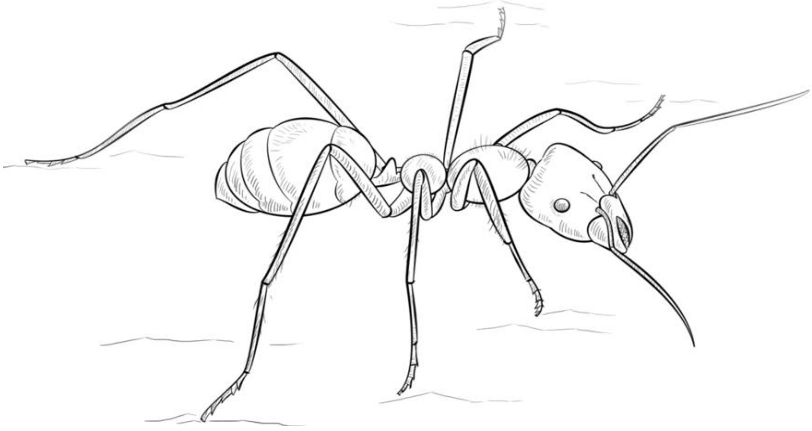

Seasonal diversity of the Formicidae (Hymenoptera) communities and their dynamics in pig carcasses in Lisbon (Portugal): putative application for *postmortem* interval estimation

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To Sara, Álvaro and João

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ABSTRACT

The value of the Formicidae (Insecta, Hymenoptera) community in forensic investigations is poorly studied in Portugal. In order to deepen the knowledge of the structure and dynamics of this group of insects in cadavers, studies were carried out in Lisbon area between autumn 2006 and summer 2007. Piglet (*Sus scrofa* L.) carcasses weighing between 7.5-8kg were used as a model of human decomposition in this study. Modified Schoenly traps were used to collect the entomofauna attracted to the carcasses over a period of about 2 months in each season of the year. The collection of ants was performed at regular intervals, daily in the first 23 days and then with intervals of 2, 3 or 5 days until the end of the experiment. Five stages of cadaveric decomposition were recognized with the ants being present in all of them. 309 specimens were captured: 7 in autumn, 6 in winter, 90 in spring and 206 in summer. These specimens belong to 3 subfamilies and 7 different species: *Tapinoma nigerrimum* (Nylander, 1856), *Plagiolepis pygmaea* (Latreille, 1798), *Aphaenogaster senilis* (Mayr, 1853), *Crematogaster scutellaris* (Olivier, 1792), *Crematogaster auberti* (Emery, 1869), *Temnothorax luteus* (Forel, 1874) and *Tetramorium semilaeve* (André, 1883). Four of these species had never been mentioned before as having forensic interest in this geographical area. Spring and summer had the greater diversity of species and a larger number of individuals. Corroborating the results of other similar studies carried out in the Iberian Peninsula, the sarcosaprophagous Formicidae community found in Lisbon is unique and different from other studied locations, which supports the need to perform regional studies.

RESUMO

O valor da comunidade da família Formicidae (Insecta, Hymenoptera) em investigações forenses está pouco estudado em Portugal. De forma a aprofundar o conhecimento da estrutura e da dinâmica deste grupo de insetos em cadáveres foram efetuados estudos na região de Lisboa, entre o outono de 2006 e o verão de 2007. Carcaças de leitões (*Sus scrofa* L.), com pesos entre os 7.5-8kg, foram usadas como modelo para a decomposição humana. Foram utilizadas armadilhas Schoenly modificadas para recolher a entomofauna atraída pelas carcaças, durante um período de cerca de 2 meses em cada uma das estações do ano. A colheita de formigas foi efetuada em intervalos regulares, diariamente nos primeiros 23 dias e depois com intervalos de 2, 3 ou 5 dias até ao final da experiência. Conseguiram-se identificar cinco estágios de decomposição cadavérica estando as formigas presentes em todos eles. Foram capturados 309 espécimes: 7 no outono, 6 no inverno, 90 na primavera e 206 no verão. Estes espécimes pertencem a 3 subfamílias e a 7 espécies diferentes: *Tapinoma nigerrimum* (Nylander, 1856), *Plagiolepis pygmaea* (Latreille, 1798), *Aphaenogaster senilis* (Mayr, 1853), *Crematogaster scutellaris* (Olivier, 1792), *Crematogaster auberti* (Emery, 1869), *Temnothorax luteus* (Forel, 1874) and *Tetramorium semilaeve* (André, 1883). Quatro destas espécies nunca tinham sido mencionadas anteriormente, com interesse forense, nesta área geográfica. A primavera e o verão apresentaram a maior diversidade de espécies e o maior número de indivíduos. Corroborando os resultados de outros estudos similares efetuados na Península Ibérica, a comunidade Formicidae sarcosaprófaga encontrada em Lisboa é única e diferente dos outros locais estudados, realçando a necessidade de se realizarem mais estudos regionais para aprofundar-se este conhecimento.

