	Saprinus detersus	2	0	8	7
	Saprinus furvus	3	0	1	0
	Saprinus georgicus	9	0	0	0
	Saprinus pharao	0	0	1	0
	Saprinus subnitescens	12	0	9	14
Hydrophilidae		1	0	0	0
Languriidae	*Cryptophilus* sp.	1	0	0	0
Lathridiidae		5	1	1	4
Leiodidae		7	6	2	0
Malachiidae		0	0	1	0
Melandryidae		0	0	1	0
Melyridae		1	0	14	3
Mycetophagidae		1	0	1	4
Nitidulidae		39	26	7	11
Pselaphidae		2	0	0	0
Ptiliidae		95	4	9	0
Ptinidae		0	0	1	0
Scarabaeidae		0	0	1	0
Scraptiidae		0	0	2	11
Scydmaenidae		0	2	1	0
Silphidae	*Silpha puncticollis*	0	0	1	0
	Thanatophilus ruficornis	4	0	3	0

FAMILY (Subfamily)	SPECIES	Autumn	Winter	Spring	Summer
	Thanatophilus sinuatus	5	12	9	0
Silvanidae		0	1	1	2
Staphylinidae	*Acrotona fungi*	38	3	0	0
(Aleocharinae)	*Aleochara curtula*	1	0	0	1
	Aleochara intrincata	2	0	1	1
	Aleochara tristis	2	0	0	0
	Aleochara verna	1	0	0	0
	Atheta laticollis	6	2	3	7
	Atheta parvicornis	0	0	3	0
	Atheta pertyi	174	25	66	0
	Atheta sodalis	29	1	0	0
	Cordalia obscura	2	0	2	1
	Dimetrota cadaverina	7	1	0	0
	Liogluta nitidula	0	0	1	2
	Oligota pusillima	28	2	3	62
(Micropeplinae)	*Micropeplus staphylinoides*	1	0	0	0
(Oxytelinae)	*Anotylus complanatus*	166	21	178	2
	Anotylus inustus	1	0	0	0
	Anotylus pumilus	33	5	0	0
(Paederinae)	*Nazericus ibericus*	0	0	4	0
(Proteininae)	*Proteinus atomarius*	25	0	3	0
(Staphylininae)	*Creophilus maxillosus*	8	30	13	6
	Ocypus aethiops	2	5	0	1
	Philonthus discoideus	1	1	0	0
	Philonthus longicornis	0	0	1	0
	Philonthus varians	16	2	41	6
	Quedius fumatus	0	0	2	0
	Quedius latinus	2	0	0	0
	Xantholinus translucidus	1	0	0	0
(Steninae)	*Stenus bifoveolatus*	0	1	0	0
(Tachyporinae)	*Bryophacis maklini*	2	1	0	0
	Mycetoporus longulus	0	0	0	1
	Mycetoporus mulsanti	5	0	0	0
	Mycetoporus nigricollis	4	0	0	0
	Mycetoporus piceolus	0	2	0	0
	Mycetoporus solidicornis	2	1	0	0
	Tachyporus nitidulus	0	0	2	0
Tenebrionidae	*Cossyphus hoffmannseggii*	0	1	0	0
Coleoptera abundance (N)		844	183	654	287
Species richness (S)		51	31	50	33

Values of Coleoptera abundance (N) and species richness (S) were significantly different among sampling seasons (N: $F_{(df=3)} = 16.9$, $P = 0$; S: $F_{(df=3)} = 9.01$, $P = 0$). The highest values were found in autumn and the lowest in winter (Figs. 3 and 4).

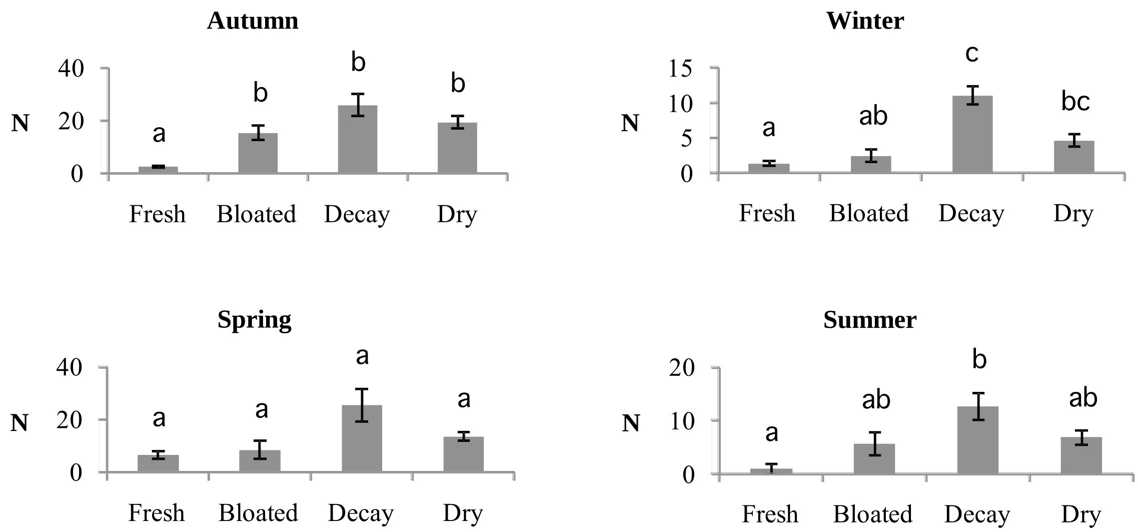


Figure 3. Mean values of Coleoptera abundance (N) along carcass' decomposition stages in each sampling season (error bars represent the standard error of the mean); a, b and c represent different groups after pair-wised Bonferroni tests between decomposition stages. Deacy and advanced decay stages are treated as one stage (Decay).

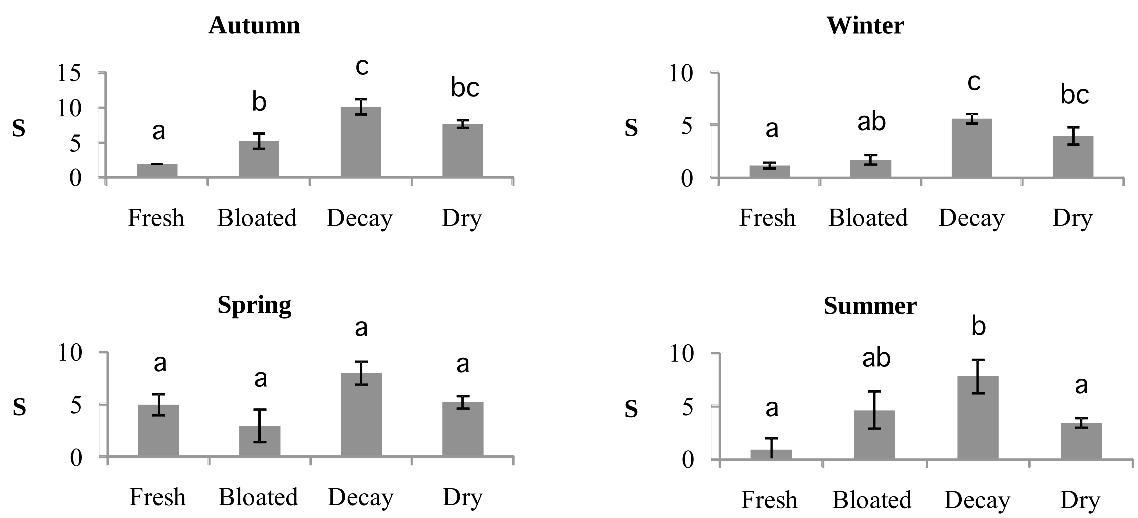
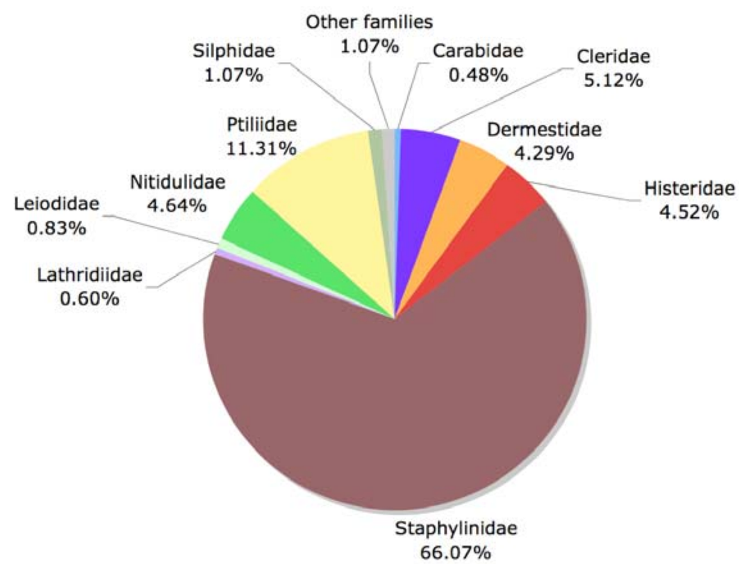
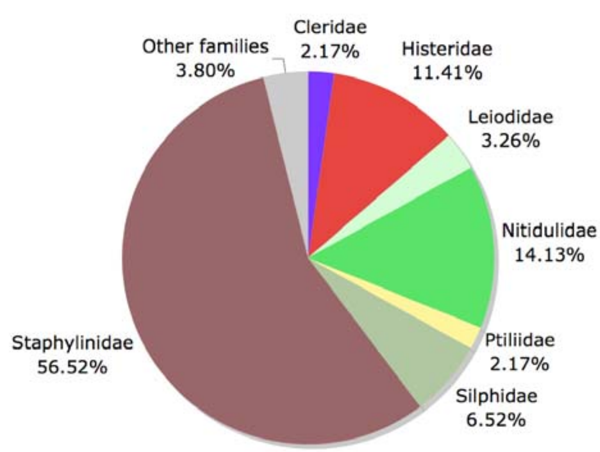


Figure 4. Mean values of Coleoptera species richness (S) along carcass' decomposition stages in each sampling season (error bars represent the standard error of the mean); a, b and c represent different groups after pair-wised Bonferroni tests between decomposition stages. Decay and advanced decay stages are treated as one stage (Decay).

