

# Temporal dynamic and key species of the sarcosaprophagous entomofauna in a Mediterranean natural environment

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## Resumen

*Dinámica temporal y especies clave de la entomofauna sarcosaprófaga en un medio natural mediterráneo*

Los insectos pueden detectar la presencia de un cadáver a gran distancia, colonizándolo rápidamente y siendo los primeros en explotar este recurso. La composición y dinámica de la comunidad, junto con su presencia en un lugar específico, son clave para su posterior aplicación forense. Por este motivo, se estudió la fauna entomosarcosaprófaga asociada a un cadáver en una zona montañosa de la provincia de Murcia (SE, España). Las especies *Chrysomya albiceps*, *Calliphora vicina* y *C. vomitoria* fueron las especies más valiosas como indicadores estacionales. Estas especies, junto con *Dermestes frischii*, *Camponotus sylvaticus* y la superfamilia Chalcidoidea, definen un patrón claro de colonización que varía según la estación del año. Finalmente, la especie *Iberoformica subrufa* pueden tener valor potencial como indicador de hábitat del área estudiada.

**Palabras clave:** Descomposición; Entomología forense; Sucesión entomológica; Insectos indicadores; Especies clave.

## Abstract

Insects can detect the presence of a cadaver at a great distance, colonizing it rapidly and being the first to exploit this resource. Its composition and dynamics, together with its presence in a specific location, are key to its subsequent forensic application. For this reason, it was studied the entomosarcosaprophagous fauna associated with a carcass in a wild mountainous area of the province of Murcia (SE, Spain). The species *Chrysomya albiceps*, *Calliphora vicina* and *C. vomitoria* were the most valuable species as seasonal indicators. These species, together with *Dermestes frischii*, *Camponotus sylvaticus* and those of the Chalcidoidea superfamily, define a clear pattern of colonization that varies according to the season of the year. Finally, the species *Iberoformica subrufa* may be of potential use as habitat indicator of the studied area.

**Key words:** Decomposition; Forensic entomology; Insect succession; Insect indicators; Key species

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## Introduction

A corpse is an important energy resource and, at the same time, an ecosystem characterized by its short period of existence. Insects have the ability to detect the presence of a corpse at a great distance, colonizing it rapidly, and being the first to exploit this resource (Charabidze & Gosselin 2014). For forensic purposes, the most important fact is that insects are attracted to the carcass in a predictable successional sequence during the process of decomposition (Richards & Goff 1997, Matuszewski *et al.* 2011). The distribution and abundance of insects depend on the geographic location, time of year, environmental characteristics and the habitat in which the corpse is located, and the faunal succession in a cadaver follows a certain order depending on these conditions (Anton *et al.* 2011, Brundage *et al.* 2011, Moretti *et al.* 2011, Bembow *et al.* 2013). Arthropod activity and their community composition commonly shift through the different stages of decomposition either because of alteration of the resource, changes in the environmental conditions or through competitive and/or predatory interactions with other invertebrates or invasive species, which also influences the pattern of succession (Hofer *et al.* 2017, Carmo *et al.* 2018). Consequently, the colonization pattern will also be influenced by the trophic relationships observed in the sarcosaprophagous community with respect to the phases of decomposition of the corpse.

A wide variety of arthropod species that play an active role in the decomposition processes are attracted by a cadaver. The orders Diptera and Coleoptera are the most important since they include the first species capable of detecting and finding a corpse, even from great distances, showing a seasonal presence, together with some species endemic to certain areas (Campobasso *et al.* 2001, Byrd & Castner 2010). In the Iberian Peninsula few studies have considered entomosarcosaprophagous fauna as a whole, the majority contributing with data on certain groups (see table S1 in [Appendix](#)). Therefore, the complete study of the wide variety of entomological species related to corpses and their sequence is of great interest and a useful tool in forensic studies. For this reason, a study all along the four seasons of the year in a wild mountainous area of the province of Murcia, south-eastern Spain, was carried out to provide information on the entomosarcosapro-

phagous fauna composition though the different decomposition stages and its temporal evolution in such environmental conditions.

## Materials and methods

### Study area and experimental design

The study was conducted in Sierra Espuña Regional Park, a protected area (Site of Community Importance ES0000173) in the centre of the province of Murcia (Murcia Region) (37°49'50"N 1°29'56"W). The area is located at about 400 masl, facing South, and characterized by an understory dominated by *Rosmarinus* sp. and *Thymus* sp., and an arboreal stratum of *Pinus* sp. (Pérez-Marcos *et al.* 2016).

Samplings were conducted between 15<sup>th</sup> September 2006 and 31<sup>st</sup> July 2007. Sampling duration for the autumn season was 60 days, (September 15<sup>th</sup>–November 13<sup>rd</sup>, 2006), for winter and spring 59 days (January 8<sup>th</sup>–March 7<sup>th</sup>, 2007 and April 2<sup>nd</sup>–May 30<sup>th</sup>, 2007, respectively), and for summer 47 days (June 15<sup>th</sup>–July 31<sup>st</sup>, 2007). In our study, domestic piglets (*Sus scrofa* L.) of about 5 kg were used as baits models (Battán Horenstein *et al.* 2010, 2012, Prado e Castro *et al.* 2012, 2013, Bembow *et al.* 2013, Mañas-Jordá *et al.* 2018). The pigs were provided by the Veterinary Faculty Farm of the University of Murcia where they were sacrificed by premedication, in accordance with the current Spanish legislation on the protection and sacrifice of animals used for experimentation and other scientific purposes (Real Decreto 1201/2005). One piglet per season was used, and in each season, the trap was set in place a week before starting the assay in order for the substrate conditions to stabilize. Samples were collected with a 60x70x70 cm modified Schoenly trap (Schoenly *et al.* 1991, Prado e Castro *et al.* 2009). This method has been confirmed as the effective device for characterizing the sarcosaprophagous succession in several geographical areas (Arnaldos *et al.* 2001, 2004, Ordóñez *et al.* 2008, Battán Horenstein *et al.* 2010, Battán Horenstein & Salvo 2012, Prado e Castro *et al.* 2012, 2013, 2014, Lyu *et al.* 2016, Mañas-Jordá *et al.* 2018). The receptacles in which arthropods were captured contained Morrill liquid as preservative (Morrill 1975, Schoenly 1981). In each season, samples were taken daily for the first 16 days, and then every 2 days. Temperature and rel-