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OUTLINE OF THE DISSERTATION

The present thesis is structured in four main parts:

PART I - GENERAL INTRODUCTION AND OBJECTIVES OF THE DISSERTATION

In Part I a general approach is made about Forensic Entomology focusing on aspects such as its definition, importance and its application. There is also a general survey of the forensic importance of the ants. The content of the introduction was mainly based on high-quality scientific publications. It was also taken into account the authors of renown in this area of studies and also the most recently published articles.

In this part the objectives of this work are presented.

PART II - MATERIALS AND METHODS

The PART II is divided in six points, with all the information important to describe al the experimental procedures.

PART III

1. RESULTS

In this section all the results of this work are presented.

2. DISCUSSION

The results obtained are analyzed critically and conclusions are drawn up duly based on the relevant bibliography in this subject.

PART IV

The references used in PART I, PART II and PARTIII are listed.

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PART I
INTRODUCTION

The entomofauna present at a crime scene represents important evidence as it can provide useful information about the place of death, the time of the year death has occurred or even assist in the calculation of the *postmortem* interval (PMI) (Catts and Goff, 1992; Goff and Win, 1997). An accurate estimate of PMI is one of the most important, complex and challenging tasks for forensic pathologists. In the first hours after death, physical (*algor mortis* and *rigor mortis*) and physicochemical (*rigor mortis*) changes constitute the main basis for the estimation of PMI (Costa, 2015; Dinis-Oliveira, 2016). In addition, other methods that use biochemical parameters have been shown to be more accurate in this estimation since they are less susceptible to the effect of external factors (Costa, 2015; Dinis-Oliveira, 2016; Ferreira, 2013; Sampaio-Silva et al., 2013). Mathematical models have recently been developed as complements for PMI estimation. These models take into account some biomarkers whose varying concentrations are strongly correlated with time after death (Costa, 2015; Dinis-Oliveira, 2016; Ortmann et al., 2016). Nevertheless, the accuracy of the various models depends on several internal and external factors that influence the cadaveric decomposition process and may be unique to each case, such as age, gender, xenobiotic administration, environmental temperature, clothing, body location, and insect activity, among many others. In an attempt to increase accuracy of the PMI estimation it is advisable to combine the different methods (Costa, 2015; Dinis-Oliveira, 2016; Madea, 2005; Muñoz Barus et al., 2008).

Arthropods have long been used to aid in the calculation of the PMI (Catts and Goff, 1992; Payne et al., 1968; Smith, 1986) and this is one of the main objectives of Forensic Entomology studies. Forensic Entomology uses arthropods, mainly from the class Insecta, in criminal investigations. It applies to several areas, namely in medical-legal cases, urban and in stored products infestations (Byrd, 2009; Catts and Goff, 1992; Smith, 1986). This work focuses mainly on the medical-legal aspect of Forensic Entomology. This area of study focuses on arthropods that are found or infest human corpses. These colonizers can be used in various ways in the investigation: to estimate the PMI through the study of their life cycle or succession patterns of colonization; to know if the body was moved; or to associate suspects with the crime scene (Amendt et al., 2011; Catts and Goff, 1992). Insects are an important tool in the resolution of crimes and in recent years Forensic Entomology has evolved immensely with recent and innovative studies (Andrade-Silva, 2015; Arnaldos et al., 2004; Byard and Heath, 2014; Cammack et al., 2016; Maciel, 2016; McIntosh et al., 2017; Paula et al., 2016; Pechal et al., 2015). A corpse is visited by a wide variety of arthropods called sarcosaprophagous which represent a community that is specific from a location and depends on its soil and climate conditions (Anderson, 2010). The in-depth knowledge of this community in a particular region is an asset for criminal investigation.

While Forensic Entomology has been progressing internationally, in the Iberian Peninsula there is still little research in this area (Arnaldos et al., 2001; Arnaldos et al., 2004; Martínez-Sánchez et al., 2011; Ubero-Pascal et al., 2015) and the studies are even more scarce in Portugal, focusing mainly on the study of flies (Diptera) and beetles (Coleoptera) on corpses (Prado e Castro, 2011; Prado e Castro et al., 2012a; Prado e Castro et al., 2013; Prado e Castro et al., 2012b). Although ants (Formicidae) are one of the most abundant groups of the sarcosaprophagous community, they are also the less studied in the forensic context (Campobasso et al., 2009), even though some works were carried in the Iberian Peninsula (Castillo Miralbes, 2002; Martinez, 2002; 1997; Prado e Castro, 2014).