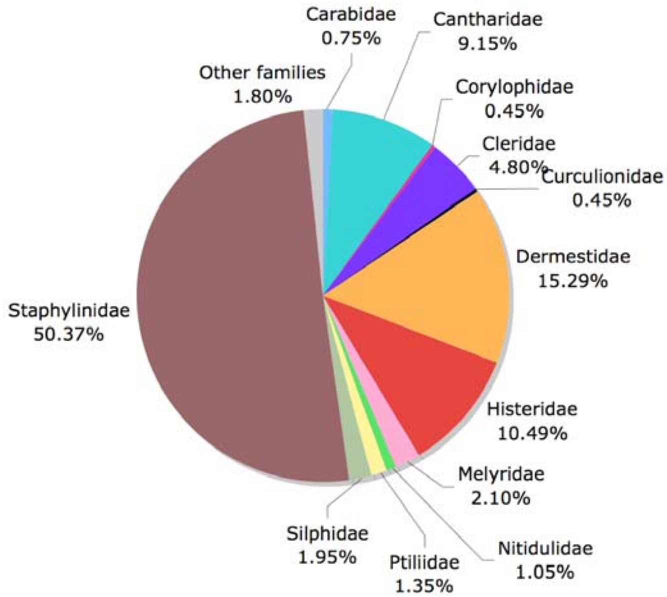
In what concerns the abundance of Coleoptera families (Fig. 5), Staphylinidae was the most abundant family, representing in all seasons (except in summer) more than 50% of the Coleoptera captured. Cleridae and Histeridae were a constant presence in every season. In autumn, Ptiliidae and Nitidulidae were well represented families; in winter, Nitidulidae was the second most abundant family and Silphidae was considerably abundant; in spring and summer, Dermestidae was very dominant.



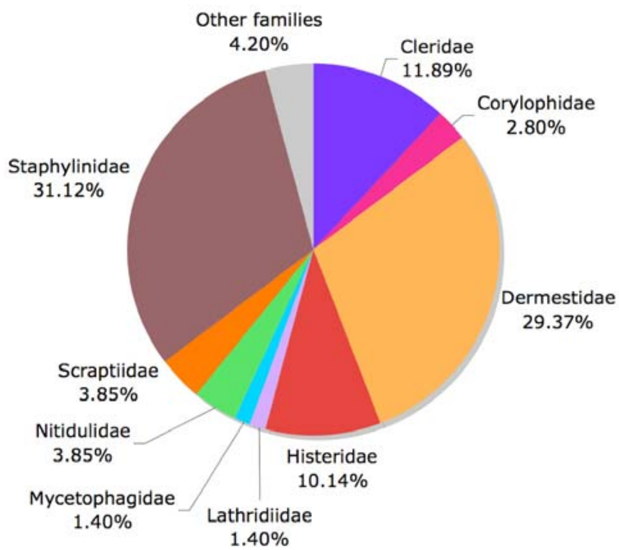
A



B



C



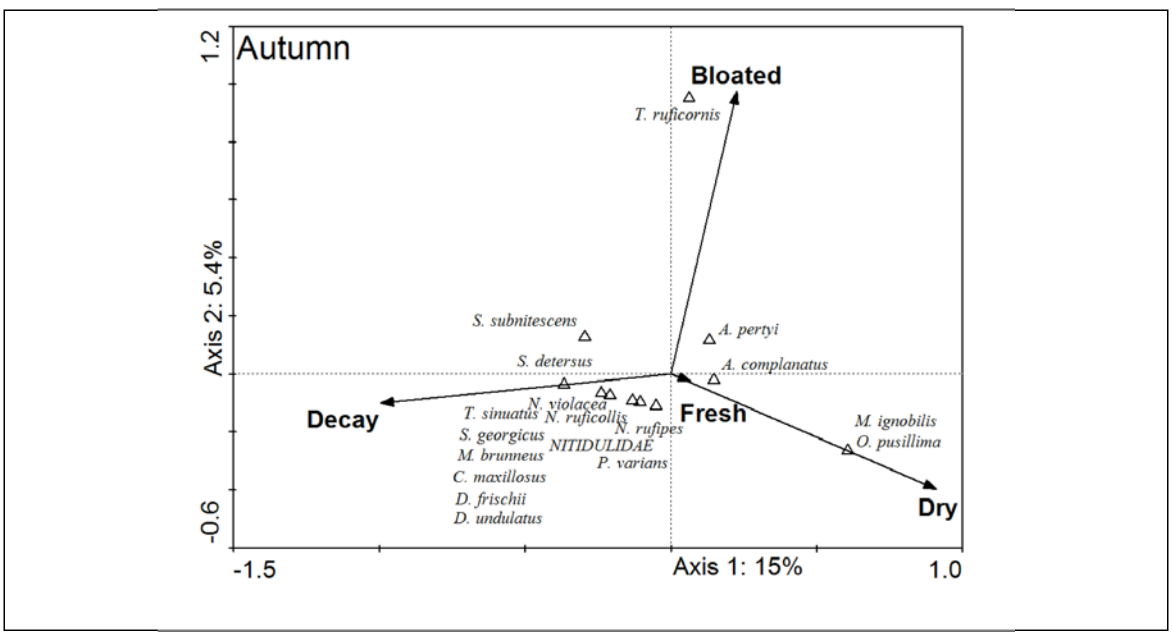
D

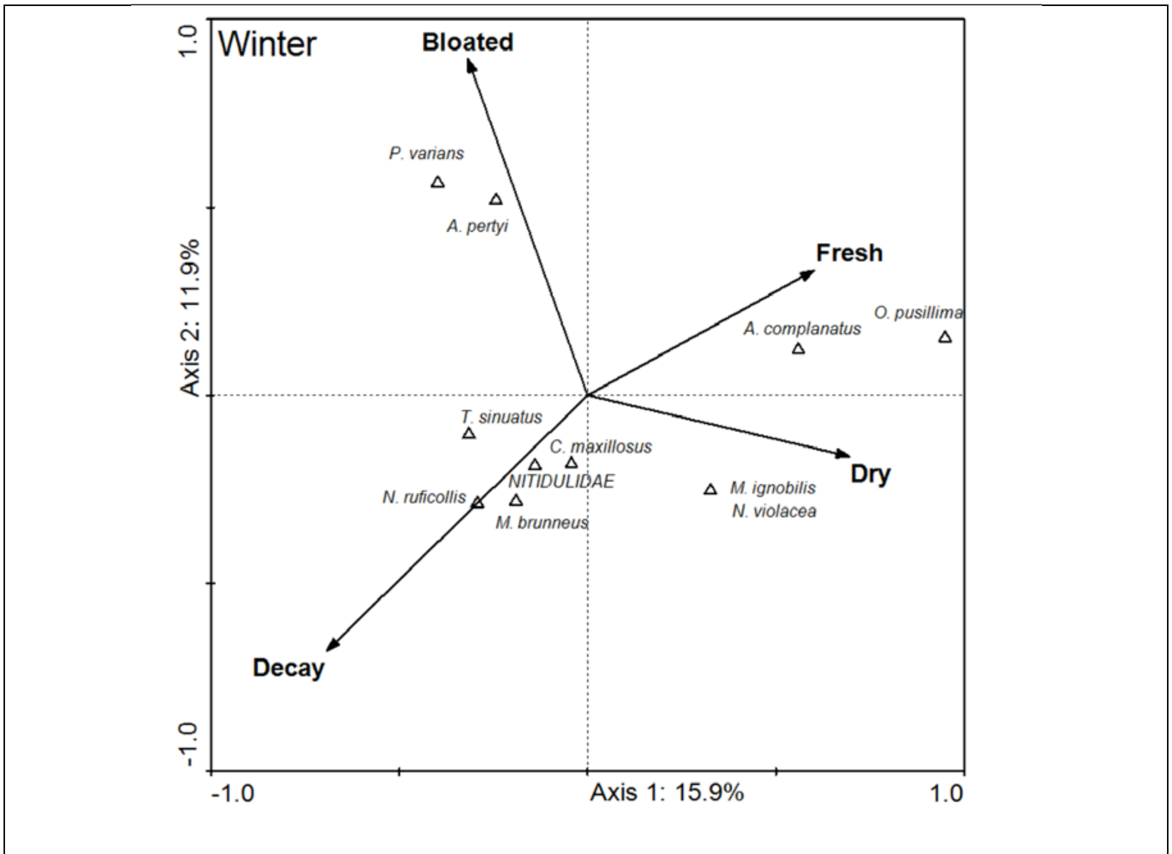
Fig. 5. Relative abundance of Coleoptera families during the study period, for each of the four seasons of the year (adult specimens). A) autumn; B) winter; C) spring; D) summer