ative humidity inside the trap were continuously recorded with a HOBO® U10-Temp /RH Data Logger, and at the bait-soil interface they were registered every sampling day with a portable hygrometer Delta OHM DO 9406 attached to a probe Delta OHM HD 8501SAT/500.

Following the classification of Anderson and VanLaerhoven (1996), five decomposition stages were considered: fresh (between the moment of death and the moment when bloating is first evident); bloated (between gases started to accumulate in the carcasses resulting in a definite bloated appearance and when the body deflates due to insects piercing the carcass); decomposition (marked by the complete deflation of the carcass due to the action of larvae breaking the skin); late decomposition (much of the flesh has been removed and maggots left the remains as prepupal insects); and dry remains (very little of the carcasses remained except bones, cartilage and some skin). This methodology was also used in Pérez-Marcos *et al.* (2016) for a preliminary study regarding the spring entomofauna of the same area. Labelled samples were taken to the laboratory, where the Morrill liquid was changed to ethanol (70%). Specimens were counted and identified to the maximum level under a stereomicroscope following Chinery (1998) for order level. McAlpine (1981) and Carles-Tolrá (2004) were followed to identify Diptera families. Diptera species were also confirmed with the keys provided by Gregor *et al.* (2002) for Muscidae family and González Mora & Peris (1988), González Mora (1989) and Peris & González Mora (1991) for Calliphoridae. Coleoptera families and species were identified following Plata (1971), Outerelo & Gamarra (1986), Zahradník (1990), Yélamos (2002) and Bajerlein *et al.* (2012). Hymenoptera families and species were identified according to Martínez *et al.* (1985) and Gómez & Espadaler (2006).

### Analysis of data

The structure of the entomosarcosaprophagous community was analyzed on depending on the different decomposition stages and on the different seasons. Differences in faunal composition, considering all the species that visited the baits though the five decomposition stages and the four seasons, were tested by PERMANOVA, using the function "adonis" by Bray Curtis dissimilarity and a random subset of 999 permutations, with the

available function in the "vegan" package (Oksanen *et al.* 2019). Second generation of Diptera emerged on the corpse were avoid in the analysis. A pairwise multilevel comparison was then carried out between the different decomposition stages and between the different seasons, with the function "pairwise.adonis" using Bray Curtis dissimilarity. In addition, a principal component analysis (PCA) was carried out using the function "prcomp" ("stats" package), to determine how samples from the different decomposition stages and from the different seasons were grouped as a function of the abundance of the different species (R-Development-Core-Team 2017). Species abundance was previously standardised by the log (x+1) to correct deviations from normality. Finally, a Similarity Percentages analysis (SIMPER) run for "permutations=999" was used to find the discriminating species between pairs of decomposition stages and seasons, using the function "simper" of the "vegan" package (R-Development-Core-Team 2017).

## Results

### Environmental conditions throughout the four seasons studied

Temperature in the trap oscillated between an absolute minimum of 6.47 °C and an absolute maximum of 35.54 °C in autumn, 0.45 and 34.16 °C in winter, 5.24 and 39.62 °C in spring, and 16.90 and 45.08 °C in summer, what illustrates the high temperatures and the great climatic variability of the area. The average daily temperatures in the trap for all the study period ranged between 4.67 and 31.63 °C in winter and summer, respectively (Fig. 1). In the bait-soil interface the temperatures had less oscillations and ranged between 7.00 and 36.30 °C also in winter and summer. The relative humidity in the trap, measured as daily average, varied between 30.29% in spring and 100% in autumn and winter (Fig. 1), while in the bait-soil interface its minimum reached 26.00% in summer and the maximum was measured in autumn and winter with a value of 100%.

### Decomposition stages duration throughout the different seasons

The different stages had quite different duration depending on the season (Fig. 2). In general, and as expected, in autumn and summer they occurred