According to the categories described by Smith (Smith, 1986) arthropods can be: Necrophagous - when feeding exclusively on parts of the corpse; Predators and parasites of necrophagous species; Omnivores - that feed on both the corpse and the species that visit it; and Adventive - species that appear randomly because the corpse is in its habitat. In forensic context, the ants, due to their eating habits are considered omnivores. They are practically ubiquitous and have different eating habits, some are seed collectors, others are predators and some create symbioses with other insects to obtain food. Ants that feed on carcasses can feed on the carcass itself, or the fauna associated with it (Andrade-Silva, 2015; Bonacci and Vercillo, 2015; Smith, 1986; Tabor et al., 2005). Several experimental studies on animal carcasses have confirmed the presence of ants in the different stages of cadaveric decomposition, being mainly opportunistic predators of eggs and larvae of other insects (Chen et al., 2014; Maciel, 2016; Paula et al., 2016; Payne et al., 1968; Prado e Castro, 2014). Even though observed in the different stages of decomposition, the value of ants is often neglected by forensic pathologists and investigators, and its effect on cadaverous remains is not much appreciated, as it can bring confusion to research. They are able to remove eggs, larvae and even adults of Diptera and Coleoptera (Arnaldos et al., 2004; Paula et al., 2016; Payne et al., 1968; Smith, 1986; Tabor et al., 2005), thus affecting the normal rate of decomposition (Campobasso et al., 2009) and interfering in the estimation of the PMI based on the succession of insects. In addition, some species feed on the corpse and can cause damage, often confused with *antemortem* or *perimortem* wounds. An experienced pathologist can distinguish it, but, in certain circumstances they are difficult to differentiate. It is necessary to be taken into account the size and orientation of the lesions and habitat where the remains were found and try to find evidence of ants activity that could support the final diagnosis (Bonacci and Vercillo, 2015; Byard, 2005; Byard and Heath, 2014; Campobasso et al., 2009; Heath and Byard, 2014). However, the effects of ants in these situations are dependent on species, abundance and geographical area (Prado e Castro, 2014). In many cases ants are the group of arthropods numerically dominant on the corpse, since they are social insects that live in colonies that can reach thousands of individuals (queen, soldiers and workers). Some species, such as *Anoplolepsis longipes* (Jerdon, 1851), have already been used to estimate the PMI, taking into account the minimum time this species needs to establish a colony (Goff and Win, 1997).

This study aims to investigate the dynamics of the Formicidae family community in cadavers during the different seasons of the year, in Lisbon (Portugal). The information on the species found will allow us to increase the knowledge about the group in this geographical area and in the Iberian Peninsula, by comparing the results found with those from other similar studies.

PART II

MATERIALS AND METHODS

Site description

The experiments were carried out from October 2006 to August 2007 in Lisbon (the capital of Portugal). The region climate is classified as type Csa (temperate Mediterranean climate with hot summers and rainy winters) according to Köppen-Geiger. Also accordingly to data from the Portuguese national weather service, Instituto Português do Mar e da Atmosfera (IPMA), the average climatological values of precipitation and temperature of the seasons are: in spring 119.5mm, 12.6ºC-14.1ºC; in summer 11.4mm, 20.2ºC-22.1ºC; in autumn 108.5mm 15.6ºC-17.0ºC and in winter 192.4mm, 9.5ºC-10.8ºC. The study was performed at Instituto Superior de Agronomia, Tapada da Ajuda (38 ° 42 41N 09 ° 11 28 6W), a forested area within the urban perimeter composed mainly of *Ailanthus altissima* (Mill.) Swingle, *Fraxinus angustifolia* (Vahl.) and *Ulmus minor* (Mill.). The study site was in the shade and away from anthropological activities.

Carcasses and experimental procedures

Four experiments were carried out with duration of 8 to 10 weeks each, to correspond to the four seasons of the year. The dates of the experiments were as follows: autumn between October 18, 2006 and January 2, 2007; winter between January 17, 2007 and April 3, 2007; spring between April 16, 2007 and June 16, 2007; and summer between June 27, 2007 and August 27, 2007. In each experiment, one piglet (*Sus scrofa domesticus* L.) was killed through an incision in the jugular vein. The pig carcass was then placed inside a modified Schoenly trap (Prado e Castro, 2009) acting as bait. This trap allows the entomofauna to be continuously collected, while allowing the cadaveric decomposition process to occur naturally. The bottles in the trap from which the arthropods were collected contained a 40% solution of ethylene glycol with formalin and detergent, which allowed them to be killed and stored temporarily. The collection and replacement of the capture bottles was performed daily in the first 23 days and then every 2, 3 and 5 days until the end of the experiment. The temperature was recorded hourly within the trap with a HOBO data logger. Day 1 corresponds to the date of death and placement of the piglet into the trap. The collected specimens were stored in 80% ethanol and deposited in the collection of the Department of Animal Biology, University of Lisbon.

Identification

All collected ants were identified to the species level. For this task a binocular stereomicroscope and dichotomous keys (Collingwood, 1998; Gómez, 2007) related to this group of insects were used.