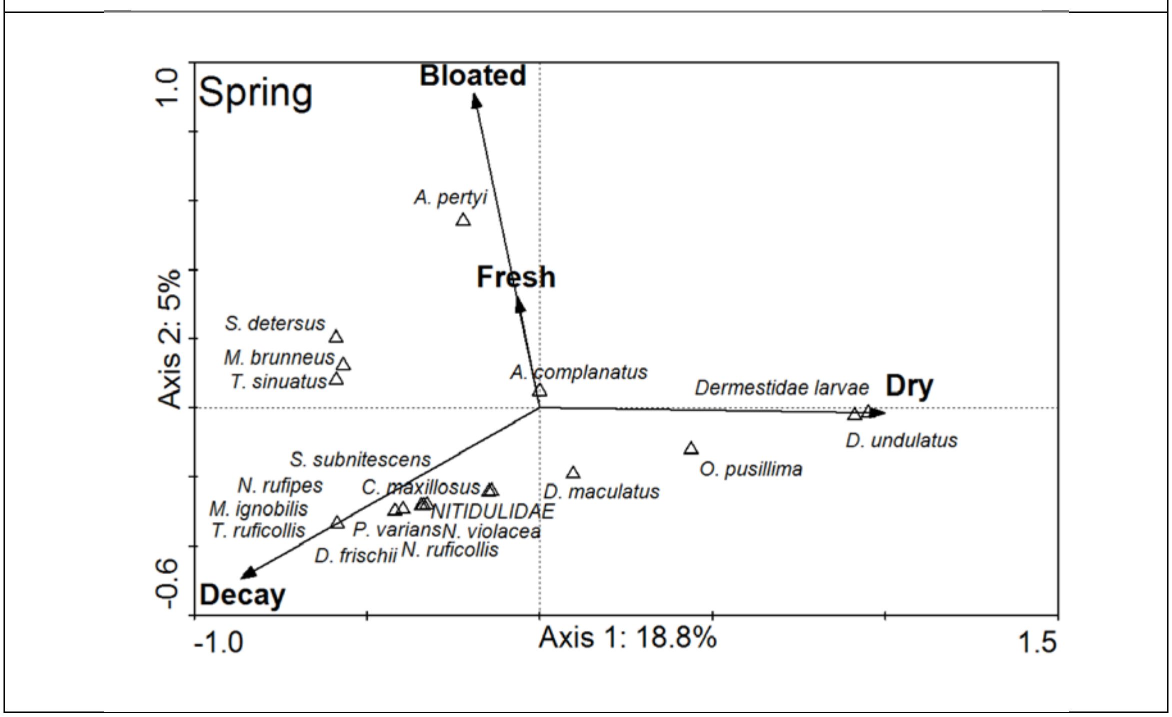
The presence of many species was markedly seasonal. For instance, *Dermestes undulatus* Brahm, 1790, very abundant in spring and summer, was scarce in autumn and absent in winter. No species of Dermestidae appeared in winter, neither *Saprinus* spp. (Histeridae) (Table 2). On the other hand, Silphidae were not present in the summer.

Regarding the stages of decomposition within each season, in general a higher abundance and species richness of Coleoptera was found in the D stage (Figs. 3 and 4) and the lowest values were found in the F stage in all seasons. Furthermore, abundance and richness values between D and F stages were generally significant except in spring (Figs. 3 and 4).

In all sampling seasons coleopteran community composition was clearly different among decomposition stages. The first two axes of CCAs explained a considerable percentage of total community variance (20.4% in autumn, 27.8% in winter, 23.8% in spring and 18.8% in summer) (Fig. 6). In overall sampling seasons, CCA biplots have strikingly separated the stages D and DR in terms of Coleoptera community composition, while stages F and B generally appeared in a more central position (Fig. 6). Most species were generally related to the D stage, particularly *Margarinotus brunneus* (Fabricius, 1775) and *Creophilus maxillosus* (Linnaeus, 1758), but also *Saprinus detersus* (Illiger 1807) and *Thanatophilus sinuatus* (Fabricius, 1775), while only few were related to the DR stage, namely *Oligota pusillima* (Gravenhorst, 1806) and Dermestidae larvae (Fig. 6). On the other hand, *Anotylus complanatus* (Erichson, 1839) and *Atheta pertyi* (Heer, 1839) were apparently more associated to the stages F and B, respectively (Fig. 6).




Winter
Bloated
P. varians
A. pertyi
Fresh
A. complanatus
O. pusillima
T. sinuatus
C. maxillosus
NITIDULIDAE
N. ruficollis
M. brunneus
M. ignobilis
N. violacea
Dry
Decay
Axis 2: 11.9%
1.0
-1.0
-1.0
Axis 1: 15.9%
1.0


Spring
Bloated
A. pertyi
Fresh
S. detersus
M. brunneus
T. sinuatus
A. complanatus
Dermestidae larvae
Dry
D. undulatus
O. pusillima
S. subnitescens
N. rufipes
C. maxillosus
M. ignobilis
T. ruficollis
NITIDULIDAE
D. maculatus
P. varians
N. violacea
D. frischii
N. ruficollis
Decay
Axis 2: 5%
1.0
-0.6
-1.0
Axis 1: 18.8%
1.5

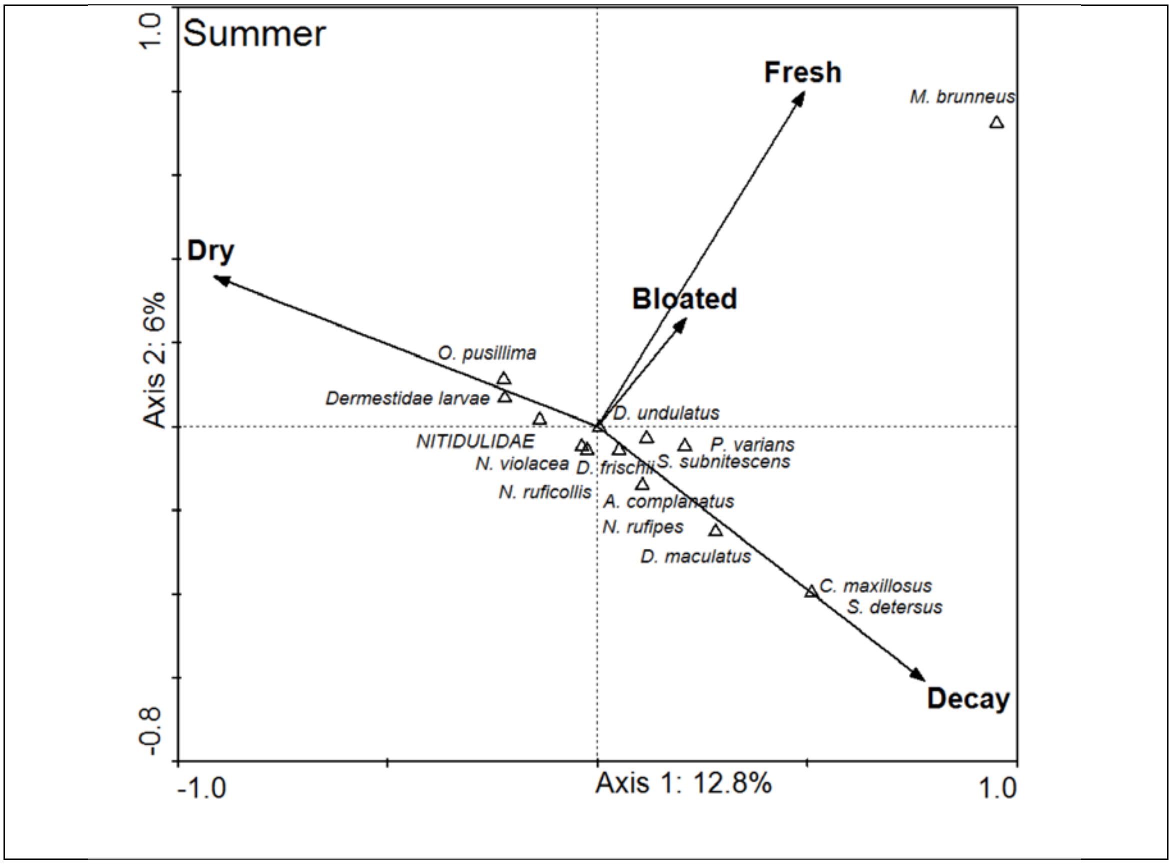


Figure 6. Scatterplots of CCAs based on Coleoptera community composition from carcass decomposition stages in each sampling season: autumn, winter, spring and summer. Significance of canonical axes: Autumn - Axis 1: F = 6.15 (p = 0.002), all axes: F = 3.08 (p = 0.002); Winter - Axis 1: F = 4.53 (p = 0.002), all axes: F = 3.69 (p = 0.002); Spring - F = 7.88 (p = 0.002), all axes: F = 3.77 (p = 0.002); Summer - F = 4.40 (p = 0.024), all axes: F = 2.62 (p = 0.008).