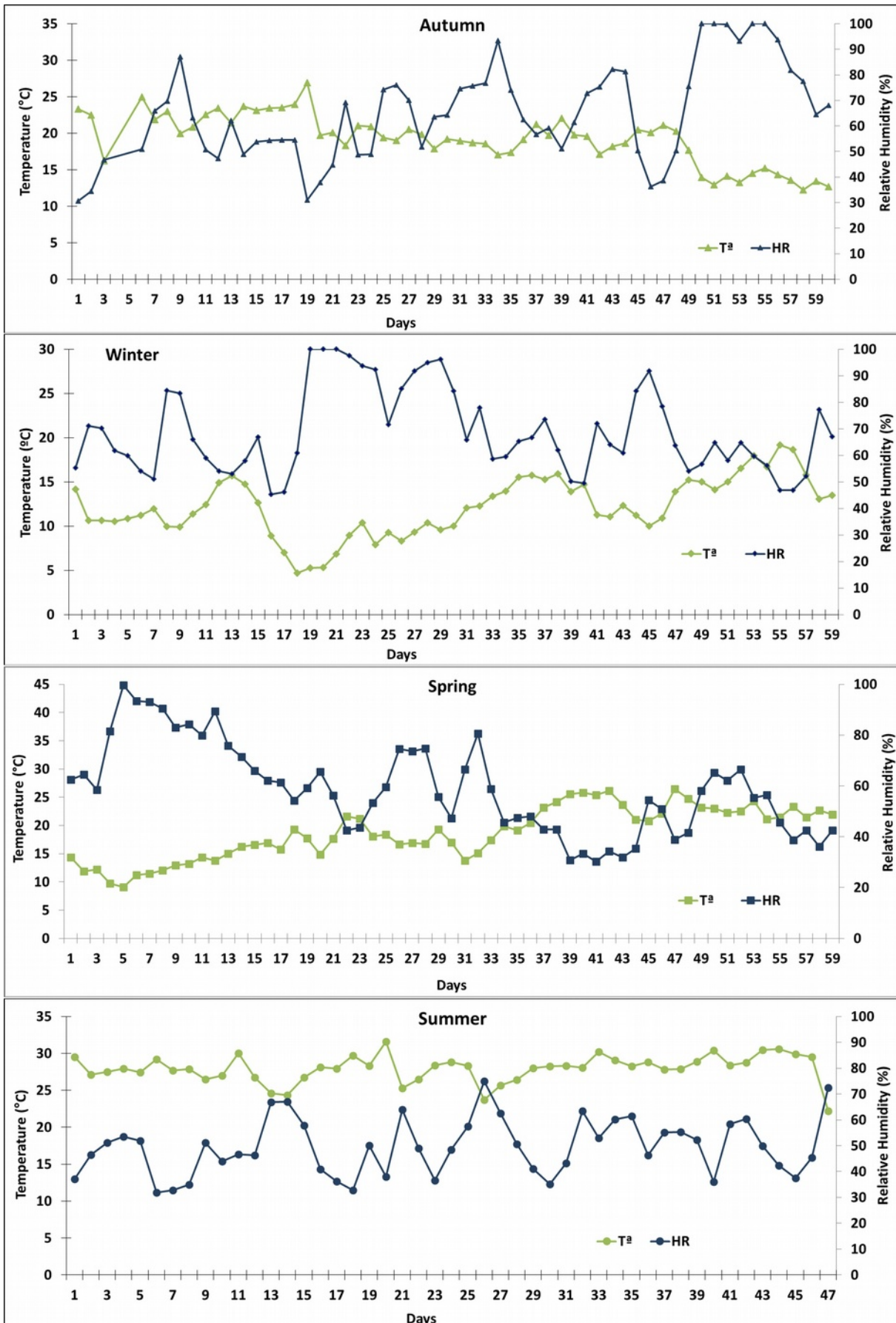


Figura 1. Variables ambientales, temperatura media ( $T^a$ ) y humedad relativa media (HR) durante las cuatro estaciones de muestreo.

Figure 1. Environmental variables, mean temperature ( $T^a$ ) and mean relative humidity (HR) during the four sampling seasons.



more rapidly than in the colder seasons, reaching the dry remains stage very early.

The fresh stage started at the time of death and corresponded to the first 2 or 3 days, with the exception of spring when it lasted 8 days. The bloated stage lasted 3 days in autumn, spring and summer and 6 days in winter, being not very apparent in winter and spring. The decay phase was very evident, lasting only 2 days in autumn and summer, 3 days in spring and 24 days in winter. The late decomposition stage lasted 2 days in autumn and summer, 9 in spring and 18 in winter. Finally, during the dry remains stage, only skin and bones remained. In autumn and summer, this occurred very early (from day 11 and 10, respectively) onwards, while in spring and winter it began on days 24 and 52, respectively (Fig.2).

**Composition and dynamic of the entomosarcosaprophagous community**

A total of 52,567 individual arthropods, belonging to the orders of greatest forensic relevance, were collected and identified (42,789 in the adult stage and 9,778 in the pre-imaginal stage). Within them, 28 families and 52 species were recognized. The order with the highest number of specimens collected was Diptera, with 90.59% of the total adults and 79.64% of the preimaginals, followed by Coleoptera (3.28% of the adults and 20.36% of the preimaginals) and, finally, by Hymenoptera that constituted a 6.13% of the total adults (Table S2, [Appendix](#)). The dynamic of the most representative families of the three orders is described in the end of the [Appendix](#).

Adults of the order Diptera were dominant in abundance in all seasons studied, while their larvae were the most abundant preimaginals in spring, winter and autumn. Among families of this order, Calliphoridae accounted for 65.19% of the total sarcosaprophagous adults recollected. Next, but with a much lower abundance, was Muscidae, representing 16.07%, and the Sphaeroceridae fa-

mily with 5.88%, meanwhile other minority Diptera families represented less than 2.38% of the total (Table S2, [Appendix](#)).

Adults of the Coleoptera constituted the second order in abundance in winter and spring, and their larvae were the most abundant preimaginal in summer. Within this order, the most abundant family was Dermestidae (1.37% of the total adults registered in the study), followed by family Histeridae (1.11%). The remaining families showed less than 0.5% of the total abundance (Table S2, [Appendix](#)).

Finally, the order Hymenoptera was recorded more abundantly in autumn and with very low abundances in winter (just 4 individuals). The most abundant taxon within this order was the superfamily Chalcidoidea (3.89%), followed by the family Formicidae (1.88%), and with much lower abundances the families Vespidae and Apidae (less than the 0.27% of the whole adult sarcosaprophagous community) (Table S2, [Appendix](#)).

**Correspondence of the stages of decomposition and seasons with the entomosarcosaprophagous community studied**

On one hand, the PERMANOVA showed a significant effect of decomposition stage on the abundance of the different species (F=12.4, df=4, 125, P<0.001). Moreover, the pairwise test showed significant differences between Decomposition and Late Decomposition; Late Decomposition and Dry remains (P<0.05); but not between Fresh and Bloated and Bloated and Decomposition stages (P=0.36 and P=0.49, respectively). On the other hand, when the seasons were considered, the PERMANOVA also showed a significant effect on the abundance of the different species (F=9.71, df=3, 126, P<0.001). Moreover, the pairwise test showed significant differences between all the seasons (P<0.05) except between Winter-Spring and Autumn-Summer (P=1.0).

