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ORIGINAL ARTICLE

Formicidae (Hymenoptera) community in corpses at different altitudes in a semiarid wild environment in the southeast of the Iberian Peninsula

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Abstract

The Formicidae are considered crucial components of the entomosarcosaprophagous fauna because they can delay the decomposition process, cause tissue postmortem damage and produce bloodstain patterns that may confuse investigations. Moreover, some studies suggest that the Formicidae can act as environmental and seasonal indicators. However, studies on this group on vertebrate carcasses are scarce, especially in the Iberian Peninsula and the southwest of Europe. Thus, comparative studies at different altitudes in a protected wild mountain area could provide useful information on its composition in such environmental conditions, their role as environmental indicators and their forensic implications. For this reason, the Formicidae sarcosaprophagous community was studied at three different altitudes, between 400 and 1,500 m, in a wild mountainous area in the southeast of Spain using a modified Schoenly trap, with two pitfall traps inside, baited with 5 kg piglets (*Sus scrofa* L.). This work illustrates an approach to the community of the Formicidae, as a representative of the sarcosaprophagous community in an altitudinal gradient, showing a great variability in its composition. Furthermore, when comparing our results with other studies carried out in the Iberian Peninsula, we are able to suggest certain species with a potential utility as geographic and environmental indicators. Thus, *Iberoformica subrufa*, *Lasius brunneus*, *Lasius cinereus* and *Camponotus sylvaticus* are species of special interest as they appeared in either one of the sampled areas or in the same region.

Key words: altitudinal gradient, ant, decomposition process, environmental indicator, forensic entomology, Schoenly trap.

INTRODUCTION

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). Understanding the sarcosaprophagous community is important in forensic practice as it can provide useful information regarding different aspects of death. The knowledge of the specific association of some species with certain habitats is of capital importance in applied forensic entomology because it could provide evidence of the postmortem transportation of a corpse (Amendt *et al.* 2011). The sarcosaprophagous community includes a great diversity of species (e.g. Arnaldos *et al.* 2004), some of them with greater forensic significance than others. The most significant elements for forensic purposes are the necrophagous, which feed on the corpse, and the necrophilous, which predate or parasitize the necrophagous. The omnivorous elements take advantage of both the carcass and

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the fauna present in it (Prado e Castro *et al.* 2014). The Formicidae (Hymenoptera) are considered to belong to this last group (Arnaldos *et al.* 2005; Battán Horenstein *et al.* 2012; Prado e Castro *et al.* 2014), being one of the most abundant within the sarcosaprophagous community (Arnaldos *et al.* 2004, 2005) and the main scavengers of some type of carcasses (Angulo *et al.* 2011). They are among the most diverse groups, including phytophagous, predatory, nectarivorous and parasitic forms (McGavin 2006), and undertake a variety of ecological functions, as well as playing an important role in the redistribution of nutrients, as evident in rainforests (Griffiths *et al.* 2018). Furthermore, they are considered to be crucial components of most terrestrial ecosystems (Arnan *et al.* 2014) and, in fact, are important within the entomosarcosaprophagous fauna (Early & Goff 1986; Hewadikaram & Goff 1991; Tantawi *et al.* 1996; Martínez *et al.* 2002; Chen *et al.* 2014; Maciel *et al.* 2016) because they can delay the decomposition process by diminishing the amount of maggots and other necrophagous insect populations (Campobasso *et al.* 2009; Byrd & Castner 2010; Lindgren *et al.* 2011; Ramón & Donoso 2015; Maciel *et al.* 2016). They are also important in forensic practice because they can cause tissue postmortem damage resembling premortem burns and produce bloodstain patterns that can confuse the investigations (Jayaprakash 2006; Campobasso *et al.* 2009; Ramón & Donoso 2015). In addition, some studies suggest that the Formicidae can act as environmental and seasonal indicators (Castro Delgado *et al.* 2008; López Gallego *et al.* 2011; Rodríguez & Lattke 2012; Prado e Castro *et al.* 2014; El Bokl *et al.* 2015; Neto-Silva *et al.* 2018).

It is well known that the sarcosaprophagous community is affected by different factors, such as the geographical region, that will define the habitat, vegetation or meteorological conditions (Anderson 2010), highlighting the need to study this community in different microclimatic environments, even if the different sites are close to each other. Many families of the sarcosaprophagous insects are ubiquitous, but the species are not, as their distribution and abundance depend on the environmental conditions where the corpse is located. Therefore, the faunal succession in a cadaver will follow a certain order depending on these conditions (Anderson 2010; Anton *et al.* 2011; Brundage *et al.* 2011; Benbow *et al.* 2013). Understanding the processes responsible for variations in species richness and community composition along gradients is a central challenge in ecology (Arnan *et al.* 2014). Studies along altitudinal gradients allow us to compare the diversity of a local fauna between different microclimatic environments (Longino & Colwell 2011) that are close to each other. Many of these studies have documented an inverse relationship between altitude and diversity (Brown Jr 1973; Fisher 1996; Brühl *et al.* 1999; Sanders *et al.* 2007); however, the diversity of ants does not necessarily decrease as a function of the altitudinal gradient and several studies have indicated a peak in species richness between 500 and 900 m (Olson 1994; Fisher 1998; Ward 2000; Longino & Colwell 2011). The organization of ant communities is affected by several factors; for example, within the physical ones, temperature, humidity, radiation and wind are important (Cros *et al.* 1997). Within the Mediterranean biogeographical region and the Iberian Peninsula, there are studies on the whole entomosarcosaprophagous fauna, but most of these studies concern data from only certain arthropod groups (Table S1). Nevertheless, the studies on the Formicidae community on vertebrate carcasses are scarce; they were mainly carried out in a periurban environment (Retana *et al.* 1992; Martínez *et al.* 1997, 2002; Prado e Castro *et al.* 2014; Neto-Silva *et al.* 2018) although in some cases they report from wild environments (López Gallego *et al.* 2011; Martínez Moñino 2011). Such communities have also been studied regarding other non-forensic ecological aspects (Cros *et al.* 1997; Cerdá *et al.* 1998; Retana & Cerdá 2000; Carpintero *et al.* 2007; Angulo *et al.* 2011; Arnan *et al.* 2012, 2014; Blight *et al.* 2014).