Coleoptera succession. The presence of adults of the different species and immatures belonging to the different families in each sampling day of the studied periods is presented on Tables 3 to 6.

Members of Cleridae family were present all over the year, but with lower abundances in winter. The three species, *Necrobia ruficollis* (Fabricius, 1775), *Necrobia rufipes* (De Geer, 1775) and *Necrobia violacea* (Linnaeus, 1758) were clearly associated with AD stage, when they generally appeared and DR stage, when they were also common.

Table 3. Coleoptera occurrence matrix for autumn. Numbers refer to adults collected (all taxa with at least 2 specimens collected are included in the table). From day 25 onwards, numbers are average number of specimens per day. Abbreviations: L= larvae.

	Autumn	**F**			**B**				**AD**																	**Dry**																
	Day of decomposition	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**25**	**27**	**29**	**31**	**33**	**35**	**37**	**40**	**43**	**46**	**49**	**52**	**55**	**58**	**61**	**66**	**71**	**76**
	CARABIDAE												1	2																				1								
C	*N. ruficollis*											2	1				2				1									1												
L	*N. rufipes*										5	2	2	2					1		1	3	1	1	1		1		1				1				1	1	1			
E	*N. violacea*										1		1				1	1	1		2								1													
R	Cleridae larvae				L																																					
	CRYPTOPHAGIDAE																													1				1								
D	*D. frischii*										8	5	2	1	3	2	7	2	2			2																				
	D. undulatus											1									1																					
H	*M. brunneus*									2	4		1		1					1		1	1																			
I	*S. detersus*										1						1																									
S	*S. georgicus*												2			2		1	3	1																						
T	*S. furvus*										1	1								1																						
	S. subnitescens						1			1	2	1	2		2	1	1	1																								
	LATHRIDIIDAE							1			1								1					1												1						
	LEIODIDAE																	1	1				1					1			1	1			1							
N	NITIDULIDAE										2	1	3	4	1	1	4	2	9	2	1	1		1			1	1	1							1		1	1			
	Nitidulidae larvae																															L				L		L	L			L
	PSELAPHIDAE		1								1																															
	PTILIIDAE					4	3	1		12	10		4				2	2		1			2	1	1	2	3	7	2	3	1	1	1		1	1	1		1	1		1
S	*T. ruficornis*						1	1			2																															
I	*T. sinuatus*									1	1	2	1																													
L	Silphidae larvae												L				L	L																								
	A. fungi	1	2	2	1		7	1	1	7	3	1						1								1							1		1		1	1			1	1
	A. intrincata																														1											
	A. tristis																						1																			1
	A. laticollis									1							1	1	1												1							1				
S	*A. pertyi*				12	4	6	6	8	8	5	2	4	3	5	3	4	1	4		11	4	9	3	2	1	3	5	2	1	2	1	2	2	1		1	2		1	1	2
T	*A. sodalis*				1			1			1			1				1	3	1	1	2	1	4	2	1	1		1	1				1	1						1	
A	*C. obscura*																			1													1									
P	*D. cadaverina*			1		1					1						1										1														1	
H	*O. pusillima*																																	1	1	1		1	1	1	3	1
Y	*A. complanatus*	1				1	2	4	9	12	24	7	5	5	3	2	4	3	4	1	1						1	5		1	1	5	3	2	1	2	1	2			1	2
L	*A. pumilus*																	1					1		6		3	3		1	2	1	1		1			1				
I	*P. atomarius*						3			1	2						1			2	5		2					1		1		1					1	1		1	1	1
N	*C. maxillosus*									2	1	4	1																													
I	*O. aethiops*																																					1		1		
D	*P. varians*								1	2	2	3	1		1	2																		1			1				1	
A	*Q. latinus*										1																													1		
E	*B. maklini*																																				1	1				
	M. mulsanti																1																							1	1	1
	M. nigricollis																											1										1				1
	M. solidicornis																																	1							1	
	Staphylinidae larvae						L					L	L		L		L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L

Table 4. Coleoptera occurrence matrix for winter. Numbers refer to adults collected (all taxa with at least 2 specimens collected are included in the table). From day 25 onwards, numbers are average number of specimens per day. Abbreviations: L= larvae.

	Winter	F																	B								D			AD							Dry					
	Day of decomposition	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	25	27	29	31	33	35	37	40	43	46	49	52	55	58	61	66	71	76
C	*N. ruficollis*																													1			1									
L	*N. violacea*																																			1		1				
H	*M. brunneus*																										1	1		1	1	1	1		1	1		1				
I	*M. ignobilis*																																1			1		1				1
S	*M. uncostriatus*																													1					1							
	LEIODIDAE																											2	1					1	1							
N	NITIDULIDAE				1													1									1	3	1	4	1	1	1	1								
N	Nitidulidae larvae	L																																								
	PTILIIDAE															1																					1	1				
S	*T. sinuatus*																									1	2	1		1	1	1	1									
S	Silphidae larvae																																			L						L
	SCYDMAENIDAE																1																								1	
S	*A. fungi*													1											1														1			
T	*A. laticollis*																																				1					1
A	*A. pertyi*													1	1						1		1	1	3	2	1	1	1	2	2											
P	*O. pusillima*				1																																	1				
H	*A. complanatus*		2			1									3	1		1							1			1					1		1	1	1		1	1	1	1
Y	*A. pumilus*										1																		1												1	1
L	*C. maxillosus*																									1	1			1		1	3	1	2	1	1	1	1		1	
	O. aethiops							1	1					1						1		1																				
	P. varians																									1					1											
	M. piceolus															2																										
	Staphylinidae larvae																L											L				L	L	L	L	L	L	L	L	L	L	L

Table 5. Coleoptera occurrence matrix for spring. Numbers refer to adults collected (all taxa with at least 2 specimens collected are included in the table). From day 25 onwards, numbers are average number of specimens per day. Abbreviations: L= larvae.

	Spring	**F**			**B**			**D**	**AD**												**Dry**																		
	Day of decomposition	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**25**	**27**	**29**	**31**	**33**	**35**	**37**	**40**	**43**	**46**	**49**	**52**	**55**	**58**	**60**
	CANTHARIDAE										1		2				1		1	1	2		2			1	1	2	2	3	2		3	1	3	1	1	1	
	CARABIDAE		1	1																								1							1				
CL	*N. ruficollis*							1	1		1	6	3	1					1					1						1									
CL	*N. violacea*							1	1	1		2	2	2		1			1		1	1			1				1										
CL	Cleridae larvae																																			L		L	L
	CORYLO/*S. lateralis*																																1	1				1	
	CURCULIONIDAE																									1									1				1
DERM	*D. frischii*							1	5	1	2	1	1								1																		
DERM	*D. maculatus*								3	1		1		1															1			1			1	1			
DERM	*D. undulatus*												1								3	6	5	1			1	3	3	2	2	1	2	4	1	1	1	1	6
DERM	*Anthrenus sp.*																												1								1		
DERM	*Dermestes* larvae																					L			L	L	L								L	L	L	L	L
HIST	*M. brunneus*		1				2	14	24	4	2																												
HIST	*M. ignobilis*							1				1																											
HIST	*S. detersus*						1	3	3	1																													
HIST	*S. subnitescens*							3	4		1											1																	
	LEIODIDAE												1												1														
	MELYRIDAE																			1											1			1	3	1			
	NITIDULIDAE								1	1		1						1		1													1	1					
	PTILIIDAE																					1	1	3	1				1	1					1				
	SCRAPTIIDAE																				1										1								
SIL	*T. ruficornis*							1	1				1																										
SIL	*T. sinuatus*						1		1	2	2	2	1																										
SIL	Silphidae larvae								L				L		L								L										L						
STAPHYLINIDAE	*A. laticollis*			2											1																								
STAPHYLINIDAE	*A. parvicornis*																			1			1												1				
STAPHYLINIDAE	*A. pertyi*		2		2	10	5	2	2	15		1	1	1	1	2	9			1	5		1		2	1							1						
STAPHYLINIDAE	*C. obscura*	1																											1										
STAPHYLINIDAE	*O. pusillima*															1													1								1		
STAPHYLINIDAE	*A. complanatus*	2	2	2		2	2	12	15	12		1	6	12	6	5	8	1	17	13	11		7	16	4	3		1		1	1			1	1	1			
STAPHYLINIDAE	*N. ibericus*																												1	1	1					1			
STAPHYLINIDAE	*P. atomarius*			1			1											1																					
STAPHYLINIDAE	*C. maxillosus*							2	3		2	1	3																				1						
STAPHYLINIDAE	*P. varians*							1	19	13			1	1			1		3		1							1											
STAPHYLINIDAE	*Q. fumatus*													1															1										
STAPHYLINIDAE	*T. nitidulus*																																1		1				
STAPHYLINIDAE	Staphylinidae larvae				L								L		L	L	L	L	L			L	L											L					

Table 6. Coleoptera occurrence matrix for summer. Numbers refer to adults collected (all taxa with at least 2 specimens collected are included in the table). From day 25 onwards, numbers are average number of specimens per day. Abbreviations: L= larvae.