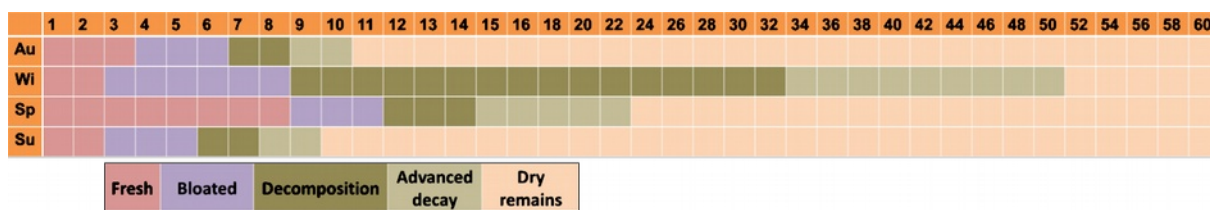


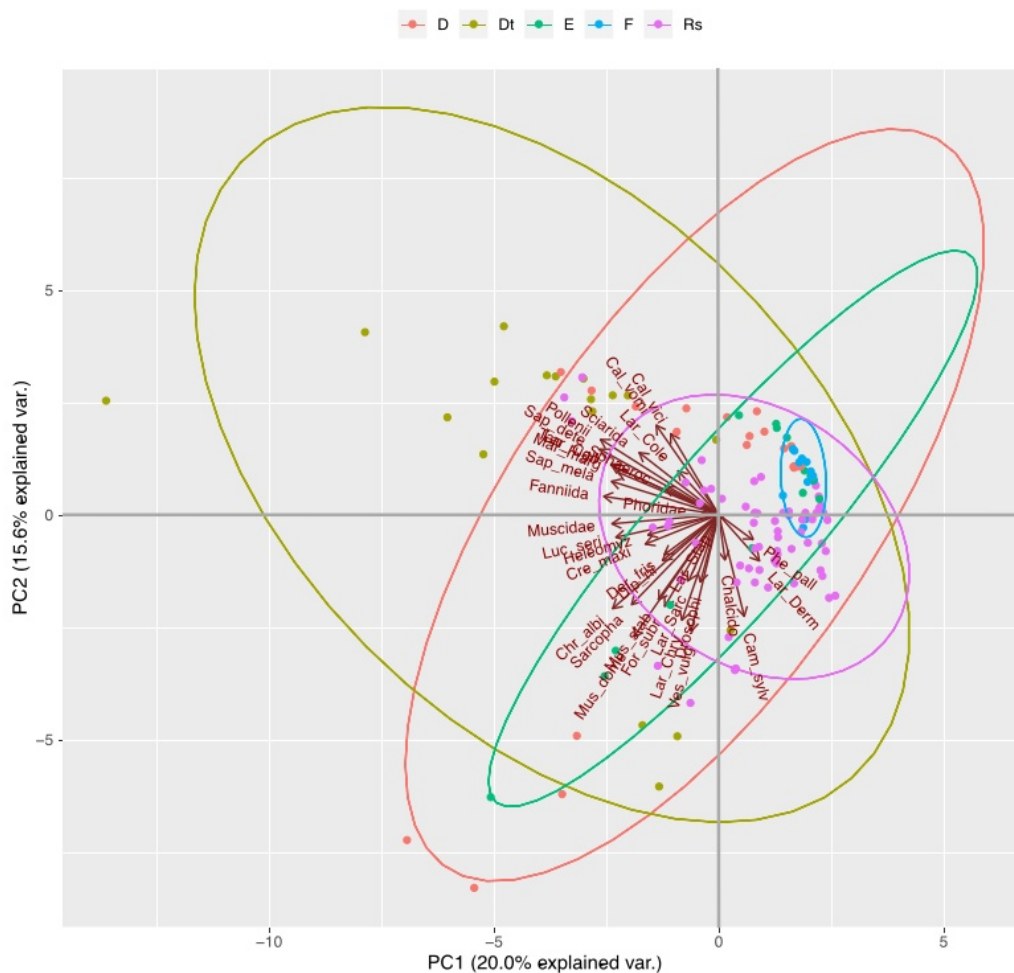
Figura 2. Duración de los diferentes estadios de la descomposición para cada estación muestreada. Au: otoño, Wi: invierno, Sp: primavera, Su: verano.

Figure 2. Duration of the different decomposition stages for each season. Au: autumn, Wi: winter, Sp: spring, Su: summer.

Considering the decomposition stages, although differences between the first stages were not significant, the SIMPER analysis showed which species contributed significantly between them (Table S3, [Appendix](#)). For instance, *Chrysomya albiceps* (Wiedemann, 1819), *Calliphora vicina* Robineau-Desvoid, 1830, *Lucilia sericata* (Meigen, 1826) and Phoridae family characterized the bloated stage (Table S2, [Appendix](#)). Moreover, *C. albiceps*, *C. vicina*, *Calliphora vomitoria* (Linnaeus, 1758) and *Musca domestica* Linnaeus, 1758 characterized the decomposition stage when compared to the bloated stage. Furthermore, *Calliphora* larvae, *C. albiceps*, Polleniinae, *Saprinus detersus* (Illiger, 1807), Muscidae, *Thanatophilus ruficornis* (Küster, 1851) and Fanniidae were the groups which contributed significantly in the differences between decomposi-