Data analysis

Biodiversity parameters were calculated, namely abundance and specific richness for each season of the year and for each stage of decomposition.

Climatic data

The variation of the temperatures in the sampling periods of each season is represented graphically in Figure 1.

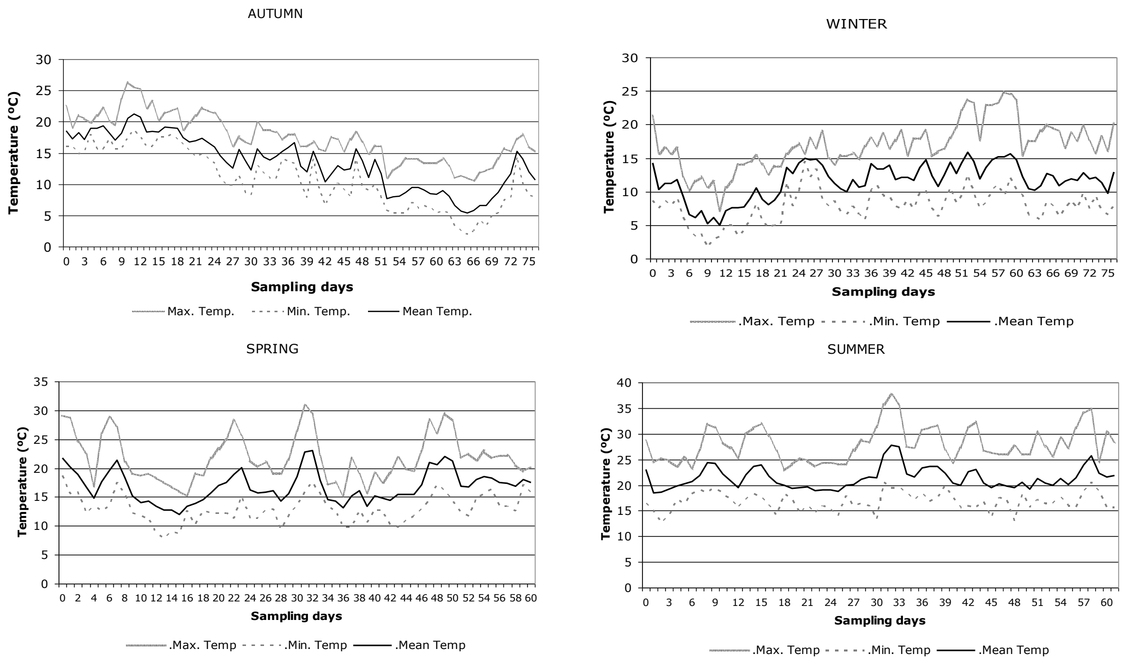

Figure 1 - Temperature data for the studied periods.

The summer, as expected was the season with the highest temperatures. In winter temperatures were considerably lower compared to other seasons. Taking into account the first two weeks of carcass exposure, the mean temperature was 18.7 ± 1.3ºC in autumn, 8.4 ± 2.7ºC in winter, 16.8 ± 3.1ºC in spring and 21.2 ± 2.0ºC in summer.

Decomposition process

The decomposition stages observed in the piglets during the experiments were evaluated according to Anderson & VanLaerhoven (Anderson, 1996). The stages can be briefly described as: Fresh (F), fresh appearance without odor; Bloated (B), bloating, initiating as slight inflation of the abdomen with odor of putrefaction; Decay (D), the carcass starts to deflate, larval masses feeding on soft tissues with strong odor of decay; Advanced decay (AD), intense migration of larvae with decrease of the odor, most of the flesh as been removed at the end of this stage; and Dry (DR), carcass consists of bones, skin and hair with little to no odor. All these stages were observed but with different durations in the different seasons of the year, with the exception of the autumn in which state D was not observed since deflation started simultaneously with larval migration. Also in autumn, differently from the other seasons, the humidity and mold were usually present in the DR stage.

PART III

RESULTS

In this study 309 specimens of ants were collected, 7 in autumn, 6 in winter, 90 in spring and 206 in summer. These specimens belong to 3 subfamilies and 7 different species as shown in Table 1. The species found are briefly described below:

Family	Subfamily	Species	Autumn	Winter	Spring	Summer	Total
Formicidae	Dolichoderinae	*Tapinoma nigerrimum*				109	109
	Formicinae	*Plagiolepis pygmaea*			7	23	30
	Myrmicinae	*Aphaenogaster senilis*		5	7	7	19
		Crematogaster auberti			3	13	16
		Crematogaster scutellaris			8	27	35
		Temnothorax luteus	4	1	58	26	89
		Tetramorium semilaeve	3		7	1	11
		Abundance	7	6	90	206	309
		Species richness	2	2	6	7	

Table 1 - Absolute seasonal abundance and species richness.