To determine the utility of Formicidae as forensic indicators, a study has been carried out at different altitudes of a wild mountain area of the Murcia region in order to provide information on its composition in such environmental conditions. The results of this study will contribute to enlarge our knowledge of Formicidae in southeastern Spain, and will also allow a comparison of the features of this community with those of other locations of the Iberian Peninsula.

MATERIALS AND METHODS

The study was carried out in the Sierra Espuña Regional Park, a protected area (Site of Community Importance ES0000173; 1°34′25″W, 37°52′35″N) in the center of the Murcia Region, at three sites with different altitudes, El Abuznel (400 m a.s.l.), Peña Apartada (900 m a.s.l.) and El Morrón (1,500 m a.s.l.), between autumn 2006 and summer 2008. El Abuznel faces south and is characterized by undergrowth dominated by a vegetation of *Rosmarinus* sp. and *Thymus* sp., and an arboreal stratum of *Pinus* sp. (Pérez Marcos 2016). Peña Apartada, oriented NE, is a shaded area with a steep slope, characterized by dense pine forest, oak groves (*Quercus* sp.) and undergrowth of rockrose

(*Cistus* sp.) (López Gallego 2016). El Morrón is a hill characterized by *Juniperus* and scrub of *Erinacea anthyllis* (Begoña Gaminde 2015).

Samples were collected with a 60 × 70 × 70 cm modified Schoenly trap (Schoenly *et al.* 1991) provided with two pitfall traps inside. The Schoenly trap has been confirmed and recommended as the most effective device to characterize the sarcosaprophagous succession (Ordóñez *et al.* 2008) and it has been successfully used to study the sarcosaprophagous fauna in different geographical areas (Arnaldos *et al.* 2001, 2004; Battán Horenstein *et al.* 2005, 2010, 2012; Prado e Castro *et al.* 2011, 2012, 2013, 2014; Mañas-Jordá *et al.* 2018; Neto-Silva *et al.* 2018). The Schoenly trap was surrounded by a fence to prevent vertebrate scavengers from approaching the bait. Arthropods were captured in receptacles containing Morrill liquid as preservative (Morrill 1975). This liquid is a killing and temporary preservative solution of 40% ethylene glycol with formalin and detergent. It is widely used because it does not attract arthropodian fauna, ensuring that the bait is the one that attracts it, and can maintain the samples up to a week without decomposing (Schoenly 1981). In our study, domestic piglets (*Sus scrofa* L.) of approximately 5 kg weight were used. Pig baits have been used by several investigators, such as Battán Horenstein *et al.* (2005, 2010, 2012), Prado e Castro *et al.* (2011, 2012, 2013), Benbow *et al.* (2013) or Mañas-Jordá *et al.* (2018). The baits were provided by the Veterinary Faculty Farm of the University of Murcia and killed following the CEE protocols and regulations (RD 1201/2005, October 10). Each trap was established 1 week before starting the experiment in order to stabilize the substrate conditions. During each season, samples were taken daily for the first 16 days, and then every 2 days. Finally, as the sarcosaprophagous community is affected by different factors, such as the geographical region (Anderson 2010), the Formicidae sarcosaprophagous communities present along the Iberian Peninsula were compared.

Data processing and analysis

Samples were properly labeled and transported to the laboratory where the preservative liquid was changed to 70% ethanol. Specimens were counted and identified at the maximum level under a stereomicroscope (Leica MZ8) following Bernard (1968), Collingwood (1978), Martínez *et al.* (1985) and Gómez and Espadaler (2007).

Differences in faunal composition between localities were tested by PERMANOVA, using the function "adonis" with Bray–Curtis dissimilarity and a random subset of 999 permutations, being the function available in the "vegan" package (Oksanen *et al.* 2019). Furthermore, the corresponding post comparisons was made between pairs of groups (pairwise test) using the function "pairwise. adonis" with Bray–Curtis dissimilarity and Bonferroni *P* adjustment method (Martinez Arbizu 2019). Kruskal's non-metric multidimensional scaling (NMDS), using the Bray–Curtis distance and K = 3, was applied to find out how localities clustered in relation to the community of Formicidae. The function "metaMDS" in the "vegan" package was used to apply NMDS on the number of Formicidae species registered on each sampling date. An analysis of similarity percentages (SIMPER) was used to show the percentage of dissimilarity between the levels of the altitude. It shows the percentage of contribution of the variables (Contrib %) that explain the dissimilarity between the levels of the altitude and the cumulative contribution (Cum. %) in order to understand how many variables explain the 90% of the dissimilarity. The analysis was carried out in R 3.6.1 (R Core Team 2019) using the "vegan" package (Oksanen *et al.* 2019).