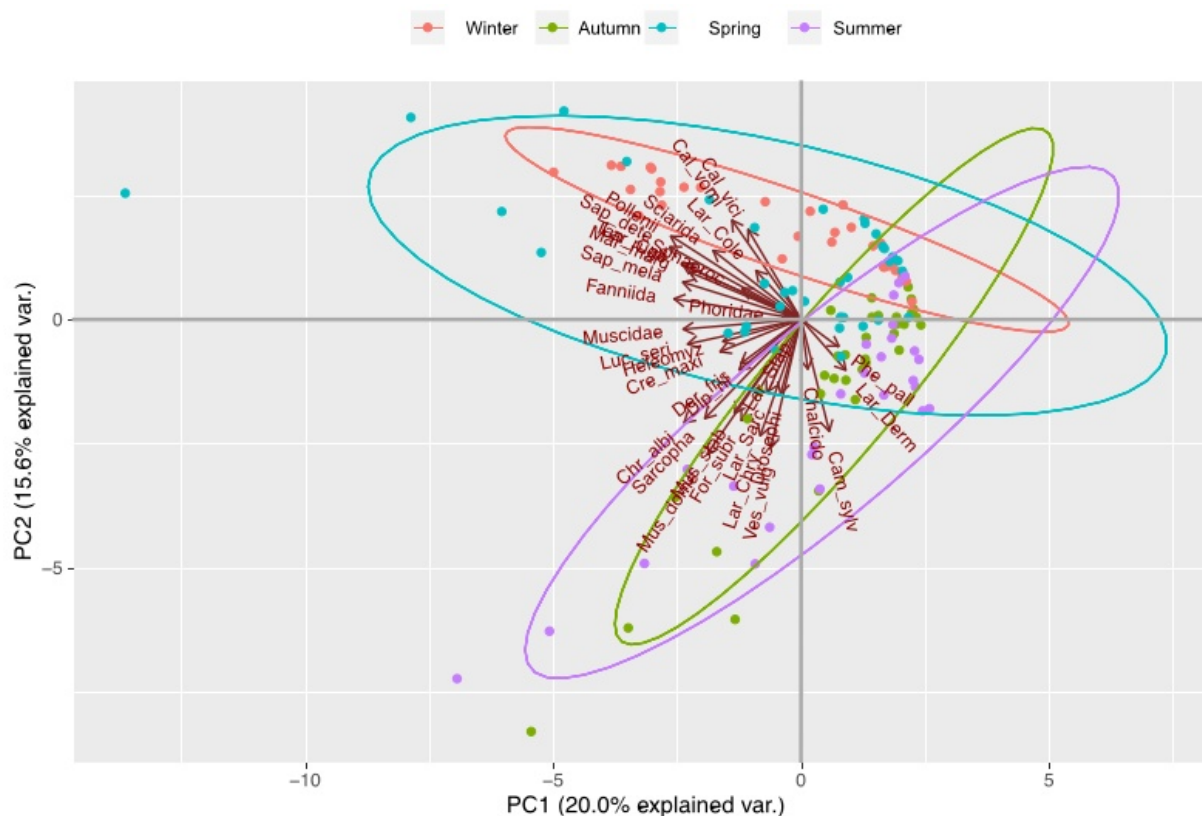
tion and late decomposition stages, being all of them more abundant in the late decomposition. Finally, *Calliphora* and *Chrysomya* larvae, Polleniinae, *S. detersus*, *T. ruficornis* and Fanniidae contributed significantly to the differences between late decomposition and dry remains, characterizing the late decomposition.

In agreement with these results, PCA showed how species are distributed according to the stages of decomposition and seasons (Figs. 3, 4). The PC1 axis explained 20.0% of the variance and was mainly correlated to Pheidole and Dermestidae larvae on the positive side and with Phoridae, Muscidae, Fanniidae or *L. sericata* on the negative side. The PC2 axis explained 15.6% and was positively correlated mainly with *C. vicina* and *C. vomitoria* and was negatively correlated to Chalcidoidea and *Camponotus sylvaticus* (Olivier, 1792)



**Figura 3.** Representación de los dos primeros ejes del PCA realizado sobre la abundancia total de cada especie muestreada en cada cadáver identificando la etapa de descomposición a la que corresponden. (F= fresco, E= enfematoso, D= descomposición, Dt= descomposición tardía, Rs= restos secos).

**Figure 3.** Biplot representations of the first two axes of the PCA performed on the total abundance of each specie sampled on each corpse identifying the decomposition stage to which they correspond. (F= fresh, E= bloated, D= decomposition, Dt= late decomposition, Rs= dry remains).



**Figura 4.** Representación de los dos primeros ejes del PCA realizado sobre la abundancia total de cada especie muestreada en cada cadáver identificando la estación a la que corresponden.

**Figure 4.** Biplot representations of the first two axes of the PCA performed on the total abundance of each species sampled on each corpse identifying the season to which they correspond.

(Figs. 3, 4). In the first case, a low segregation between the samples of the first decomposition stages was observed, meanwhile there was a higher segregation of the decomposition in relation to the late decomposition stage and late decomposition and dry remains stages (Fig. 3).

On the other hand, when considering the seasons, there is a low segregation between the samples of winter and spring and autumn and summer, meanwhile there was a higher segregation between the colder and the warmer seasons (Fig. 4). However, although the differences between the cold seasons were not significant, the SIMPER (Table S4, [Appendix](#)) showed how there were some species that marked differences between them, but mainly because spring was related with higher abundances than winter. The same occur between autumn and summer, not having significant differences between them but being summer related with higher abundances than autumn. However, the differences between both kind of seasons, warm and cold, could be explained because the presence of some species. For instance, the colder seasons were related with *C.*

*vicina*, *C. vomitoria* or Histeridae and the warmest seasons were related with *C. albiceps*, *M. domestica*, Chalcidoidea and the presence of Formicidae as also confirms the PCA analysis.