Hymenoptera: Formicidae, Dolichoderinae:
Tapinoma nigerrimum (Nylander, 1856)

Polymorphic species, with individuals ranging from 3.2mm to 5.2mm in length, characterized by their uniform black color. They usually form columns of mass workers during foraging. They are active at moderate temperatures and with high relative humidity. Without these conditions they are more easily found in the shade or in night activities (Redolfi, 2002). It is an omnivorous species, generalist and opportunistic (Cerda, 1989), which can feed on aphid exudates and may also be predator of larvae, pupae and adults of other insects (Pereira 2004). The species is distributed throughout the Mediterranean and very frequent in center and south of the Iberian Peninsula (Gómez, 2007) being very resistant to human activities.

Hymenoptera: Formicidae, Formicinae:
***Plagiolepis pygmaea* (Latreille, 1798)**

The ants of the genus *Plagiolepis* are unmistakable with other genera in the Iberian Peninsula by having 11 segments in the antennas. They are very small with a length of less than 3mm of brown color. They can live among putrefied wood, under rocks and in wooded areas in underground colonies. It presents several queens per colony forming a network between the nests of the different queens known as polydomous colony. It is an omnivorous species that feeds on sugary exudates as well as on eggs and larvae from other insects and also from meat and fruit remains (Thurin, 2008). It occurs throughout the Southern part of Europe, being common in the Iberian Peninsula.

Hymenoptera: Formicidae, Myrmicinae:
***Aphaenogaster senilis* (Mayr, 1853)**

Black ants with matte appearance, covered by white and thick hairiness with a length of 5.5mm to 7mm. Observed in open sunny places, forest edges, fields with shrubs and urban areas. Colonies often move their nests to open areas in the spring and autumn and to shady areas in the summer. Workers are individual foragers moving quickly (Barroso, 2013). They are omnivores feeding on both seeds and animal remains. The species is very common in the Iberian Peninsula (Gómez, 2007).

***Crematogaster scutellaris* (Olivier, 1792) and *Crematogaster auberti* (Emery, 1869)**

The ants of this genus are unmistakable because the post-petiole joins the abdomen dorsally being this unique feature, as well as the heart-shaped abdomen. Ants with approximately 4mm in length, in which the distinction of *Crematogaster scutellaris* from the species *Crematogaster auberti* is based on the coloration –*C. scutellaris* has the head and part of the abdomen red and *C. auberti* is unicolored brown (Gómez, 2007). Omnivorous species, feeds on aphid exudates and predates eggs (López-Sebastián, 2004), larvae and other insects (Ottonetti, 2008). These ants are distributed throughout the Mediterranean region, very abundant in the center, south and coast of the Iberian Peninsula (Gómez, 2007).

***Temnothorax luteus* (Forel, 1874)**

The classification of ants of the genus *Temnothorax* (Mayr, 1861) has undergone several modifications mainly due to morphological variations caused by different temperatures during the pupal stage and by the different types of diet. These different conditions originate in several cases hybridizations, which make the taxonomy of this group quite complex (Bolton, 2003; Seifert, 2014). Workers are usually small (<3mm). Colonies are typically monogynous with less than 100 workers. They may be arboreal living within hollow stems, old beetle or termite galleries. *Temnothorax* species appear to be trophic generalists, feeding on a wide variety of scavenged items (Snelling et al., 2014). It is found in the Western Mediterranean region and is considered a cryptic species of *Temnothorax racovitzai* (Bondroit, 1918) with which it is often confused taxonomically (Seifert, 2014).

***Tetramorium semilaeve* (André, 1883)**

Small ants of about 2.5mm to 3mm, light yellow, slightly darker on the head and back of the thorax. Monomorphic species with striated and rough tegument. It has preference for hot and dry open areas, also occurring in forest edges, urban areas and enclosed spaces. They are omnivores being aggressive and with great resource dominating capacity (Retana, 1992). They are distributed throughout the Mediterranean region with a presence throughout the Iberian Peninsula.

Although with different abundances and different species, the Formicidae family was present in all stages of decomposition of the piglet carcasses (Table 2) in the four seasons of the year.

Season	Species	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
Autumn	*Temnothorax luteus*										1													2	1																	
	Tetramorium semilaeve	1											1												1																	
Decay stage		F			B				AD																	DR																
Winter	*Aphaenogaster senilis*																																	4					1			
	Temnothorax luteus																																			1						
Decay stage		F																B									D			AD							DR					
Spring	*Aphaenogaster senilis*							2	1													1	1											1	1							
	Crematogaster auberti																				3																					
	Crematogaster scutellaris																				5							1							1			1				
	Plagiolepis pygmaea								1	1									1								1		1						1		1					
	Temnothorax luteus	2	8				3	6	2		2		2						1		4		2	1				1	4			2	3	3	4	6		2				
	Tetramorium semilaeve							1						1										1				1						1		1	1					
Decay stage		F			B			D	AD												DR																					
Summer	*Aphaenogaster senilis*	1		1	1						1		1				1			1																						
	Crematogaster auberti						3	5	5																																	
	Crematogaster scutellaris	13	3		4	1		1					1	1		1		1																					1			
	Plagiolepis pygmaea	1	1			2						1		1		1	1	1		1					1			1	3				3			1	4					
	Tapinoma nigerrimum																	19	5	11	5	2	7	3	3	9	12	9	10	3	6	1	4									
	Temnothorax luteus	2	2		3	1					2				1	1	1	1		2		1		1					4	1			1	2								
	Tetramorium semilaeve																		1																							
Decay stage		F		B			D	AD						DR																												

Table 2 - Species found by season, day and stage of decomposition.