The structure of the Formicidae community along the decomposition process was studied examining taxonomic richness (number of species), abundance and diversity; this last measured with Shannon diversity (Margalef 1974) and Pielou evenness indices (Magurran 1989). Shannon index considers that individuals are sampled randomly from an indefinitely large population, and assumes that all taxa are represented in the sample. It is calculated from the equation:

$$H' = -\Sigma p_{\mathrm{i}} \log_2 p_{\mathrm{i}}$$

where p_i is the probability that a random individual belongs to the taxon *i*. The value of the Shannon diversity index usually is between 1.5 and 3.5 and only rarely exceeds 4.5 (Magurran 1989). Values higher than 2.5 could be considered a well-structured community, whereas values less than 1 indicate either a very productive system, an environmentally stressed system or pioneer communities (Margalef 1974). Meanwhile, Pielou evenness, which represents the distribution of the relative abundances of taxa in a community, is calculated as:

$$J' = H'/H_{max} \text{ (or } H'/\log_2 S).$$

where H' is Shannon diversity, S the total number of taxa and H_{max} the diversity that could occur if all taxa were equally abundant. This relationship between the observed and maximum diversity can be taken as a measure of uniformity. The value of J' is between 0 and 1, where 1 represents a situation in which all taxa are equally abundant. As in H', this measure of uniformity considers that all taxa in the community have been counted in the sample (Magurran 1989). All analyses were undertaken with the statistical program PRIMER 6.0 (Clarke & Warwick 2001) and in R 3.6.1 (R Core Team 2019).

RESULTS

Dynamics and community structure of Formicidae

A total of 5,850 Formicidae specimens, belonging to 24 species, were collected (Table 1). As expected, the seasons with the highest abundance were summer and spring, although in autumn, especially in El Abuznel, some species were also abundantly collected (Table 1). Even so, it should be noted that the number of ants sampled in El Morrón in summer exceeded the sum of all other samplings.

Regardless of the season, Formicidae arrived earlier to the corpse in El Abuznel than at the other locations, and in all the three cases, appeared earlier in summer (days 1–4) and autumn (days 2–7). During these two seasons, the abundances were observed evenly distributed throughout the sampling period, except in El Morrón where, during summer, they reached their maximum levels towards the end of the sampling period (Fig. 1). Finally, in spring they were observed since after day 3, reaching their maximum abundance between days 15 and 36 (Fig. 1).

The most abundant species at the lowest altitude, represented by El Abuznel, were *Pheidole pallidula* (Nylander) (830 specimens), *Camponotus sylvaticus* (Olivier) (784 specimens) and *Iberoformica subrufa* (Roger) (345 specimens) and at Peña Apartada (PApartada) were *Camponotus cruentatus* (Latreille) (145 specimens) and *Lasius brunneus* (Latreille) (134 specimens). At the highest altitude of El Morrón, *Crematogaster auberti* Emery (2,762 specimens) was the most abundant species, and *Aphaenogaster iberica* Emery (313 specimens) could also be found, although with much lower abundance (Table 1).

The PERMANOVA showed a significant effect of the localities on the composition of the different

Table 1 Formicidae species along the three altitudes of Sierra Espuña, Iberian Peninsula, and their abundances during each season

Locality	El Abuznel (400 m)				Peña Apartada (900 m)				El Morrón (1,500 m)			
Species/season	Win.	Aut.	Spr.	Sum.	Win.	Aut.	Spr.	Sum.	Win.	Aut.	Spr.	Sum.
Aphaenogaster iberica Emery	0	0	0	0	1	24	9	37	0	11	22	280
Aphaenogaster gibbosa (Latreille)	0	0	0	0	0	0	0	0	1	13	2	43
Camponotus sylvaticus (Olivier)	4	311	253	216	0	0	5	39	0	0	1	0
Camponotus cruentatus (Latreille)	0	0	0	0	0	3	1	141	0	0	0	0
Camponotus piceus (Leach)	0	0	0	0	0	0	0	0	0	1	1	4
Camponotus pilicornis (Roger)	0	1	0	0	2	3	3	5	0	0	0	1
Camponotus sp. Mayr	0	0	0	0	0	0	1	9	0	0	0	0
Crematogaster auberti Emery	0	0	0	0	0	0	0	0	14	34	179	2,535
Goniomma blanci (André)	0	0	0	0	0	0	0	0	0	0	0	1
Hypoponera punctatissima (Roger)	0	0	0	1	0	0	0	0	0	0	0	1
Iberoformica subrufa (Roger)	0	121	77	147	0	0	0	0	0	0	0	0
Lasius brunneus (Latreille)	0	0	0	0	0	0	101	33	0	0	0	0
Lasius cinereus Seifert	0	0	0	0	0	0	0	0	0	0	0	1
Lasius sp. Fabricius	0	0	0	0	0	0	0	0	0	7	0	0
Messor capitatus (Latreille)	0	0	0	0	0	0	0	0	0	1	4	21
Pheidole pallidula (Nylander)	0	7	473	350	1	0	5	32	1	0	1	1
Plagiolepis grassei LeMasne	0	0	1	0	0	0	0	0	0	0	0	0
Plagiolepis pygmaea (Latreille)	0	0	0	0	1	2	29	40	0	0	0	0
Solenopsis sp. Westwood	0	0	0	0	0	0	0	1	0	1	0	0
Tapinoma madeirense Forel	0	0	0	0	0	0	0	0	0	0	0	1
Temnothorax formosus (Santschi)	0	0	0	0	0	0	0	0	0	0	1	9
Temnothorax sp. Mayr	0	0	0	0	0	0	0	0	1	0	6	12
Tetramorium caespitum cfr. (Linnaeus)	0	0	0	0	0	0	0	0	0	0	1	4
Tetramorium semilaeve André	0	31	59	3	0	0	0	0	0	6	13	33
TOTAL	4	471	862	717	5	32	154	337	17	74	231	2,946

Aut, autumn; Spr., spring; Sum., summer; Win., winter.