#### Overall characterization of the community and indicator species of the decomposition stages

In a global analysis of the community, the data point to *C. vicina* as being an indicator of the earlier stages in the coldest seasons, winter and spring. Despite of it, this species was not collected during the fresh stage in winter, when the family Phoridae was the most abundant group (Table 1). *C. albiceps* and *M. domestica* characterised the bloated stage in the warmer seasons (autumn and summer). In turn, *C. albiceps* and Muscidae characterised the phase of decomposition for the colder periods (winter and spring) acting as secondary flies (Table 1). The late decomposition stage was fundamentally different mainly because of the migration of Diptera larvae. It should be noted that the larvae of *C. albiceps* pupate under the corpse, while those of *Calliphora* spp. move

| Season | Decomposition stages                                    |  |                                       |  |   |
|--------|---|--|---------------------------------------|--|---|
|        | Fresh   | Bloated  | Decomposition                         | Late decomposition                         | Dry remains   |
| Autumn |   | <i>Chrysomya albiceps</i><br><i>Musca domestica</i>                  | <i>Creophilus maxillosus</i>          | Diptera larvae                             | <i>Dermestes frischii</i><br>Chalcidoidea<br>Dermestidae larvae |
| Winter | Phoridae  | <i>Calliphora vicina</i>   | <i>Chrysomya albiceps</i><br>Muscidae | Diptera larvae<br><i>Saprinus detersus</i> | <i>Dermestes frischii</i><br>Coleoptera larvae                  |
| Spring | <i>Calliphora vicina</i><br><i>Calliphora vomitoria</i> |  | <i>Chrysomya albiceps</i><br>Muscidae | Diptera larvae<br><i>Saprinus detersus</i> | <i>Dermestes frischii</i><br>Dermestidae larvae                 |
| Summer |   | <i>Chrysomya albiceps</i><br><i>Musca domestica</i><br>Sarcophagidae |                                       | Diptera larvae                             | <i>Dermestes frischii</i><br>Chalcidoidea<br>Dermestidae larvae |

**Table 1.** Principales taxones indicativos de las diferentes estados de descomposición.

**Table 1.** Main taxa indicative of the different stages of decomposition

away from it, being reflected in the different number of larvae registered in the trap. This phase was also characterized by the presence of *S. detersus* (Table 1). Finally, the dry remains stage was mainly defined by the presence of *Dermestes frischii* Kugelann, 1792 and coleopteran larvae in all four seasons, and the superfamily Chalcidoidea in autumn and summer (Table 1).

## Discussion

Temperature and humidity are one of the main environmental variables determining the duration of the decomposition stages, since they both have an essential influence on the decomposition processes (Ioan *et al.* 2017) and the presence and biology of arthropod species (Campobasso *et al.* 2001). These environmental variables are conditioned by the climate, which in our study area is semiarid, with high temperatures and an important daily variability, and with scarce rainfall. Throughout the decomposition process of the cadaver studied in the different seasons, the fresh stage was very similar in all seasons except spring, which is attributable to the very low temperatures during the first days of this season. The bloated stage was the most difficult to define since during winter and spring the carcass was not clearly swollen, possibly also due to the low temperatures, being the gases produced by the metabolic processes gradually expelled. The other stages were different regarding the moment they started and how long they lasted. In this respect, autumn and summer were the most similar seasons, with similar temperatures, and winter was when decomposition and late decomposition stages started later and lasted longer; in fact, in this season the dry remains stage really started at the end of the sampling period. The general trend in which low

temperatures were related with longer decomposition stages coincides with other studies performed in the same geographical region at a periurban location (Arnaldos-Sanabria 2000) or at a higher altitude (Begoña-Gaminde 2015).

Likewise, the colonization pattern and the insect abundance depend on the time of year, geographic location, environmental characteristics and the habitat in which a corpse is located (Anton *et al.* 2011, Brundage *et al.* 2011, Moretti *et al.* 2011, Bembow *et al.* 2013). And, for forensic purposes, the knowledge of the composition and the dynamic of the entomosarcosaprophagous community found in a corpse are one of the most important data since insects play an active role in the decomposition process (Richards & Goff 1997, Byrd & Castner 2010, Amendt *et al.* 2011, Tomberlin *et al.* 2011). Although the community found in the corpse was very extensive, we are only going to focus on those species or families that have an outstanding value.

Regarding our work and as other works suggested, Calliphoridae was the most abundant family (e.g. Anderson & VanLaerhoven 1996, Arnaldos *et al.* 2001). Within the family, *Chrysomya albiceps* was the most abundant and its dynamics coincided with those mentioned by other authors for the Iberian Peninsula (Arnaldos-Sanabria 2000, Martínez-Sánchez *et al.* 2000, Baz *et al.* 2007, Prado e Castro *et al.* 2012, Martín-Vega & Baz 2013), being the most abundant species during the warmest sampling months, confirming a high temperature preference of this species. On the contrary, *Calliphora* species were related to the coldest seasons according to their thermophobic character (Martínez-Sánchez *et al.* 2000, Arnaldos *et al.* 2001, Greco *et al.* 2014, Díaz-Aranda *et al.* 2018). On the one hand, *C. vicina* was the first species in arriving to the



cadaver and was predominant in winter and spring. On the other hand, *C. vomitoria* had a seasonal distribution similar to *C. vicina* but always appears delayed than the other species, which coincide with other studies in the Iberian Peninsula (Arnaldos *et al.* 2001, Prado e Castro *et al.* 2012, Martín-Vega & Baz 2013, Begoña-Gaminde 2015, Díaz-Aranda *et al.* 2018). Furthermore, its great abundance registered in our study may be explained because *C. vomitoria* is known to prefer rural environments at the same time as the mentioned low temperatures (Smith 1986). The preimaginal stages of the dipterans are considered to form part of the necrophagous group as they feed directly from the carcass, being one of the main causes of the loss of tissue (Prado e Castro *et al.* 2012) and they are a very important element in the decomposition process. However, it should be considered that second and third stage of *C. albiceps* larvae may, depending on the nutrient substrate, be predators of other larvae, so that they can be at the same time considered a necrophagous and necrophilic component of the sarcosaprophagous community (Smith 1986, Battán Horenstein 2008).