	Summer	F		B			D	AD						Dry																							
	Day of decomposition	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**25**	**27**	**29**	**31**	**33**	**35**	**37**	**40**	**43**	**46**	**49**	**52**	**55**	**58**	**61**
	CANTHARIDAE			1		1																																	
C	*N. ruficollis*							2		2		1	1	2	1			2		1					1	1				1			1						1
L	*N. rufipes*						1			1								1	1																				
E	*N. violacea*									1		1	2		1		3						2					1	1					1					
R	Cleridae larvae																L						L								L	L						L	L
	COCCINELLIDAE									1																							1					1	
	CORYLO/*S. lateralis*																						1		1											1			1
D	*D. frischii*					1		2	6	2			1	1		1				1							1		1	1	1		1						1
E	*D. maculatus*							1		1		1	1		2																								
R	*D. undulatus*				2	2	2	3	2	5	1	2	1	3	1	1		1			1					1	2	1	1	2	3	1	1						1
M	*Dermestes* larvae							L		L		L	L	L	L	L	L			L			L			L	L	L	L	L	L	L	L		L		L	L	L
H	*M. brunneus*		1			1	5	1																															
I	*S. detersus*						2	2	3																														
S	*S. subnitescens*					2	2	3	1						1		2						1															1	1
	LATHRIDIIDAE																							1									1				1		
	MELYRIDAE		1	1																				1															
	MYCETOPHAGIDAE												1																			1	1		1				
N	NITIDULIDAE									1			1	1	2		1						1						1				1						1
	Nitidulidae larvae																																			L			L
	SCRAPTIIDAE				1	1	1						2		1								1						1			1					1	1	
	SILVANIDAE											1			1																								
S	*A. laticollis*			1				1				1													1		1	1											
T	*L. nitidula*											1			1																								
A	*O. pusillima*					1		1																									1	1	1	3	9	3	4
P	*A. complanatus*									1																			1										
H	*C. maxillosus*						3	1	1	1																													
Y	*P. varians*					1	1			2																			1										
L	Staphylinidae larvae													L				L		L	L												L						

Among Dermestidae species, *Dermestes frischii* Kugelann, 1792 was dominant in autumn, being present in the cadaver from day 10 to 21 (AD stage). *Dermestes undulatus* was the most abundant dermestid in spring and summer, together with *D. frischii* and *Dermestes maculatus* De Geer, 1774. The three species were present from D to DR stage; *D. undulatus* was particularly more numerous during DR stage. In summer, *D. undulatus* and *D. frischii* could reach the carcass earlier, in the B stage (days 4 and 5, respectively). Dermestidae larvae were present in the carcass from day 21 in spring and day 7 in summer, until the end of the experiment and they were not found in the other seasons. It is also worth to mention that adults were absent in winter.

Ten histerid species were found on carcasses, of which *M. brunneus* and *Saprinus subnitescens* Bickhardt, 1909 were the most abundant. Most species were present mainly from the end of B stage to the end of AD.

Nitidulidae was present all over the year, with particular abundance in autumn and winter, when it was more associated to D and AD stages.

Ptiliidae, very abundant in autumn, was present throughout the study period.

Thanatophilus sinuatus was the most abundant silphid species, particularly in winter, being clearly associated with D stages. *Thanatophilus ruficornis* (Kuster, 1851), only present in autumn and spring was scarce and coincident in time with *T. sinuatus*, but appearing some days earlier in autumn. This family was completely absent in the summer experiment. Silphidae larvae were also not abundant and were present latter than the adults, however in spring they were also present at the same time as adults.

Regarding Staphylinidae, the most abundant and speciose Coleoptera group collected in the experiments, it is noticeable the abundance of *A. pertyi* and *A. complanatus* in autumn, winter and spring. These species were present throughout the study period. *Creophilus maxillosus* was present during all year, mainly in D and AD stages of the different trials, but it was particularly abundant in winter. In spring, *Philonthus varians* (Paykull, 1789) was very numerous in the beginning of AD stage. Summer was dominated by *O. pusillima*, in DR stage.

Discussion

The duration of the decomposition stages differed in the four seasons of the year, especially in winter, due to the lower temperatures that retarded decomposition and insect activity. In autumn and spring, the decomposition pattern was similar, due to similar temperatures, especially in the first week. However, in autumn temperatures were decreasing with time, which lead to a longer AD stage. Summer was the season in which decomposition was faster, because of higher temperatures. The carcass was dry in less than 2 weeks.

Winter was the season with the lowest activity of beetles (lower values of abundance and richness), as expected, since in general the cold reduces the activity of insects. Other European studies on carrion communities characterization (Arnaldos *et al.*, 2004; Matuszewski *et al.*, 2010) demonstrated that spring was the richest season of the year. In our case, autumn yielded the highest values of Coleoptera abundance and richness. Among decomposition stages, in general, D stage presented the highest abundance and richness regarding Coleoptera communities.

The composition of Coleoptera communities changed along with the carcass decomposition process and the different *a priori* defined decomposition stages partly explained this variability. Particularly, our results showed that D and DR decomposition stages uphold substantially different Coleoptera communities in terms of species composition and their relative abundance. Other factors, however (e.g. related to the vegetation structure in the area) could also have some influence on community composition of Coleoptera occurring in the carcass. Still, the *a priori* visually defined decomposition stages were consistently supported by specific groups of Coleoptera. This indicates that their presence in the carcasses is truly connected with specific decompositional stages and thus, its use as forensic indicators of postmortem interval is valuable.

The most abundant and diverse group of beetles were the Staphylinidae, in all seasons of the year. The high number of specimens collected in carcasses was also referred in Easton & Smith (1970), Johnson (1975) and Lefebvre & Gaudry (2009). *Creophilus maxillosus* was frequently observed predating blowfly larvae, which explains their presence on carcasses during D and AD stages. *Creophilus maxillosus* is the most commonly cited staphylinid in forensic entomology studies (e.g. Reed, 1958; Anderson, 1995; Anderson & VanLaerhoven, 1996; Bourel *et al.*, 1999; Castillo Miralbes, 2002; Kocárek, 2003; García-Rojo, 2004; Matuszewski *et al.*, 2008, 2010; Özdemir & Sert, 2009; Michaud *et al.*, 2010), with identical observations. *Philontus varians* was mainly related to D and AD stages, as well. This species, described as coprophilous and fitodetritivore in Vogel (1989), demonstrates also a clear necrophilous behaviour. Staphylinidae are, in general, predators of other species; many opportunistically visit carrion, but are not restricted to it (Fernández *et al.*, 2010). This is also the case of *A. pertyi* and *A. complanatus*, both humicolous and saprobic, as well as *O. pusillima*, fitodetritivore and fungicolous (Vogel, 1989). The species composition within this family in this study has very little in common with similar studies from diverse geographical areas. Taking into account the 5 most abundant species, only *C. maxillosus* is common; *P. varians* was also mentioned in Kentner & Streit (1990), Anderson & VanLaerhoven (1996), Kocárek (2003) and Michaud *et al.* (2010) studies and *A. pertyi* was listed in Castillo Miralbes (2002), in Spain. *Anotylus complanatus* and *O. pusillima*, to the best of our knowledge were not found in any similar study in the Iberian Peninsula or elsewhere.

Regarding Histeridae species, most are shared with other regions of Portugal (Grosso-Silva & Soares-Vieira, 2009) and many with other regions of Spain (Castillo Miralbes, 2002; García-Rojo, 2004). Studies from other geographical areas share very few species (e.g. Tantawi *et al.*, 1996; Matuszewski *et al.*, 2008, 2010; Özdemir & Sert, 2009; Michaud *et al.*, 2010) or no species at all (e.g. Reed, 1958; Bourel *et al.*, 1999; Kocárek, 2003; Kentner & Streit, 1990). Similarly to Matuszewski *et al.* (2008) and Özdemir & Sert (2009), after Staphylinidae, Histeridae was the Coleoptera family having the largest number of species on the carcasses and they were found mainly in stages that present intense larval activity (D and AD stages). The presence of Histeridae in carcasses is related to the predatory habits of both their adults and larvae that feed on eggs, larvae and pupae of Diptera (Yélamos, 2002).

As for Staphylinidae and Histeridae, most Silphidae species are geographically localized, differing from region to region. While *T. sinuatus* is common throughout Europe (e.g. Kentner & Streit, 1990; Bourel *et al.*, 1999; Kocárek, 2003; Matuszewski *et al.*, 2008, 2010), *T. ruficornis* and *T. sinuatus* together appear to be characteristic from the Iberian Peninsula, (Bahillo de la Puebla & López-Colón, 2000; Castillo Miralbes, 2002; García-Rojo, 2004; Grosso-Silva & Soares-Vieira, 2009). Adults of *Thanatophilus* are specialists with respect to habitat (they occupy carrion only), but generalists concerning their diet (maggots as well as decaying meat) (Kocárek, 2003). In our study they were not very abundant and were mostly associated to D and AD stages, periods clearly characterized by a large amount of larvae available. Similar results were reported by Kocárek (2003) and Matuszewski *et al.* (2008).