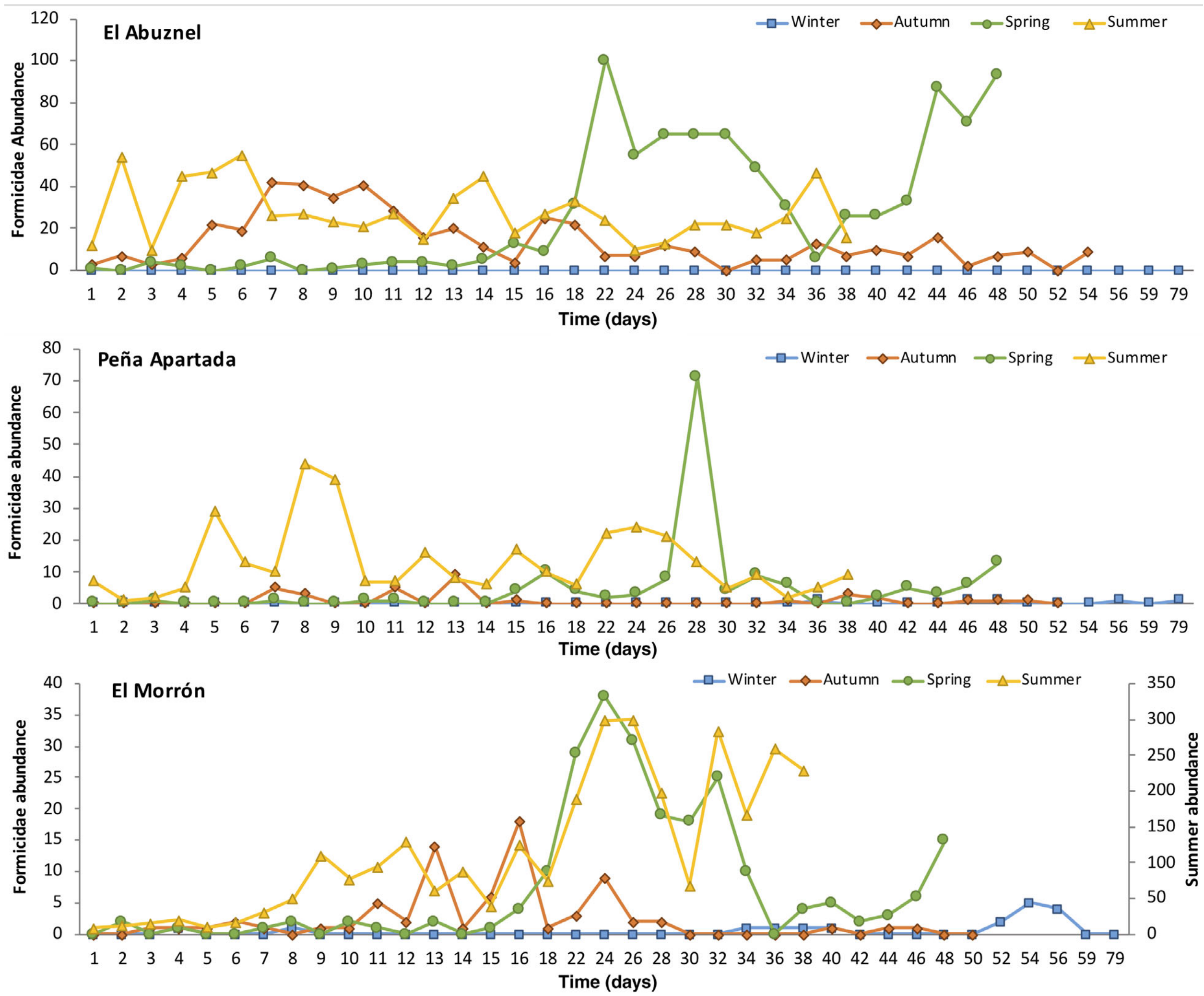

Figure 1 Abundance and dynamics of Formicidae during the four sampling seasons in three localities in the Iberian Peninsula.

Formicidae species (pseudo-F = 49.65, d.f. = 2. 222, $P < 0.01$). Moreover, the three localities were grouped separately in the NMDS analysis (stress value = 0.055) in relation to the Formicidae species present (pairwise test; Abuznel *vs*. Morron: pseudo-F = 68.37, d.f. = 1, $P < 0.01$; Abuznel *vs*. PApartada: pseudo-F = 48.59, d.f. = 1, $P < 0.01$; Morron *vs*. PApartada: pseudo-F = 32.05, d.f. = 1, $P < 0.01$; Table 1, Fig. 2). Furthermore, the SIMPER analysis (Table 2) indicates that the differences between the different localities, when comparing two at a time, are mainly due to the presence of *Cr. auberti, Ca. sylvaticus* and *P. pallidula* between El Abuznel and El Morrón, *Ca. sylvaticus*, *P. pallidula*, *I. subrufa* and *Ca. cruentatus* between El Abuznel and Peña Apartada and *Cr. auberti, A. iberica, Ca. cruentatus* and *L. brunneus* between El Morrón and Peña Apartada (Table 2). According to Figure 2, *I. subrufa, Ca. sylvaticus* and *P. pallidula* characterized El Abuznel, *Plagiolepis pygmaea* (Latreille) and *Ca. cruentatus* (Latreille) represented Peña Apartada and *Messor capitatus* (Latreille) and *Cr. auberti* El Morrón (Table 2, Fig. 2). *Tetramorium semilaeve* André is shared by El Abuznel and El Morrón and *A. iberica* by Peña Apartada and El Morrón (Table 2, Fig. 2).