Within Muscidae family, *M. domestica*, which was the most representative species, was present in all seasons except winter and, regarding the decomposition stages, this species acted as secondary fly. These findings agree with the results of previous works (Arnaldos-Sanabria 2000, Arnaldos *et al.* 2001, Battán-Horenstein *et al.* 2010, Prado e Castro *et al.* 2012).

Moreover, it should be emphasized that the family Phoridae was the second in abundance during the winter. It appeared from the fresh stage which coincided with the results of Anton *et al.* (2011) who pointed to the Phoridae as an indicator of this season. From the forensically point of view, this is especially interesting since winter is the season that presents, in general, the lowest abundances and diversity of insects.

Finally, as regards Sarcophagidae presence in the different decomposition stages, our results are consistent with what is known about the family. They do not appear in the fresh stage (Smith 1986), probably as a result of their larviparous vital strategy, which enable them to lay live larvae in cadavers with a certain degree of success when the decomposition process is already underway and other flies are present (Prado e Castro *et al.* 2012).

In what refers to the Coleoptera order, the most abundant family during the whole sampling period was Dermestidae, which agrees with Sánchez-Piñero (1997) and Arnaldos-Sanabria (2000). This family is usually associated with the last decomposition stages (Early & Goff 1986) since their larvae feed on the dried tissue and skin of many animals (Bonacci *et al.* 2017). In our work, it was present in autumn and summer mainly from the decomposition stage onward, and in winter and spring from late decomposition onwards, and in all cases, it was more abundant in the late decomposition and dry remains stages. However, many studies mention adults of this group in the earliest decomposition stages (Arnaldos-Sanabria 2000, Castillo-Miralbes 2001, Arnaldos *et al.* 2004, López Dos Santos 2008, Pérez-Bote *et al.* 2012, Prado e Castro *et al.* 2013, Begoña-Gaminde 2015) throwing doubt on the generalization implied in the initial premise. In this regard, it should be borne in mind that the adults, especially those of genus *Dermestes* are occasional predators and cannibalistic and will eat young larvae and puparia (Byrd & Castner 2010), which would explain their presence in the mentioned earlier decomposition stages. Furthermore, the larvae of Dermestidae are mainly necrophagous, since they feed directly from the corpse, and are associated with the latter stages of decomposition (Arnaldos-Sanabria 2000, Castillo-Miralbes 2001, Martín-Vega & Baz 2013, Begoña-Gaminde 2015). Our results coincide and confirm this previous affirmation. The seasonality of the larvae coincided also with other works done in the same region (Arnaldos-Sanabria 2000, Begoña-Gaminde 2015).

The distribution of Histeridae in all seasons, mainly in the colder ones, coincided with the results obtained by Prado e Castro *et al.* (2013), however, it does not coincide with those obtained in other studies on the southern of Spain (Sánchez-Piñero 1997, Arnaldos-Sanabria 2000, Begoña-Gaminde 2015) where Histeridae were collected more frequently during the summer, with lower or zero abundances in autumn and winter. On the other hand, this family was collected mainly between the decomposition and dry remains stages, coinciding with the Diptera larvae (Arnaldos-Sanabria 2000, Arnaldos *et al.* 2004, Aballay *et al.* 2012, Begoña-Gaminde 2015, Zanetti *et al.* 2015). However, in other areas, they were captured from the earliest stages of the

decomposition (Castillo-Miralbes 2002, Prado e Castro *et al.* 2013), which could indicate that this group is not consistent in their seasonal nor decomposition stage appearance. *Saprinus detersus* was the most abundant species as well as occurred in Begoña-Gaminde (2015) and López-Gallego (2016) in different altitudes of the same area, however, in Central Spain, it was the second species in abundance (Martín-Vega *et al.* 2015), in Lisbon, although present, this species was not very abundant (Prado e Castro *et al.* 2013) and in a periurban area in Murcia it was not found (Arnaldos-Sanabria 2000). All the above could point to the possible interest of *S. detersus* as a wild habitat indicator species; so further studies on this species could be very interesting to accurately define this possible indicator role.

Finally, in the case of the Hymenoptera, the superfamily Chalcidoidea, has been considered as a necrophilic component of the community due to its parasitoid nature, being able to attack the larval and pupal stages of Diptera (Smith 1986, Frederickx *et al.* 2013). According to Meskin (1986), *Chrysomya albiceps* is one of the most parasitized species by this group, because *Chrysomya* pupates under the corpse, making it more vulnerable, which could explain the dynamics exhibited by the Chalcidoidea in our study. Nonetheless, it is advisable to consider this group at the species level to properly understand such results.