Autumn and winter were the seasons with lower specific richness and lower abundance of formicides that visited the carcasses. In autumn only two species appeared: *Tetramorium semilaeve* that was present in the F and AD stages; and *Temnothorax luteus* that was present in the AD state (Table 2). In winter two species were also captured: *Aphaenogaster senilis* in the AD and DR states; and *Temnothorax luteus* represented only by one individual in the AD state.

Spring and summer were the seasons that presented the greater specific richness and abundance. In spring there were 6 different species: *Temnothorax luteus* was present in all stages of cadaveric decomposition; *Tetramorium semilaeve* and *Aphaenogaster senilis* were found in stages D, AD and DR; *Plagiolepis pygmaea* appeared in the AD and DR stages; and *Crematogaster auberti* and *Crematogaster scutellaris* were only captured in DR stage. The most abundant species during spring was *Temnothorax luteus* representing 64% of all individuals collected (Figure 2).

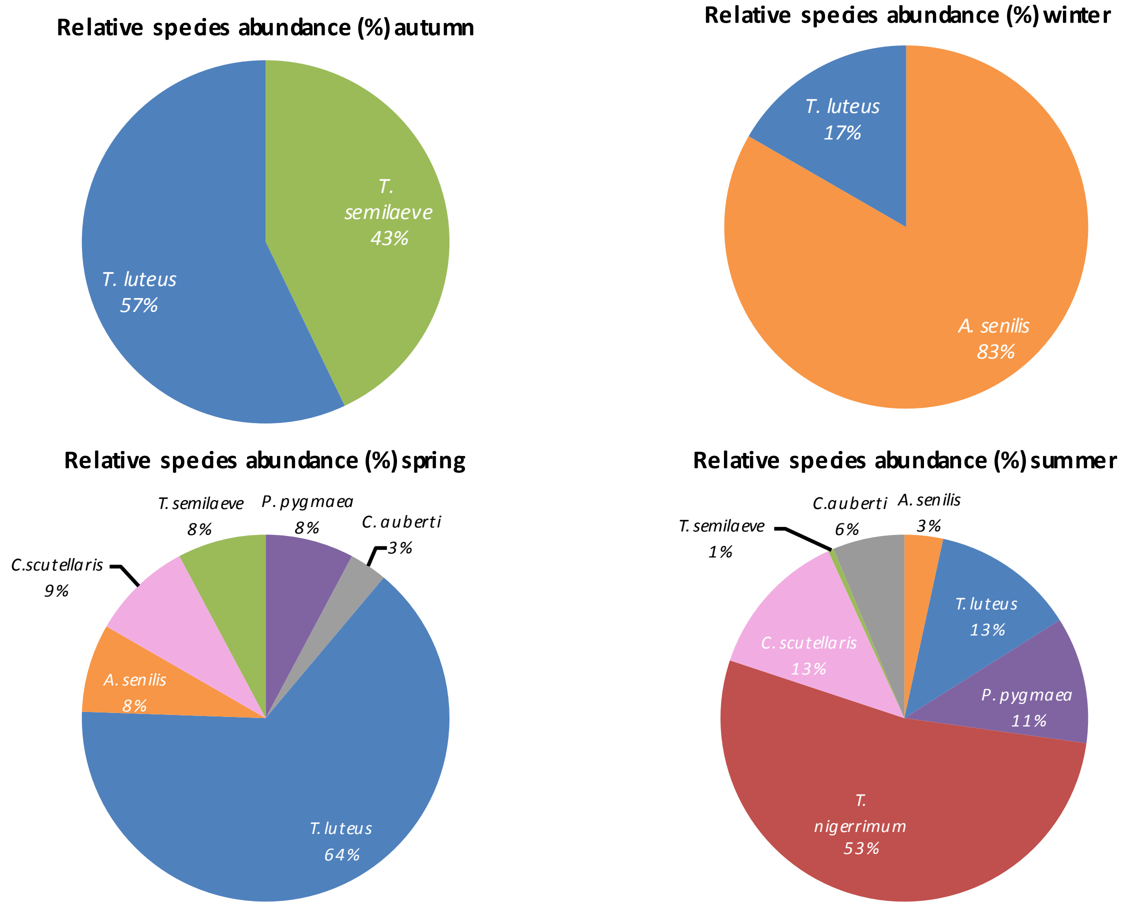

Figure 2 - Relative species abundance by season.