Considering the community structure, the highest abundance was registered in El Morrón (3,268 individuals), followed by El Abuznel (2,050) and Peña Apartada (527). In addition, the locality with the highest richness was El Morrón, where 17 different species were found, followed by Peña Apartada (6) and El Abuznel (6). Otherwise, the locality with the highest diversity was Peña Apartada (H' = 1.817), its value

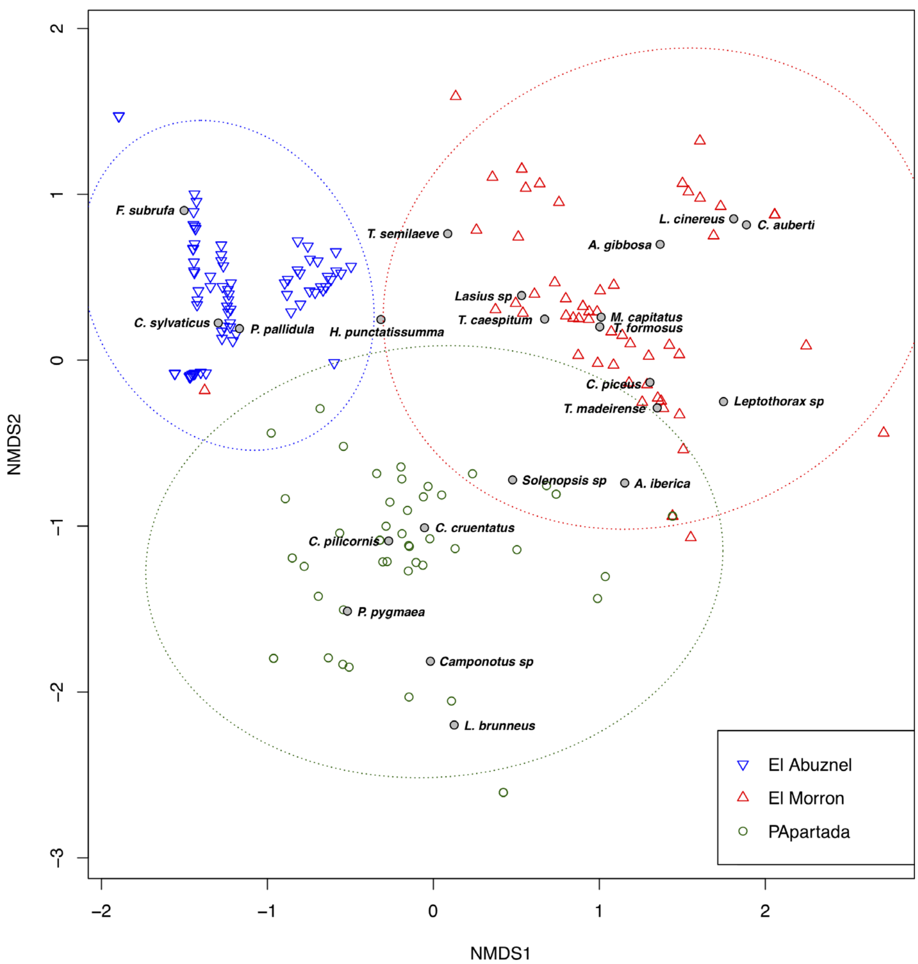

Figure 2 Non-metric multi-dimensional scaling (NMDS) representation of all the Formicidae species distributed among the localities (Iberian Peninsula) using Bray–Curtis dissimilarity. Stress value: 0.055. Every symbol represents a sample and the species label indicates the centroid of the different species. Blue inverted triangle, El Abuznel, 400 m; circle, Peña Apartada, 900 m; red triangle, El Morrón, 1,500 m.

being supported by the high evenness index (*J'* = 0.827), meaning that all the species tended to have similar abundance. The values of the Shannon and Pielou indices for El Abuznel and El Morrón were *H'* = 1.181 and *J'* = 0.659 and *H'* = 0.647 and *J'* = 0.228, respectively.

Richness and its variation in the Iberian Peninsula

In the Iberian Peninsula, 41 species have been reported in relation to decomposing remains (Table 3). El Morrón is still the place with the highest richness, followed by Murcia city. The remaining localities have a very similar richness (6–9 species). *Pheidole pallidula* and *Tet. semilaeve* are the species with the greatest range of distribution, followed by *P. pygmaea*. It seems clear that there are specific species in almost each locality. For instance, *I. subrufa* in El Abuznel, *L. brunneus* in Peña Apartada, *Lasius niger* (Linnaeus) in Murcia city, *Lasius grandis* Forel in Huesca, *Temnothorax nylanderi* (Foerster) and *Temnothorax recedens* (Nylander) in Coimbra, and *Aphaenogaster senilis* Mayr, *Tapinoma nigerrimun* (Nylander) and *Temnothorax luteus* (Forel) in Lisbon (Table 3) (Castillo Miralbés 2002; Martínez *et al.* 2002; López Gallego *et al.* 2011; Prado e Castro *et al.* 2014; Neto-Silva *et al.* 2018). Moreover, there are species shared within Sierra Espuña but not present in Murcia city, such as *Camponotus pilicornis* (Roger), and species shared in all the samplings undertaken in the Region of Murcia, such as *Ca. sylvaticus* or the genus *Solenopsis* (Westwood), but not present in other regions.

