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First report on sarcosaprophagous Formicidae from Portugal (Insecta: Hymenoptera)

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Summary. The community structure, dynamics and succession patterns of the sarcosaprophagous Formicidae (Insecta, Hymenoptera) at the end of spring and summer, 2004, have been determined following a study carried out in Coimbra (Central Portugal) using piglet carcasses. A modified Schoenly trap was used to collect the fauna. Five decomposition stages were observed and 1061 ant workers, corresponding to six species, were collected and identified. The dominant species was *Linepithema humile* (Mayr 1868), for which its distribution throughout the decomposition process is shown. Comparisons are made with data from literature at two other localities in Spain. The information presented in this study represents the first report on the sarcosaprophagous Formicidae community that is present in Portugal. These data will contribute to enlarging our knowledge on the biology of this fauna, will provide interesting references for some species, and will be very useful for establishing a database that is critical in forensic practice.

Résumé. Premier rapport sur les Formicidae sarcosaprophages du Portugal (Insecta : Hymenoptera). Une étude a été menée à Coimbra (centre du Portugal), avec des carcasses de porcelets, pour y déterminer la structure de la communauté, la dynamique et les tendances de la relève des Formicidés sarcosaprophages (Insecta : Hymenoptera) au cours de la fin du printemps et en été 2004. Un piège Schoenly modifié a été utilisé pour recueillir la faune. Cinq stades de décomposition ont été identifiés et 1061 fourmis ouvrières, correspondant à 6 espèces ont été recueillies et identifiées. L'espèce dominante était *Linepithema humile* (Mayr 1868), pour laquelle la distribution dans tout le processus de décomposition est affichée. Des comparaisons sont faites avec les données bibliographiques d'études réalisées en deux différentes localités d'Espagne. L'information donnée est le premier rapport sur la communauté des fourmis sarcosaprophages du Portugal. Il accroît les connaissances biologiques sur cette faune, en offrant des références intéressantes pour certaines espèces, et sera très utile pour établir une base de données indispensable à la pratique médico-légale.

Keywords: ant; decomposition process; faunistics; forensic entomology

The entomosarcosaprophagous community includes a great diversity of species (e.g. Arnaldos et al. 2004, 2005). Those more significant for forensic purposes are the necrophagous organisms, which feed on the corpse, and necrophilous ones, which predate or parasitize the necrophagous species. Also of interest are omnivorous species, which take advantage of the corpse itself, as well as the fauna present on the corpse.

The structure of the sarcosaprophagous community is influenced by many factors. The most important is the geographic region or bio-geo-climatic zone, since it defines the habitat, vegetation, soil type, and meteorological conditions in which the remains are found (Anderson 2010). All these factors determine which insect species may be present. Many families of sarcosaprophagous insects are ubiquitous, but the particular species are not. Thus, in forensic practice, data corresponding to a particular region should not be used to derive conclusions from a different region. For this purpose, the features of local or regional sarcosaprophagous communities should be determined.

Within the sarcosaprophagous community, Formicidae is one of the most abundantly represented groups (Arnaldos et al. 2004, 2005). In tropical countries Formicidae can be the most abundant invertebrates found on a dead body, and occur at all stages of decay (Gunn 2009). Together with scarabeids (Coleoptera), Formicidae have been considered one of the most important groups in decomposing vertebrate corpses (Cornaby 1974; Scott et al. 1987). This group are opportunistic arthropods that can be present during all the decomposition stages of a corpse (Payne 1965; Gunn 2009). Their role in succession varies from predators of insect eggs and larvae to necrophagous, as they can also directly feed on the corpse. Thus, Formicidae may be considered omnivorous (Arnaldos et al. 2005; Battán Horenstein et al. 2012) and

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will often feed on human skin and body tissues when the remains are left outside and exposed. Some species (e.g. *Solenopsis invicta* Buren 1972) often cause tissue postmortem damage that resembles premortem burns, and produce bloodstain patterns that can confuse investigations (Jayaprakash 2006). Formicidae have frequently been observed to prey on primary colonizers, such as fly eggs and maggots, during the earlier stages of decomposition, and can delay the decomposition process by decreasing the maggot populations (Byrd & Castner 2010; Lindgren et al. 2011). Formicidae can be used to estimate the postmortem interval, by considering the time required for development of the colony of the species associated with carrion (Goff & Win 1997).