On the other hand, the importance of the family Formicidae within the entomosarcosaprophagous fauna appears reflected in several works (Early & Goff 1986, Martínez *et al.* 2002, Pérez-Marcos *et al.* 2020). They are considered omnivores and, when they are abundant, they can affect the population of necrophages, reaching to alter the natural process of decomposition. *Camponotus sylvaticus*, which was the most abundant Formicidae species in our work, was also observed in a periurban environment in Murcia (Martínez *et al.* 2002) and at the highest altitude of Sierra Espuña (Begoña-Gaminde 2015), but with very low abundance, whereas it was not collected in other places of the Iberian Peninsula (Castillo-Miralbes 2002, Prado e Castro *et al.* 2014, Neto-Silva *et al.* 2017). In addition, in our case, *Iberoormica subrufa* Roger, 1859 was of great interest since it has been not found in other areas, related or not to the cadaveric ecosystem (Castillo-Miralbes 2002, Martínez *et al.* 2002, Cantarineu-Guillen & Tinaut 2012, Prado e Castro

*et al.* 2014, Begoña-Gaminde 2015, Neto-Silva *et al.* 2017, Pérez-Marcos *et al.* 2020). This fact gives it a potential value as a geographical and environmental indicator. The other Formicidae species were collected in very low numbers, which, in certain cases, strongly contrasts with other data from the same area. For instance, considering the work conducted in a periurban site in the same region (Martínez *et al.* 2002, Arnaldos *et al.* 2004), the most abundant species was *Pheidole pallidula* (Nylander, 1849) which was present in all seasons and in all decomposition stages while, in our case, it was present in very low numbers. Moreover, at the highest altitude of Sierra Espuña (Begoña-Gaminde 2015), this species was not collected and the most abundant was *Crematogaster auberti* Emery, 1869, which was not collected in our case. These data, as well as those referring to other areas of the Iberian Peninsula, underline the great variability existing in the composition of the Formicidae community related to carcasses, confirming the forensic importance of its species as potential geographical and seasonal indicator (Pérez-Marcos *et al.* 2020).

All these differences both between the decomposition stages and between the seasons as it was previously shown, were confirmed on the analysis results. For instance, the PCA and the SIMPER analysis showed which taxa differentiated them. In the first case, although no differences between first stages of decomposition were found, SIMPER analysis showed some species which contributed significantly. For instance, *C. albiceps*, *C. vicina*, *L. sericata* and Phoridae family characterized the bloated stage; *C. albiceps*, *C. vicina*, *C. vomitoria* and *M. domestica* characterized the decomposition stage when compared to the bloated stage; *Calliphora* larvae, *C. albiceps*, Polleniinae, *S. detersus*, Muscidae, *T. ruficornis* and Fanniidae were the groups which contributed significantly in the differences between decomposition and late decomposition stages, being all of them more abundant in the late decomposition; and *Calliphora* and *Chrysomya* larvae, Polleniinae, *S. detersus*, *T. ruficornis* and Fanniidae contributed significantly to the differences between late decomposition and dry remains, characterizing the late decomposition. However, there were some cases where these differences may not be sufficient to make them significant, because each pair of stages had similar fauna or, in many cases, was composed of species that are not associated

with any specific phase (Matuszewski 2017). Moreover, because similar species arrive first to the corpse in low abundances, there were found some similarities between the different seasons in the fresh stage. Therefore, as Martín-Vega & Baz (2013) stated, more studies to confirm the observed patterns are advisable.

When comparing the whole community considering the four seasons, cold and warm seasons were grouped separately. Even so, although the differences between the cold seasons are not significant, the SIMPER shows how there are some species that mark differences between them, but mainly because spring was related with higher abundances than winter. And the same occur between autumn and summer, not having significant differences between them but being summer related with higher abundances than autumn. The differences between warm and cold seasons could be explained due to the presence of some species. For instance, the colder season were related with *C. vicina*, *C. vomitoria* or Histeridae family because of their thermophobic character as other authors suggested (Martínez-Sánchez *et al.* 2000, Arnaldos *et al.* 2001, Greco *et al.* 2014, Díaz-Aranda *et al.* 2018) and the warmest seasons were related with *C. albiceps*, *M. domestica*, Chalcidoidea and the presence of Formicidae because of their high temperature preference (Meskin 1986, Smith 1986, Arnaldos-Sanabria 2000, Martínez-Sánchez *et al.* 2000, Baz *et al.* 2007, Prado e Castro *et al.* 2012, Frederickx *et al.* 2013, Martín-Vega & Baz 2013).

## Conclusions

Our study confirms the numerical dominance of different groups or species in the decomposing remains, their diversity and their importance in forensic studies. Knowledge of the specific association of some species with certain habitats is of capital importance in applied forensic entomology because it could indicate the postmortem transportation of a corpse (Amendt *et al.* 2011). In our case, it has been observed that in a natural area in a semi-arid environment with low rainfall and high temperatures, *C. albiceps*, *C. vicina* and *C. vomitoria* are valuable as seasonal indicators. *Calliohora vicina* and *C. vomitoria* appeared predominantly in winter and spring, while *C. albiceps* appeared in autumn and summer. These results point to the value of these taxa as seasonal

indicators. Moreover, the community species define a clear pattern of colonization that varies according to the season of the year. In the warmer seasons, the species *M. domestica* and *C. albiceps* mainly characterized the bloated stage. In the colder seasons, spring and winter, the dominant species in the early stages were *C. vicina* and *C. vomitoria*. The advanced stages, in turn, were characterized by *D. frischii* and coleopteran larvae in the four seasons, *C. sylvaticus* in all seasons except winter, and Chalcidoidea in autumn and summer. Finally, the species *Iberoformica subrufa* may be of potential use as habitat indicator of the studied area.

To conclude, the fact that in some cases differences in the species certainly exist, emphasises the need to study the sarcosaprophagous community in as many different conditions as possible to avoid applying unadjusted data in the evaluation of carrion decomposition and the sarcosaprophagous community related to a specific place (Sanchez Piñero 1997, Martín-Vega & Baz 2013, Prado e Castro *et al.* 2014). Furthermore, taxonomical studies, like this one, are necessary to know accurately the specific composition of the populations of several groups not always considered when studying the sarcosaprophagous community. This knowledge would allow explaining the dynamics of certain taxa and the role they play within such community, increasing their forensic importance as geographical, environmental or seasonal indicators.

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