Cleridae is another group of predacious beetles; adults and larvae feed mainly on the immature stages of beetles, while other feed on maggots (Byrd & Castner, 2010). *Necrobia ruficollis*, *N. rufipes* and *N. violacea* are the three *Necrobia* Olivier, 1795 present in the Iberian Peninsula, all presenting a cosmopolitan distribution (Grosso-Silva & Soares-Vieira, 2009). Their presence in cadavers is very common and, as in the present study, associated to late decomposition stages (e.g. Kocárek, 2003; Özdemir & Sert, 2009).

Dermestids feed on a wide variety of dried animal matter and they can be very numerous feeding on skin, sinews and bone (Smith, 1986). Adults and larvae are common on corpses and most species are cosmopolitan (Midgley *et al.*, 2010). Their association to AD is well documented (e.g. Reed, 1958; Payne, 1965; Anderson & VanLaerhoven, 1996; Schroeder *et al.*, 2002; Grassberger & Frank, 2004), which is in line with our results. Tantawi *et al.* (1996) and Özdemir & Sert (2009) refer their appearance in the B stage, which has also happened in our summer trial.

Some taxa may have the potential to be seasonal indicators, as they just appear in a particular time of the year. *Atheta pertyi* and *A. complanatus* were absent in summer, as well as Silphidae. However at least *T. sinuatus* was captured in this season in Portugal (Grosso-Silva & Soares-Vieira, 2009). *Saprinus* spp. were absent in winter, which is in line with other observations in the Iberian Peninsula (Castillo Miralbes, 2002; Grosso-Silva & Soares-Vieira, 2009). *Dermestes* spp. were also absent in winter, but the same was not observed in similar studies in Spain (Castillo Miralbes, 2002; Arnaldos *et al*., 2004). Further studies could be useful to highlight the phenology of these species in Portugal.

Seasonal studies of insect succession on cadavers offer information on abundance of carrion species and a perspective of the shifting species composition of the community with changing seasons (Kocárek, 2003). The information presented in this paper is the first regarding cadaver's colonization patterns by Coleoptera in Portugal and will certainly provide valuable clues to forensic investigations in this geographical area. In order to achieve more useful data, this type of study implemented for Lisbon region must be applied for other regions.

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Chapter 9

STAPHYLINID FORENSIC COMMUNITIES FROM LISBON WITH NEW RECORDS FOR PORTUGAL (COLEOPTERA: STAPHYLINIDAE)

Prado e Castro, C., García, M.D., Serrano, A., Gamarra, P. & Outerelo, R. (2010) Staphylinid forensic communities from Lisbon with new records for Portugal (Coleoptera: Staphylinidae). *Boletín de la Asociación española de Entomología*, **34**, 87-98.

ARTÍCULO DE INVESTIGACIÓN

Staphylinid forensic communities from Lisbon with new records for Portugal (Coleoptera: Staphylinidae)

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ABSTRACT

From experiments conducted to study insect species composition and seasonal succession in piglet carcasses, 35 Staphylinidae species were collected, of which 5 are reported for the first time in Portugal: *Anotylus pumilus, Stenus (Metastenus) bifoveolatus, Bryophacis maklini, Mycetoporus mulsanti* and *Mycetoporus piceolus.* Results of this study improve the knowledge of entomosarcosaprophagous fauna in Portugal, especially relevant to forensic science.

Key words: Coleoptera, Staphylinidae, faunistic, new records, forensic entomology, Portugal

RESUMEN

Los Staphylinidae de la comunidad sarcosaprófaga de Lisboa, con nuevas citas para Portugal (Coleoptera)

A partir de experiencias desarrolladas para conocer la composición específica y la sucesión estacional de la fauna asociada con cadáveres de cerdos, se han recogido 35 especies de Staphylinidae, de las cuales 5 son nuevas para la fauna portuguesa: *Anotylus pumilus, Stenus (Metastenus) bifoveolatus, Bryophacis maklini, Mycetoporus mulsanti* and *Mycetoporus piceolus.* Los resultados obtenidos incrementan el conocimiento en relación con la fauna entomosarcosaprófaga en Portugal, de especial relevancia en la práctica forense.

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Palabras clave: Coleoptera, Staphylinidae, faunística, nuevas citas, entomología forense, Portugal

INTRODUCTION

Staphylinidae is one of the most diverse Coleoptera families, with 45724 species described worldwide (HERMAN, 2001) and 1547 in the Iberian Peninsula (GAMARRA & OUTERELO, 2005, 2007, 2008a, b, c, 2009a, b, c, d, e, f, g). This group comprises species with a variety of feeding habits, but most of them are predators (KOCH, 1989). Particularly, staphylinid species have been considered the commonest predators found on cadavers (SMITH, 1986). This finding was also corroborated by PRADO E CASTRO *et al*. (2009b).

Despite the forensic importance of rove beetles, their faunistic knowledge in Portugal remains incipient, regardless all contributions already made to study this Coleoptera family in Portugal (e.g., OLIVEIRA, 1894; SEABRA, 1943; FERREIRA, 1962; COIFFAIT, 1963; SERRANO, 1981; AGUIAR & SERRANO, 1995; BOIEIRO *et al.*, 1999; MARTINS DA SILVA *et al.*, 2006).

The occurrence and distribution of species from 16 Staphylinidae subfamilies (Aleocharinae, Paederinae, Staphylininae, Omaliinae, Oxytelinae, Leptotyphlinae, Osoriinae, Tachyporinae, Steninae, Habrocerinae, Phloeocharinae, Trichophyinae, Euaesthetinae, Pseudopsinae, Micropeplinae and Proteininae) in the Iberobalear Region has been recently compiled in catalogues (GAMARRA & OUTERELO, 2005, 2007, 2008a, b, c, 2009a, b, c, d, e, f, g) that also include lists of all the bibliographic references available for the Iberian Peninsula, in each of the subfamilies.

The aim of this paper is to provide information about 35 Staphylinidae species collected in cadavers in Lisbon and thus contribute to a better knowledge of this Coleoptera family in Portugal. New records are provided as well as some data about species distribution.

MATERIAL AND METHODS

From October 2006 to August 2007, in Lisbon (Portugal), Staphylinidae specimens were collected using a modified Schoenly trap (PRADO E CASTRO *et al*., 2009a) in order to sample the fauna associated with pig carrion. Four piglets were used in four different experiments of 10 weeks each, in order to cover all seasons of the year. The experiments consisted on leaving a dead animal as bait inside the trap and during periodical visits collecting the insects that colonized the carcass and bred on it. This was made daily in the first three weeks and afterwards in alternate days. A 40% ethylene glycol solution with formalin and detergent was used in the trap as killing and temporary preservative agent for the arthropods, after which they were removed to 80% ethanol. The experiments were performed in Instituto Superior de Agronomia, Tapada da Ajuda, Lisbon (UTM coordinate 29SMC8384). The location is a forested "island" inside the urban perimeter, mainly composed of *Ailanthus altissima* (Mill.) Swingle, *Fraxinus angustifolia* Vahl and *Ulmus minor* Mill., in an altitude of 80 m a.s.l..

The specimens, collected by C. Prado e Castro, are deposited in the collection of the Department of Animal Biology of the University of Lisbon.

RESULTS AND DISCUSSION

This study has allowed the collection of specimens from 35 species belonging to 8 different Staphylinidae subfamilies. Some of them represent the first record of unknown *taxa* for Portugal, thus enlarging the faunistic knowledge of this group. In the following list, the new records are signalized with an asterisk. For each species the biogeographical distribution, as well as date of collection and number of specimens collected are presented below. Geographic distribution of species is based on GAMARRA & OUTERELO (2005, 2007, 2008a, c, 2009c, d, g) catalogues.

Subfamily Aleocharinae

***Acrotona fungi* (Gravenhorst, 1806)**

Species with a broad distribution, present in the Palaearctic, Oriental and Ethiopic regions.

Material examined: from 19.10.2006 to 19.3.2007, 41 specimens.

***Aleochara (Aleochara) curtula* (Goeze, 1777)**

A holarctic and neotropical species that, in the Iberian Peninsula, has been referred from the northern half.

Material examined: 29.10.2006, 1 specimen; 6.7.2007, 1 specimen.

***Aleochara (Baryodma) intrincata* Mannerheim, 1831**

Species distributed throughout the Palaearctic region.

Material examined: 24.11.2006, 2 specimens; 25.04.2007, 1 specimen; 20.07.2007, 1 specimen.

***Aleochara (Xenochara) tristis* Gravenhorst, 1806**

Palaearctic species.

Material examined: 9.11.2006, 1 specimen; 2.1.2007, 1 specimen.

***Aleochara (Coprochara) verna* Say, 1836**

A euronearctic species, spread throughout the Iberian Peninsula.

Material examined: 1.11.2006, 1 specimen.

***Atheta (Mycetota) laticollis* (Stephens, 1832)**

Eurosibiric species, spread throughout the Iberian Peninsula.

Material examined: from 27.10.2006 to 15.12.2006, 6 specimens; 13.3.2007, 1 specimen; 3.4.2007, 1 specimen; from 19.4.2007 to 30.4.2007, 3 specimens; from 30.6.2007 to 28.7.2007, 7 specimens.

***Atheta (Microdota) parvicornis* (Rey, 1873)**

Westerneuropean species, in the Iberian Peninsula is present about the northern half.

Material examined: 5.5.2007, 1 specimen; 8.5.2007, 1 specimen; 4.6.2007, 1 specimen.