Summer was the season with the highest number of captured ants (206 specimens) and also with the highest specific richness with 7 different species captured: *Aphaenogaster senilis*, *Crematogaster scutellaris*, *Plagiolepis* *pygmaea* and *Temnothorax luteus* which were present in all states of cadaveric decomposition with the exception of stage D; The species *Crematogaster auberti* was present in stages D and AD; and *Tapinoma nigerrimum* appeared exclusively in this season of the year and was the most abundant species with 53% of relative species abundance (Figure2), nevertheless it only appeared in the DR stage of decomposition.

Temnothorax luteus was the only species that was present in the four seasons being more abundant during the spring and summer (Table 1). *Aphaenogaster senilis* and *Tetramorium semilaeve* were present in three seasons however the number of specimens collected was very low. *Plagiolepis pygmaea* and the species of the genus *Crematogaster* were only observed in spring and summer. The only species that was found in just one season was the *Tapinoma nigerrimum* and it was in the summer (Table 1). In autumn and winter the two species found were more abundant in AD stage. In spring, in the DR stage, we observed the highest specific richness with the presence of the 6 species found. In the summer it was also the DR stage that presented the highest specific richness with 6 species.

DISCUSSION

New studies and new approaches have emerged in recent times to make the PMI estimation more accurate (Costa, 2015; Madea, 2005; Muñoz Barus et al., 2008; Ortmann et al., 2016; Sampaio-Silva et al., 2013), nevertheless it is advisable to use new methods in conjunction with the traditional ones (Madea, 2005; Muñoz Barus et al., 2008). The knowledge of carrion entomofauna should also be used to aid the calculation of the PMI (Catts and Goff, 1992; Goff and Win, 1997; Payne et al., 1968; Smith, 1986), mainly using the knowledge of the life cycles of dipterans or of the entomological succession that appears in the cadaver in a predictable way. Most of the experiments on arthropods that visit corpses use animal models to simulate humans. In this case, the domestic pig was considered the most appropriate species for a comparative study since it has physiological patterns of decomposition similar to humans and better extrapolations can be expected (Arnaldos, 2006; Catts and Goff, 1992).

The environmental conditions of spring and summer regarding temperature, humidity and food availability explain a greater diversity of species and a larger number of individuals present in the environment (Smith, 1986). Indeed, these seasons presented the greater specific richness and abundance (Table 1). Of the seven species found, four have already been mentioned several times in studies performed on cadavers (*Tapinoma nigerrimum, Crematogaster scutellaris, Plagiolepis pygmaea* and *Tetramorium semilaeve*). *Tapinoma nigerrimum* is known for causing skin lesions or modifying existing wounds in bodies by feeding itself, leading to misinterpretations by forensic pathologists (Bonacci and Vercillo, 2015; Martínez, 2014). In addition, this species should be considered as an opportunistic member of the sarcosaphagous community since it is able to remove eggs and larvae of dipterans, which may slow the decomposition process and make an incorrect assessment of the PMI (Bonacci and Vercillo, 2015; Campobasso et al., 2009). In this study this species was only found in the summer and exclusively at the DR decomposition stage and it even became the most abundant species in this season (Table 1). The temperatures provided by the summer in shady areas are favorable to the action of these ants. They were captured in large numbers when compared to the other species, probably due to the mass forage activity of workers, characteristic of this species (Redolfi, 2002).

The *Crematogaster* species were present in spring and summer. In the spring the two species only appeared in DR stage. In the summer, *C. auberti* were collected in the D and DR stages while *C. scutellaris* appeared in the different stages, with the exception of D, presenting in large numbers at the beginning of stage F. The presence of this genus in spring and in the DR stage can be explained by the existence of some larval activity, pupae and adults of certain groups of dipterans (*e.g.*, Piophilidae) which would attract it to the carcass remains (Prado e Castro et al., 2012b). The availability of food provided by the carcass, both in stages F (skin and carcass flesh) and in stages D and AD (large numbers of eggs and Diptera larvae) can justify the presence of this genus in summer. *C. scutellaris*, like the *Tapinoma nigerrimum*, has already been mentioned as important in the forensic context, since it feeds on corpses, causing *postmortem* lesions that can be misinterpreted with *antemortem* and *perimortem* lesions and also as an active predator of eggs and larvae thus influencing the calculation of the PMI (Bonacci, 2011; Campobasso et al., 2009).