In an attempt to determine the utility of Formicidae as forensic indicators, the community related to corpses in a not yet explored setting of the Iberian Peninsula was studied in order to understand: (1) its composition; (2) its dynamics in relation to the decomposition stages of animal matter; and (3) possible differences between this community and those found in other settings of the Iberian Peninsula.

Materials and methods

The study was carried out in Coimbra city, at the Botanical Garden of the University of Coimbra (40°12′N, 8°25′W), in a forested zone inaccessible to visitors. The shrubby and arboreous vegetation is comprised mainly by *Ailanthus altissima* (Simaroubaceae), *Laurus nobilis* (Lauraceae), *Celtis australis* (Cannabaceae), *Olea europaea* (Oleaceae), and *Eucalyptus spp*. (Myrtaceae) (Prado e Castro, Sousa, et al. 2011). Two locations separated by 100 m were chosen, one in a sunny place (a clearing with direct sunlight) and the other in a shaded area (due to the presence of several trees) (Figure 1). Two Schoenly (1981) modified traps (Prado e Castro et al. 2009) were used (Figure 1), baited with 5 kg piglets (*Sus scrofa* L.), euthanized by carotid injury. The traps are designed to collect adult arthropods, both those that are attracted to the bait and those that emerge from the decaying remains, enabling their recordings while minimizing interference with the natural decomposition process and faunal succession. The baited Schoenly traps have shown significant improvement in between-carcass similarity of taxa and quality of sampling (i.e. higher species richness, abundance and continuity in succession) over the conventional methodology, which involves direct manual capture (using common entomological methods) of the fauna associated with the carcass at the time that the sampler visits (Ordóñez et al. 2008). Thus, the results obtained with a Schoenly trap can be assumed to be sufficiently comparable to those obtained using several traps and baits.

Samples were taken during a four-month period, from May to September 2004 (Prado e Castro, Arnaldos, et al. 2011; Prado e Castro, Sousa, et al. 2011), daily during the first month, and every 2, 3, 4, 5 or 7 days, afterwards until the end of the experiment. Five decomposition stages were identified: fresh, bloated, active decay, advanced decay, and dry/remains (Prado e Castro, Arnaldos, et al. 2011), with different durations in each site (Table 1). Decomposition stages, as described by Anderson & VanLaerhoven (1996), are characterized as follows. The fresh stage begins at the moment of death and continues until bloat is evident. No odor is associated with the remains. The bloated stage begins when gases start to accumulate in the carcasses, resulting in a definite bloated appearance and ends when the body deflates due to insects piercing the carcass. The active decay stage starts when the carcass completely deflates, being very wet, with a strong smell. At the advanced decay stage maggots move away from the remains, and there is less odor. In the dry/remains stage, very little of the carcass remains, except bones, cartilage and some skin, and little or no odor is associated with the remains.

Figure 1. Schoenly traps used in the sunny (A) and shaded (B) locations.

Table 1. Duration of the different decomposition stages of the corpses in sunny and shaded locations.

	Days since death	
	Sun	Shade
Fresh	1–2	1–2
Bloated	3–6	3–6
Active decay	7	7
Advanced decay	8–14	8–42
Dry/remains	15–end	43–end

Results

A total of 1061 Formicidae specimens, belonging to six species of five different genera, uniformly distributed in both sunny and shaded locations (Table 2), were collected. A short review of the species is hereby presented.

Linepithema humile (Mayr 1868)

This species is considered one of the most invasive species globally (Global Invasive Species Database, 2009). It has a great capacity for invading new areas, thus enlarging the colonized area (Casellas Fabrellas 2004). It is abundant in agricultural and urban habitats with a Mediterranean climate, and prefers coastal and low altitude places (Espadaler & Gómez 2003). The location under study here appears to be suitable for its establishment due to environmental humidity provided by the characteristics of an urban park (Ruiz Heras et al. 2011). In Portugal, the species has recently been found in gardened areas of the Algarve region (Obregón Romero & Reyes López 2012).

Regarding the sarcosaprophagous community, *L. humile* has been referred as omnivorous that colonizes corpses in other regions (Martínez et al. 2002; Iannacone 2003).