DISCUSSION

A limited number of studies concerning ant communities on altitudinal gradients have been undertaken and/or documented to date (Sanders *et al.* 2007; Castro Delgado *et al.* 2008; Guerrero & Sarmientom 2010; Rodríguez & Lattke 2012; Arnan *et al.* 2014). From the point of view of forensic interest there are some

Table 2 Results obtained after a SIMPER analysis on the different Formicidae species found, showing the percentage contribution of each species in each locality, Iberian Peninsula

Group Abuznel

Average squared distance = 4.45

	Av. value	Contrib. %	Cum. %
Tetramorium semilaeve	0.237	8.07	8.24
Iberoformica subrufa	0.788	19.38	27.62
Camponotus sylvaticus	1.330	31.65	59.27
Pheidole pallidula	0.934	40.73	100

Group Peña Apartada

Average squared distance = 1.82

	Av. value	Contrib. %	Cum. %
Pheidole pallidula	0.101	7.00	12.35
Camponotus sylvaticus	0.165	10.41	22.76
Aphaenogaster iberica	0.274	14.49	37.24
Plagiolepis pygmaea	0.282	14.51	51.76
Lasius brunneus	0.279	21.09	72.85
Camponotus cruentatus	0.279	27.15	100

Group Morrón

Average squared distance = 4.64

	Av. value	Contrib. %	Cum. %
Messor capitatus	0.115	2.36	7.61
Tetramorium semilaeve	0.233	4.07	11.68
Aphaenogaster gibbosa	0.227	5.07	16.75
Aphaenogaster iberica	0.540	20.12	36.86
Crematogaster auberti	1.340	63.14	100

Groups Abuznel and Peña Apartada

Average squared distance = 9.26

	Group Abuznel Av. value	Group Peña Apartada Av. value	Contrib. %	Cum. %
Camponotus sylvaticus	1.33	0.165	31.72	31.72
Pheidole pallidula	0.934	0.101	28.28	60.00
Iberoformica subrufa	0.788	0.000	15.95	75.95
Camponotus cruentatus	0.000	0.279	6.14	82.09
Lasius brunneus	0.000	0.279	4.95	87.04
Tetramorium semilaeve	0.237	0.000	4.45	91.50

Groups Abuznel & Morrón

Average squared distance = 14.4

	Group Abuznel Av. value	Group Morrón Av. value	Contrib. %	Cum. %
Crematogaster auberti	0.000	1.34	32.70	32.70
Camponotus sylvaticus	1.330	0.01	21.87	54.58
Pheidole pallidula	0.934	0.02	18.40	72.98
Iberoformica subrufa	0.788	0.00	10.26	83.24
Aphaenogaster iberica	0.000	0.54	8.46	91.70

Groups Morrón & Peña Apartada

Average squared distance = 8.68

	Group Morrón Av. value	Group Peña Apartada Av. value	Contrib. %	Cum. %
Crematogaster auberti	1.340	0.000	54.21	54.21
Aphaenogaster iberica	0.540	0.274	14.49	68.7

(*Continues*)

Table 2 Continued

Camponotus cruentatus	0.000	0.279	6.54	75.25
Lasius brunneus	0.000	0.279	5.28	80.52
Plagiolepis pygmaea	0.000	0.282	3.93	84.46
Aphaenogaster gibbosa	0.227	0.000	3.28	87.74
Tetramorium semilaeve	0.233	0.000	2.78	90.52

Av., average; Contrib. %, percentage of contribution; Cum. %, cumulative percentage.

studies on ant communities in the Iberian Peninsula (Castillo Miralbés 2002; Martínez *et al.* 2002; López Gallego *et al.* 2011; Martínez Moñino 2011; Prado e Castro *et al.* 2014; Neto-Silva *et al.* 2018). However, most of these studies have been mainly carried out in periurban environments. Given the importance of this group of species from a forensic point of view, the goals of the present study were aimed to illustrate the distribution patterns of Formicidae along the altitudinal gradient in a wild environment of the southeastern Iberian Peninsula.

In agreement with other studies (Martínez *et al.* 2002; Arnaldos *et al.* 2004; Neto-Silva *et al.* 2018), and as expected according to the phenology of this family (Mederos-López *et al.* 2012), the maximum captures of Formicidae occurred during the warmer seasons (i.e. spring and summer). Nevertheless, a great number of specimens were collected at El Abuznel during autumn, most likely due to the high temperatures recorded in that locality during this season.