Tetramorium semilaeve appeared in all seasons, except in winter, practically in all stages of decomposition, but always in very small numbers. *Plagiolepis pygmaea* was only captured in the spring and summer, being more abundant during the summer in the DR stage. These two species are very common in the Iberian Peninsula and have already been reported in other studies of Iberian sarcosaprophagous fauna (Arnaldos, 2006). The cause of their presence is probably due to its opportunistic habits of obtaining food, being the carcass and its entomofauna attractive for these two omnivorous species (Retana, 1992; Thurin, 2008). Like the previous species, *Aphaenogaster senilis* also appeared in very small numbers, in winter, spring and summer and in several stages of decomposition. The individual way the workers of this species carry their forage activity (Barroso, 2013) may explain this reduced number of collected specimens. The genus *Aphaenogaster* has already been cited as belonging to the sarcosaphagous community (Martinez, 2002), however the species *A. senilis* is mentioned in this paper, for the first time, as part of this community.

Temnothorax luteus was the only species captured in all seasons. It belongs to a genus that is currently under review and has little information available. Although, the genus has already been cited in Iberian studies of sarcosaprophagous fauna (Arnaldos, 2006), this species is mentioned for the first time in this context. *T. luteus* was also the most abundant species in the spring, being found in all stages of decomposition.

Differently to several families belonging to Diptera and Coleoptera orders, which present, respectively, abundance peaks at the beginning and at the end of the decomposition stages, in this study, the ants were found, without a definite pattern, throughout the whole process (Table 2). The absence of a consistent pattern can be explained by the opportunistic feeding habit of the species found (Martinez, 1997; Smith, 1986). Our results also evidenced that they do not present an entomological succession pattern. Thus ants cannot be used directly in PMI estimations as many flies and beetles can.

Comparing the species of ants found in Lisbon with the species found in other studies carried out so far in the Iberian Peninsula (Table 3 modified from (Prado e Castro, 2014)) we observed that four species (*Aphaenogaster senilis*, *Crematogaster auberti*, *Tapinoma nigerrimum* and *Temnothorax luteus*) had never been mentioned before with forensic interest in this geographical area. On the other hand there are three species that are common with the communities of the other studies. Indeed, *Crematogaster scutellaris* also belongs to the community of Huesca; *Plagiolepis pygmaea* was also found in Coimbra and Murcia; and the communities of Lisbon, Coimbra and Huesca shared the species *Tetramorium semilaeve*. It was possible to verify that the Formicidae community from Lisbon is unique and different from the other areas considered, thus corroborating that the communities of sarcosaprophagous ants can provide a potential application in forensic practice as a geographic indicator (Arnaldos, 2006; Prado e Castro, 2014) and emphasizing the importance of this type of studies.

	Lisbon	Coimbra	Huesca	Murcia
Aphaenogaster iberica				X
Aphaenogaster senilis	X			
Camponotus aethiops			X	
Camponotus sylvaticus				X
Cataglyphis ibericus				X
Crematogaster auberti	X			
Crematogaster scutelaris	X		X	
Formica rufibarbis			X	
Lasius grandis			X	
Lasius niger				X
Linepithema humile		X		X
Messor barbarus				X
Myrmica specioides			X	
Pheidole pallidula			X	X
Plagiolepis pygmaea	X	X		X
Plagiolepis schmitzii				X
Plagiolepis xene				X
Ponera coarctara		X		
Pyramica membranifera				X
Solenopsis s.				X
Tapinoma nigerrimum	X			
Temnothorax luteus	X			
Temnothorax nylanderi		X		
Temnothorax recedens		X		
Tetramorium semilaeve	X	X	X	

Table 3 - Formicidae species found in four different studies in the Iberian Peninsula. The shaded area indicates the common species between this work and the other studies.

Regarding the global distribution, the number of ant species decreases with increasing latitudes, altitudes and aridity (Hölldobler, 1990). Therefore comparing to our study (Table 1), it is predictable that in tropical areas the specific richness of ants found in cadavers is superior (Andrade-Silva, 2015; Paula et al., 2016). In addition to the specific richness, the voracity of the species found is also an important point to take into account, as voracious species will have a greater impact on the entomofauna from which they feed and hence in the cadaveric decomposition.

In conclusion, this study is a first approach to the seasonal dynamics of the Formicidae family as a part of the sarcosaprophagous community, in Lisbon. It is advisable to carry out more studies with several samples *per* season and at several points in the same region aiming to carry out more complex statistical calculations that improve the credibility to the extrapolation for humans. This study confirms the forensic importance of the ants, since they interfere with the cadaveric fauna and may be useful as a geographic indicator. None of the species found proved however to be useful for the direct calculation of PMI, since they are common in this geographical area, have omnivorous habits and were present without a definite pattern. Among the species found, none was endemic or of limited geographical distribution, which would be interesting and of great value for forensic investigation, since it would, for example, allow to detect movements of the body *postmortem*.

DISCLOSURE STATEMENT

The author has no relevant affiliations or financial involvement with any organization or entity with a financial interest or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties. No writing assistance was utilized in the production of this manuscript.

PART IV

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