Plagiolepis pygmaea (Latreille 1798)

This species is frequent in the Iberia Peninsula, preferring arid or low vegetation areas. It is polygynous, with small subterranean colonies (Ruiz et al. 1999). *P. pygmaea* is of small size and can act as a predator (Collingwood & Prince 1998), although it often prefers sugary food sources. It is generally considered omnivorous, and has previously been found in sarcosaprophagous communities (Martínez et al. 2002). According to Abril & Gómez (2009) this is the only native ant species shown to be able to resist the invasion of *L. humile*, at least in a natural ecosystem in the north-east of the Iberian Peninsula. This may be due to the peaceful and submissive nature of *P. pygmaea*.

Tetramorium semilaeve André 1883

This small sized species is common throughout the Mediterranean region and widely distributed in the Iberian Peninsula. It replaces other species of the genus in sunny and dry places (warmer), that do not rise much in altitude. Its nests are under stones (Retana et al. 1992). *Tetramorium semilaeve* is omnivorous, with crepuscular and nocturnal habits (Retana et al. 1992) and has previously been found to be sarcosaprophagous on vertebrate corpses (Martínez et al. 1997) and on arthropod remains, where it may be a significant competitor for other species (Retana et al. 1991).

Ponera coarctata (Latreille 1802)

There are several references to this species in Spain but, in Portugal, the only known reference does not appear to have a precise location (south Portugal) (Collingwood & Yarrow 1969; Collingwood & Prince 1998; Salgueiro 2002). In some European countries, it is widely distributed, living in wooded areas, as well as in xeric habitats (Csösz & Seifert 2003) and showing a wide altitudinal distribution. It can be found from sea level to 2000 m (Tinaut et al. 2007). Like other Ponerinae, it has hypogeal habits, it is small, and it has a very low number of workers, which means a low probability of being present in samples. The present reference is the second one for continental Portugal, but the first with a precise location, which enlarges its known distribution within this country.

Table 2. Number of specimens collected from each species, in sunny and shaded locations, and in each decompositional stage.

	Linepithema humile		*Plagiolepis pygmaea*		*Tetramorium semilaeve*		*Ponera coarctata*		*Temnothorax nylanderi*		*Temnothorax recedens*	
	Sun	Shade	Sun	Shade	Sun	Shade	Sun	Shade	Sun	Shade	Sun	Shade
Total	516	520	16	0	3	0	1	0	0	4	0	1
Fresh	21	5	6	0	0	0	0	0	0	0	0	0
Bloated	127	217	7	0	0	0	0	0	0	2	0	0
Active decay	16	46	0	0	0	0	0	0	0	0	0	0
Advanced decay	40	201	0	0	0	0	0	0	0	2	0	1
Dry/remains	312	51	3	0	3	0	1	0	0	0	0	0

Temnothorax nylanderi (Foerster 1850)

This is a small sized species, and its colonies are not very populous, possessing only up to 200 workers and a single queen per colony. The type of foraging strategy is tandem recruitment (Beckers et al. 1989), meaning that one individual follows closely behind another (Hölldobler & Wilson 1990). Thus, fewer individuals are implied in recruitment and the process is slower that in the recruitment in mass or in any other type in which numerous individuals migrate to the food source. Thus, it is less frequently collected than other species. It is common throughout the Mediterranean region, and has been found in Central and South Europe. In the Iberian Peninsula it has been found in numerous localities, mainly in the northern half.

Temnothorax recedens (Nylander 1856)

This species, very common in the Mediterranean region, has very similar characteristics to its congener *T. nylanderi.* Its diet is omnivorous, showing crepuscular activity and individual foraging (Espadaler & Rodà 1984). It is widely distributed in the Iberian Peninsula.