Different studies on the ecology of necrophagous fauna define the carcass as an ephemeral resource. However, it has been shown that it supports high levels of diversity compared with other resources (Woodcock *et al.* 2002; Martín-Vega & Baz 2013). This pattern was also observed in our study, where large numbers of some Formicidae species were collected. The most abundant species at the lowest altitude, El Abuznel, was *P. pallidula*, a Mediterranean species very abundant in the Iberian Peninsula (Martínez *et al.* 2002), that showed low abundance at Peña Apartada and El Morrón. *Pheidole pallidula* also represented the most abundant species in previous studies undertaken in Murcia (Martínez *et al.* 2002; Arnaldos *et al.* 2004), where it was found to be present in all seasons, although in our study it was completely absent during winter. The second most abundant species at El Abuznel was *Ca. sylvaticus*, that was cited for the first time as related to the sarcosaprophagous fauna by Martínez *et al.* (2002). It was also collected in the periurban environment in Murcia (Martínez *et al.* 2002; Arnaldos *et al.* 2004) and, with very low abundance, in Peña Apartada and El Morrón, but it has not been found in other places of the Iberian Peninsula (Castillo Miralbés 2002; Prado e Castro *et al.* 2014; Neto-Silva *et al.* 2018). As it concerns El Abuznel, *I. subrufa* appears to be of great interest because it has not been found in other areas related to the cadaveric ecosystem (Castillo Miralbés 2002; Martínez *et al.* 2002; Prado e Castro *et al.* 2014; Begoña Gaminde 2015; Neto-Silva *et al.* 2018). *Camponotus cruentatus* and *L. brunneus* were the most abundant species at Peña Apartada. Both species are also of great interest as they have not been found in other areas (Castillo Miralbés 2002; Martínez *et al.* 2002; Prado e Castro *et al.* 2014; Begoña Gaminde 2015; Pérez Marcos 2016; Catarineu *et al.* 2018; Neto-Silva *et al.* 2018). In El Morrón, the most abundant species was *Cr. auberti*, also found in Lisbon (Neto-Silva *et al.* 2018), but not related to the sarcosaprophagous fauna in other peninsular areas (Castillo Miralbés 2002; Martínez *et al.* 2002; Prado e Castro *et al.* 2014). Other species with much lower abundance are represented by *A. iberica*, also collected in Peña Apartada.

There exists the possibility that species, such as *Tet. semilaeve* and *Hypoponera punctatissima* (Roger), captured at the lowest and highest altitudes of Sierra Espuña, and/or *A. iberica*, found in Murcia city and in Peña Apartada and El Morrón, could be also found along the altitudinal gradient. Thus, it would be necessary to carry out more samplings to test this hypothesis. However, the results presented in this study could have also been determined by both abiotic and biotic factors which could be interacting and would limit the settlement of their colonies in each site, or could be the result of insufficient sampling (Guerrero & Sarmientom 2010). Bernadou *et al.* (2015) refer to species turnover in the communities so that the composition of ant communities at high elevation differs from that at low elevation. This species turnover has also been observed in our study despite the fact that the altitudinal gradient (400–1,500 m) was not so pronounced as in other studies, reinforcing the interest of learning about such communities for forensic purposes.

All the results about the distribution of the Formicidae species as related to the three localities were supported by the PERMANOVA ($P < 0.01$), NMDS (stress value = 0.055, indicating that it is a reliable representation) and the SIMPER analysis, which showed how the three sites were separated and only overlapping for some species. The considerably higher abundance of *P. pallidula* in El Abuznel should not be

Table 3 Formicidae species present in different studies in the Iberian Peninsula

Species	Lisbon[†]	Coimbra[‡]	Huesca[§]	Murcia[¶]	El Abuznel	Peña Apartada	El Morrón
Aphaenogaster gibbosa (Latreille)							X
Aphaenogaster iberica Emery				X		X	X
Aphaenogaster sensilis Mayr	**X**						
Camponotus aethiops (Latreille)			**X**				
Camponotus cruentatus (Latreille)						**X**	
Camponotus piceus (Leach)							**X**
Camponotus pilicornis (Roger)					X	X	X
Camponotus sp. Mayr					X	X	
Camponotus sylvaticus (Olivier)				X	X	X	X
Cataglyphis ibericus (Emery)				**X**			
Crematogaster auberti Emery	X						X
Crematogaster scutellaris (Olivier)	X		X				
Formica rufibarbis Fabricius			**X**				
Goniomma blanci (André)							**X**
Hypoponera punctatissima (Roger)					X		X
Iberoformica subrufa (Roger)					**X**		
Lasius brunneus (Latreille)						**X**	
Lasius cinereus Seifert							**X**
Lasius grandis Forel			**X**				
Lasius niger (Linneus)				**X**			
Lasius sp. Fabricius							**X**
Linepithema humile (Mayr)		X		X			
Messor capitatus (Latreille)							**X**
Messor barbarus (Linneus)				**X**			
Myrmica specioides Bondroit			**X**				
Pheidole pallidula (Nylander)			X	X	X	X	X
Plagiolepis grassei Le Masne					**X**		
Plagiolepis pygmaea (Latreille)	X	X		X		X	
Plagiolepis schmitzii Forel				**X**			
Plagiolepis xene Starcke				**X**			
Ponera coarctata (Latreille)		**X**					
Pyramica membranifera (Emery)				**X**			
Solenopsis sp. Westwood				X	X	X	X
Tapinoma madeirense Forel							**X**
Tapinoma nigerrimum Nylander	X						
Temnothorax formosus (Santschi)							**X**
Temnothorax luteus (Forel)	**X**						
Temnothorax nylanderi (Foerster)		**X**					
Temnothorax recedens (Nylander)		**X**					
Temnothorax sp. Mayr							**X**
Tetramorium caespitum cfr. (Linnaeus)	X						X
Tetramorium semilaeve Andre	X	X	X		X		X