***Atheta (Atheta) pertyi* (Heer, 1839)**

This is a euromediterranean and macaronesic species, spread throughout the Iberian Peninsula.

Material examined: from 22.10.2006 to 23.2.2007, 199 specimens; from 18.4.2007 to 29.5.2007, 66 specimens.

***Atheta (Tetropla) sodalis* (Erichson, 1837)**

Species of euroturanic and nearctic distribution, in the Iberian Peninsula is only known from Portugal.

Material examined: from 22.10.2006 to 28.12.2006, 29 specimens; 5.2.2007, 1 specimen.

***Cordalia obscura* (Gravenhorst, 1802)**

A westpalaearctic and neartic species, spread throughout the Iberian Peninsula.

Material examined: 6.11.2006, 1 specimen; 30.11.2006, 1 specimen; 17.4.2007, 1 specimen; 19.5.2007, 1 specimen; 6.7.2007, 1 specimen.

***Dimetrota cadaverina* (Brisout, 1860)**

European species.

Material examined: from 21.10.2006 to 16.11.2006, 5 specimens; 28.12.2006, 2 specimens; 15.2.2007, 1 specimen.

***Liogluta nitidula* (Kraatz, 1856)**

Species of euroturanic distribution.

Material examined: 7.6.2007, 1 specimen; 8.7.2007, 1 specimen; 11.7.2007, 1 specimen.

***Oligota (Oligota) pusillima* (Gravenhorst, 1806)**

Species widely distributed in Euroasiatic, Nearctic and Neotropical regions.

Material examined: from 3.12.2006 to 21.1.2007, 29 specimens; 16.3.2007, 1 specimen; from 1.5.2007 to 27.8.2007, 65 specimens.

Subfamily Micropeplinae

***Micropeplus staphylinoides* (Marsham, 1802)**

Holomediterranean species, spread throughout the Iberian Peninsula.

Material examined: 27.11.2006, 1 specimen.

Subfamily Oxytelinae

***Anotylus complanatus* (Erichson, 1839)**

Widespread species, referred from Palaearctic, Australian and Neotropical regions.

Material examined: from 19.10.2006 to 3.4.2007, 187 specimens; from 17.4.2007 to 30.7.2007, 180 specimens.

***Anotylus inustus* (Gravenhorst, 1806)**

Westpalaearctic species.

Material examined: 27.10.2006, 1 specimen.

***Anotylus pumilus* (Erichson, 1839)**

New species record for Portugal. Species was known from the western Palaearctic region. In the Iberian Peninsula up to now it had been referred from the center area, Cádiz and Mallorca (Balearic Islands).

Material examined: from 4.11.2006 to 3.4.2007, 38 specimens.

Subfamily Paederinae

***Nazeris ibericus* Kock, 1940**

This species has an iberic distribution, being present in the western third of the Iberian Peninsula.

Material examined: from 19.5.2007 to 7.6.2007, 4 specimens.

Subfamily Proteininae

***Proteinus atomarius* Erichson, 1840**

Although it is a holarctic species, in the Iberian Peninsula is only known from Portugal, Cádiz province and Balearic Islands.

Material examined: from 24.10.2006 to 28.12.2006, 25 specimens; from 19.04.2007 to 3.5.2007, 3 specimens.

Subfamily Staphylininae

***Creophilus maxillosus* (Linné, 1758)**

A well known species, present in Palaearctic, Nearctic and Oriental regions.

Material examined: from 27.10.2006 to 30.10.2006, 8 specimens; from 13.02.2007 to 29.03.2007, 30 specimens; from 23.04.2007 to 06.07.2007, 19 specimens.

Ocypus (Pseudocypus) aethiops **(Waltl, 1835)**

This species has a holotirrenic distribution, being spread throughout the Iberian Peninsula

Material examined: from 15.12.2006 to 07.02.2007, 7 specimens; 04.07.2007, 1 specimen.

Philonthus (Philonthus) discoideus **(Graavenhorst, 1802)**

Cosmopolitan species.

Material examined: 29.10.2006, 1 specimen; 10.03.2007, 1 specimen.

Philonthus (Philonthus) longicornis **Stephens, 1832**

Cosmopolitan species.

Material examined: 30.4.2007, 1 specimen.

Philonthus (Philonthus) varians **(Paykull, 1789)**

Cosmopolitan species.

Material examined: from 26.10.2006 to 23.2.2007, 18 specimens; from 23.4.2007 to 17.5.2007, 41 specimens; from 2.7.2007 to 27.8.2007, 6 specimens.

Quedius (Raphirus) fumatus **(Stephens, 1832)**

A westmediterranean species, in the Iberian Peninsula is known from the northern half.

Material examined: 29.4.2007, 1 specimen; 19.5.2007, 1 specimen.

Quedius (Raphirus) latinus **Gridelli, 1938**

Species of tirrenic distribution.

Material examined: 28.10.2006, 1 specimen; 23.12.2006, 1 specimen.

Xantholinus (Idiolinus) translucidus **Scriba, 1870**

This is a holotirrenic species.

Material examined: 12.12.2006, 1 specimen.

Subfamily Steninae

***Stenus (Metastenus) bifoveolatus* Gyllenhal, 1827**

New species record for Portugal. This species, with euroturanic distribution, was only known, in the Iberian Peninsula, from Sierra de Guadarrama, thus much enlarging the distribution of the species.

Material examined: 01.3.2007, 1 specimen.

Subfamily Tachyporinae

***Bryophacis maklini* (Shalberg, 1871)**

New species record for Portugal. This species, with euroturanic distribution, is considered by some authors as a synonymy of *B. rugipennis* (Pandellé, 1869); species neither referred from Portugal. In the Iberian Peninsula it was known from southeastern area.

Material examined: 12.12.2006, 1 specimen; 15.12.2006, 1 specimen; 19.2.2007, 1 specimen.

***Mycetoporus longulus* Mannerheim, 1830**

It is a species with euroturanic distribution. In the Iberian Peninsula it was known from the northwest and central areas of Spain and from central Portugal.

Material examined: 9.7.2007, 1 specimen.

***Mycetoporus mulsanti* Ganglabuer, 1895**

New species record for Portugal. Although this species is broadly distributed by the Mediterranean basin, as well as in the Iberian Peninsula, up to now was unrecorded from Portugal. So, this is the first reference for this country.

Material examined: 3.11.2006, 1 specimen; 23.12.2006, 1 specimen; 28.12.2006, 1 specimen; 2.1.2007, 2 specimens.

***Mycetoporus nigricollis* Stephens, 1835**

Holomediterranean species.

Material examined: 18.11.2006, 1 specimen; 15.12.2006, 1 specimen; 1.1.2007, 2 specimens.

******Mycetoporus piceolus*** **Rey, 1883**

New species record for Portugal. This species has a European distribution. In the Iberian Peninsula it has been referred from the north to the center.

Material examined: 01.2.2007, 2 specimens.

Mycetoporus solidicornis **Wollaston, 1864**

This species, tirrenic in distribution, in the Iberian Peninsula is found in the northern half, as well as in Balearic Islands.

Material examined: 3.12.2006, 1 specimen; 28.12.2006, 1 specimen; 3.4.2007, 1 specimen.

Tachyporus (Palporus) nitidulus **(Fabricius, 1781)**

Cosmopolitan species.

Material examined: 29.5.2007, 1 specimen; 4.6.2007, 1 specimen.

From a faunistic point of view, our data contribute to broaden the knowledge concerning the presence and distribution of the Staphylinidae family in Portugal. As regards the sarcosaprophagous community, most staphylinid species belong to its necrophilic component (e.g. ARNALDOS *et al.*, 2005) because they are predators of other elements of the community, being attracted to carrion to feed on maggots and the larvae of other insects (BYRD & CASTNER, 2010). Since many staphylinid species feed on fungi (BOHAC, 1999), a usual resource in a decomposing cadaver, they also belong to the opportunistic component. Among the predacious species, some are specialists, while others have generalist diets (BYRD & CASTNER, 2010). Although these categories of sarcosaprophagous arthropods do not allow an accurate direct estimation of the postmortem interval, its study and evaluation provides some valuable environmental data that may contribute to the estimation of time since death and other interesting factors for forensic purposes. Regarding the knowledge of Staphylinidae related to corpses in Portugal, it is only known the recent contribution of GROSSO-SILVA & SOARES-VIEIRA (2009), who only refer the species *Creophilus maxillosus* (Linnaeus, 1758) as belonging to this community, being present soon after dead. Thus, the data provided here are interesting not only from the faunistic but also from the forensic science' point of view.

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Chapter 10

GENERAL DISCUSSION

General Discussion

The days when insects found in a cadaver were seen by the investigators as a simple and nauseating sign of putrefaction, which had to immediately be cleaned, have passed (Goff, 2002). Acceptance of insects as indicators of a variety of critical forensic parameters has increased, and now the courts recognize insect evidence, especially in cases where time of death is the key-issue (Greenberg & Kunich, 2002). The forensic pathologist depends on a series of signals as the body temperature, body stiffness, postmortem lividity, but as the time since death increases, the above methods become less useful and more accurate results are often obtained using ecological information; insects offer a much longer time scale measured in hours, days, weeks or season, and sometimes years that can lead to the identification of a missing person whose decomposed body is not recognizable anymore (Greenberg & Kunich, 2002; Wells & LaMotte, 2010). Cases involving accidents, homicides and suspicious deaths are the ones that most probably will use the help of forensic entomology (Greenberg & Kunich, 2002). In the estimation of PMI from succession data, it is essential to have information of local carrion fauna and their colonization patterns at the different times of the year (Wells & LaMotte, 2010).