[†]Neto-Silva *et al.* (2018)
[‡]Prado e Castro *et al.* (2014)
[§]Castillo Miralbés (2002)
[¶]Martínez *et al.* (2002); Martínez Moñino (2011)
X - Underlined species were recorded in a previous sampling and in very low abundances (less than five specimens).
X - The species in bold and shaded in purple were species found in just one locality.
Species shaded in red are found in five localities, yellow in four localities, green in three and purple in just one locality.

surprising due to the type of mass recruitment that this species has towards food sources of considerable size (Martínez *et al.* 2002). However, the low abundance of this species in the other two locations is interesting. The high abundance of *Cr. auberti* in El Morrón could be due to the fact that this species does not usually live in trees (Ruiz *et al.* 2006), like their relatives (i.e. *Crematogaster scutellaris* (Olivier)), but they tend to establish themselves in meadows under stones heated by the sun, and this locality was characterized by being exposed to the sun. In contrast, *L. brunneus*, a feature of Peña Apartada, usually nest inside old trees, mainly oaks, and has also been recorded from hedgerows, in agreement with the vegetation present in that locality. Finally, there are no apparent reasons why *A. iberica* does not appear in El Abuznel, so it would be interesting to carry out more studies on the ecology of this species.

Considering Formicidae diversity, the highest abundance was registered in El Morrón, followed by El Abuznel; the lowest abundance was found in Peña Apartada. This could be explained by the steepness of this locality. However, the increase of species richness with altitude could indicate that richness is explained by altitude. In our study, the largest number of species was registered in El Morrón and the highest diversity index in Peña Apartada, supported by the high evenness index, meaning that all species tended to have similar abundance. Our results agree with the study in the Spring Mountains, Nevada (USA) by Sanders *et al.* (2003). However, other studies of ant diversity in altitudinal gradients have reported a decrease in species richness with altitude (Brown Jr 1973; Fisher 1996; Brühl *et al.* 1999; Bernadou *et al.* 2015). Nevertheless, the ant diversity does not necessarily decrease consistently as a function of the altitudinal gradient, as other studies indicate a peak in richness at medium altitudes (Olson 1994; Samson *et al.* 1997; Fisher 1998; Ward 2000; Sanders 2002; Longino & Colwell 2011; Rodríguez & Lattke 2012). Thus, it would be interesting to undertake further studies at more altitudes to confirm the trend shown in our data.

It has been shown that there are specific species for almost each locality in the Iberian Peninsula, confirming that the geographical region, among other factors, affects the community (Anderson 2010). *Pheidole pallidula* and *Tet. semilaeve* are the species with a greater range of distribution, followed by *Pl. pygmaea*. The species appearing in just one of the studied localities, such as *I. subrufa* in El Abuznel, *L. brunneus* in Peña Apartada, *L. grandis* in Huesca, *L. niger* in Murcia city, *Tem. nylanderi* and *Tem. recedens* in Coimbra and *A. senilis*, *Tap. nigerrimum* and *Tem. luteus* in Lisbon, are of interest as they did not appear in other sampled areas (Castillo Miralbés 2002; Martínez *et al.* 2002; Prado e Castro *et al.* 2014; Neto-Silva *et al.* 2018). Moreover, there are species that were shared in Sierra Espuña but not in Murcia city, such as *Ca. pilicornis* (Roger). Other species shared in all the samplings undertaken in the Region of Murcia, such as *Ca. sylvaticus* or the genus *Solenopsis*, were not present in other regions. All these facts make all these species of great interest as they are likely indicators of the habitat in the studied area.

A putative scenario concerns the emergence of new invasive species that could affect the community of formicids already established in the area. However, Catarineu (2019) mentions in his work that none of the six invasive species in the studied region appear to be capable of expanding through the arid lands of the area, beyond degraded ecosystems, near the coast, or urban regions, where they are currently present. Even so, it would be interesting to undertake studies which aim to determine if the community of formicids has been altered over time.

In summary, this study represents the first approach to characterize the community of the Formicidae family as a representative of the sarcosaprophagous community in an altitudinal gradient in Murcia. As indicated by Prado e Castro *et al.* (2014), and considering the Formicidae species from all the data collected in the different areas of study, it is possible to conclude that a great variability exists in the composition of the Formicidae community. The present study, together with those carried out in different areas of the Iberian Peninsula, presents data of species with potential utility as geographic and environmental indicators. The species *I. subrufa, L. brunneus, L. grandis, L. niger, Tem. nylanderi, Tem. recedens, A. senilis, Tap. nigerrimum* and *Tem. luteus* are of interest as they just appeared in one of the sampled areas (Castillo Miralbés 2002; Martínez *et al.* 2002; Prado e Castro *et al.* 2014; Neto-Silva *et al.* 2018). This specific association of some species with certain habitats is of capital importance in applied forensic entomology as suggested by Amendt *et al.* (2011), because it could provide indications on the postmortem transportation of a corpse. Moreover, the apparent vertical restriction of some species (Rodríguez & Lattke 2012) emphasizes the need to study the sarcosaprophagous community in as many different places as possible. This is necessary to prevent using uncorrelated data in the evaluation of carrion decomposition and the sarcosaprophagous community in a specific place. This information would assist to explain the dynamics of certain taxa and to clarify the role that they play within such a community, increasing their forensic importance as geographical, environmental or seasonal indicators.

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the Supporting Information section at the end of the article.

Table S1. Studies of the entomosarcosaprophagous fauna in the Mediterranean biogeographical region and the Iberian Peninsula, and the taxa studied in each work.