Despite being well established in many countries, forensic entomology is giving its first steps in Portugal. A few years ago, studies were initiated to obtain knowledge on Diptera sarcosaprophagous community (Prado e Castro, 2005). As a result, valuable information was produced on the knowledge of Diptera cadaveric fauna in our territory (Prado e Castro *et al.*, 2010b, 2011a, 2011b). Despite this achievement, basic information on the biology and ecology of most cadaver colonizers was still lacking, a serious hindrance for the establishment of forensic entomology in Portugal.

The present study focus on seasonal insect succession on carrion, which is mainly characterized by the members of two orders – Diptera and Coleoptera. With this contribution, families and species belonging to these two groups were identified in Lisbon, Portugal. The periods of time spent on the carcass and in which decomposition stage, as well as their distribution over the year was determined. This was not yet investigated in Portugal and according to Anderson (2010), to develop a valid database, studies in all seasons of the year are needed, to know the seasonality or relative abundance variations among certain species, and their potential differences in times of colonization of the remains.

As a result of this study, it was possible to observe that the decomposition rate of pig carcasses was affected primarily by feeding of Calliphoridae larvae, whose activity and development was conditioned by the different temperature regimes, in the different seasons. Thus, winter's lowest temperatures retarded carcass colonization by insects and decompositional process was clearly slower, in comparison with the other seasons. The similar temperatures registered in autumn and spring during the first week (when most of the blowfly larval development occurred) explains the similarity in the duration of decomposition stages. In summer, the decomposition was faster due to the higher temperatures. Not surprisingly, abundance and richness values were significantly lower in winter, both for Diptera and Coleoptera. Autumn was the richest season for both groups, while Diptera was more abundant in spring and Coleoptera in autumn (chapters 3 and 8).

Twelve Diptera species were considered of major forensic importance: *Calliphora vicina* Robineau-Desvoidy, 1830, *Calliphora vomitoria* (Linnaeus, 1758), *Chrysomya albiceps* (Wiedemann, 1819), *Lucilia ampullacea* Villeneuve, 1922, *Lucilia caesar* (Linnaeus, 1758), *Lucilia sericata* (Meigen, 1826) (Calliphoridae); *Hydrotaea ignava* (Harris, 1758), *Muscina prolapsa* (Harris 1780), *Synthesiomyia nudiseta* (van der Wulp, 1883) (Muscidae), *Piophila megastigmata* McAlpine, 1978, *Stearibia nigriceps* (Meigen, 1826) (Piophilidae) and *Nemopoda nitidula* (Fallén, 1820) (Sepsidae). These species used carrion as breeding substrate and showed a clear seasonal shift in their presence. While in autumn all the species could be found, winter was dominated by *C. vicina*, *C. vomitoria* and *N. nitidula*. Spring yielded, again, a great diversity of species, however, *C. albiceps*, *S. nudiseta* and *P. megastigmata* were not active, which turns this season very different from autumn and summer, mainly because *C. albiceps*, when present, is very dominant. Summer was the only season when *Calliphora* spp. were not able to breed on the cadaver and in which Sarcophagidae spp. were abundant colonizers (chapter 3).

Two especially important events, both concerning Calliphoridae, should be highlighted from the experiments. The first one is larval migration, which establishes the time limit in which no more larvae (or a very small number) will be found in the corpse. In autumn and summer migration mainly ended on day 10, in winter on day 43 and in spring on day 9. The second event is adult emergence (appearance of tenerals of the species that bred on the corpse), which marks the point when empty pupae can be found in the proximity of the remains. This occurred about 2 weeks after death, when *C. albiceps* was present (autumn and summer), 1 month in spring and 2 months in winter (chapter 3). These information are extremely useful in order to establish the PMI in forensic practice.

Another result of this work is the new record of more than 50 Diptera species in Portugal, an important contribution for the knowledge of this fauna in the country. These species belong to families Calliphoridae (Prado e Castro & García, 2009; Prado e Castro *et al*., 2010a) - chapters 4 and 5; Piophilidae (Prado e Castro & García, 2010) - chapters 6 and 7; Carnidae, Lauxaniidae, Sphaeroceridae (Carles-Tolrá & Prado e Castro, in preparation), Fanniidae, Muscidae, and Scatopsidae (unpublished data).

Regarding Coleoptera, in general, the ones that fed on the carcasses, as *Dermestes* spp. (Dermestidae), were present in later stages of decomposition (decay to dry stage); in spring and summer its larvae were clearly dominant in dry stage. Predator groups such as Histeridae and Silphidae were more concentrated in periods of intense blowfly larval activity (end of bloated stage to the middle of advanced decay). *Necrobia* spp. (Cleridae) were, however, present in drier stages, showing that blowfly larvae are not the preferred food source, but most likely immature stages of other beetles. Among Staphylinidae, while some species were not related to any particular stage of decomposition, *Creophilus maxillosus* (Linnaeus, 1758) and *Philonthus varians* (Paykull, 1789) were abundant in decay and advanced decay stages. Dry stage was preferred by *Oligota pusillima* (Gravenhorst, 1806) (chapter 8).

The presence of many Coleoptera was also noticeably seasonal, e.g. *Atheta pertyi* (Heer, 1839), *Anotylus complanatus* (Erichson, 1839) and Silphidae spp. were absent in summer; *Saprinus* spp. were absent in winter, as well as *Dermestes* spp. The presence of *Dermestes* larvae only in spring and summer is also very relevant (chapter 8). The active seasonality of certain species might provide useful information concerning time of death because when remains are discovered a considerable time after death, sometimes years, the presence of insect remnants can easily indicate the season of death.

Within Coleoptera, 2 species belonging to Corylophidae family (Faria e Silva *et al.*, 2009) and 5 to Staphylinidae were also recorded in Portugal for the first time (Prado e Castro *et al.*, 2010c) – chapter 9.

Diptera and Coleoptera communities clearly changed along carcasses decomposition. The occurrence and abundance of families/species formed specific groups that separated the stages of decomposition. These results, besides supporting the underlying assumption that different communities may segregate the *a priori* visually defined stages of decomposition, indicate that their presence in the carcasses is truly connected with specific decompositional stages and thus, its use as forensic indicators of postmortem interval is valuable (chapters 3 and 8).

The methodology used in the present work (chapter 2) is highly effective and appropriate for the collection of sarcosaprophagous Diptera (Ordóñez *et al.* 2008). The study of the whole community attracted to a cadaver is an advantage, especially from the faunistic point of view, given that an enormous diversity of species is taken into account. In Portugal, where Diptera is still a poorly known group, the use of a modified Schoenly trap (Prado e Castro *et al.*, 2009) clearly contributed to a substantial increase in the faunistic knowledge. Additionally, consistent information on the periods of activity of species was gathered. Nevertheless, from a forensic perspective, it is not fundamental to study all the Diptera attracted to a corpse, but the species that actually breed on the cadaver are considered the most important. One of the reasons for this is that immature stages of flies are the most commonly recovered evidence from corpses and its developmental stage is what is taken into account in PMI estimation. Simpler (i.e. less laborious) experiments on insect succession may be performed in the future using only tweezers for the collection of immature Diptera (to breed) and Coleoptera, in addition to pitfall traps.

As far it was possible to compare with other regions of the Iberian Peninsula, an interesting result from our data is that Calliphoridae community is very similar in Lisbon, Coimbra and in the north of Spain (Basque Country), with *Lucilia caesar* and *L.*

ampullacea as central species in the community, whereas in most Spanish territory, *L. sericata* is the dominant *Lucilia* (chapter 3). This suggests an Atlantic influence on the communities from the studied regions in Portugal and in the north of Spain; it would be interesting to know if the same pattern is maintained in all the northwest of the Iberian Peninsula. To the south of Lisbon (Algarve and Alentejo), where Mediterranean climate is more pronounced, it would be important to evaluate the difference/similarities with all the previous mentioned studies.

Also, due to the fact that a significant number of corpses are found indoors (Amendt *et al*., 2010) it would be significant to carry out experimental work in our geographical area, to determine the differences in the species composition and colonization, in an indoor environment.

Finally, it would be important to carry out developmental studies in the laboratory with local species. Not only the assessment of possible variation in developmental time for geographically distinct populations would be relevant (Amendt *et al*., 2011), but also the collection of developmental data for *L. caesar*, species for which no information is yet available. Additionally, among Coleoptera, many taxa remain unexplored (Midgley *et al*., 2010) and developmental studies should be planned to obtain data useful for PMI estimation.

In conclusion, the presented study is the first completed in Portugal on Diptera and Coleoptera fauna, covering an annual period, providing a dynamic profile of seasonal abundance of individual carrion species and a perspective on the shifting species composition of the carrion community with changing seasons. Data obtained will be useful in the interpretation of insect evidence in forensic cases in Lisbon and its surroundings, in the future.

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