Discussion

Among all the collected species, only *Linepithema humile* achieved significant numbers, followed distantly by *Plagiolepis pygmaea. L. humile* is abundant as a consequence of its unicoloniality that allows it to achieve a large number of workers per colony, a high nest density, and a low intraspecific aggressiveness level, against very high interspecific aggression (Cammell et al. 1996). It is an introduced species from South America, so its dominance is important, as it is capable of displacing native species of arthropods (Cole et al. 1992; Holway 1995; Human & Gordon 1996), including seed dispersers, and also vertebrates (Suárez & Case 2002) that live in the same territory. In areas where *L. humile* has been identified it can represent 95.4% of the total collected species, and a significant reduction was observed in Formicidae specific richness compared to areas not invaded by this species (Gómez et al. 2003). In particular, *Temnothorax nylanderi, T. recedens, Plagiolepis pygmaea* and *Tetramorium semilaeve*, species that may be present in areas where *L. humile* is not, will disappear when the areas are invaded by the later (Gómez et al. 2003). Its alimentary regime is considered omnivorous, and therefore it does not reject any protein source to which it has access.

Linepithema humile was especially abundant during the first days of decomposition, mainly in the shaded, more humid site, reaching its maximum at day six (Figure 2) during the bloated stage, when numerous Diptera eggs and small larvae were present on the corpse, in agreement with other studies (Wolff et al. 2001). After this it was not especially abundant at any time, but was present all along the decomposition process, with a secondary maximum during the advanced decay stage. At the sunny location, where decomposition occurred faster, the numbers of this species showed another peak in the dry/remains stage, when an important fauna accessible to Formicidae was present on the corpse.

The observed dynamics of *L. humile* supports the omnivorous character of Formicidae present in the sarcosaprophagous community, which are attracted to the corpse by the fauna installed on it, especially abundant in some decomposition stages. Despite that, and according

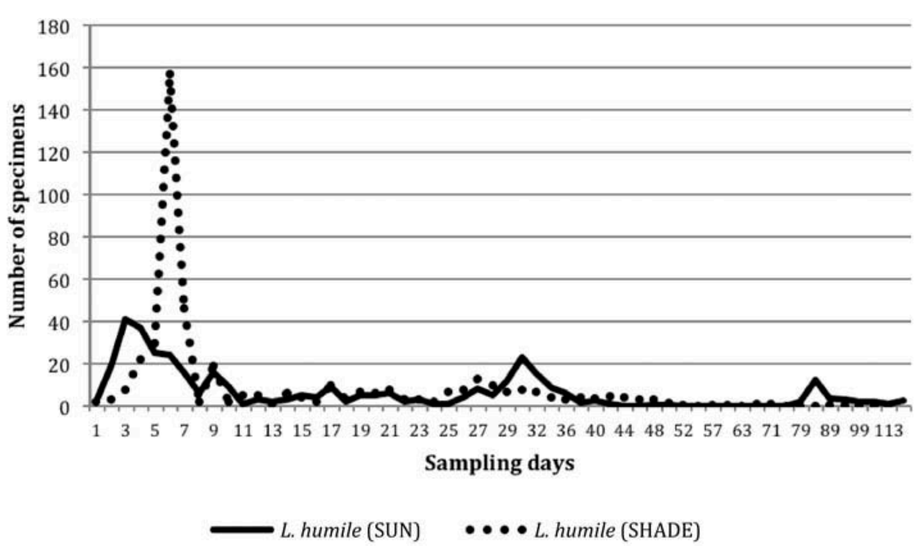

Figure 2. Distribution of *Linepithema humile* with time. After day 30, numbers of specimens refer to average collected number.

to Payne (1965) and Shi et al. (2009), Formicidae is present in the community during all decomposition stages. Most species of ants are scavengers and can consume vertebrate carrion (Clark & Blom 1991), which is rich in nutrients, such as nitrogen and phosphorus. These nutrients are limited in the vegetable diet in arid and semiarid areas (which is not the case for this area of Portugal). However, there is evidence suggesting that the composition of the elaiosomes of vegetable seeds evolved to attract ant species (Hughes et al. 1994). In some ants, oleic acid induces the behaviour of transporting corpses (Wilson et al. 1958; Haskins & Haskins 1974; Howard & Tschinkel 1976) and the burial behaviour in *Myrmecia vindex* (Haskins & Haskins 1974). Paper impregnated with oleic acid has even been observed to be treated as food (Gordon 1983). Fatty acids provide a valuable supply of energy for the larvae, contributing to the effectiveness of the colony (Boulay et al. 2007). Thus, predator and granivore (seed dispersal) as well as omnivorous ant species will access corpses producing fatty acids.

When comparing the fauna of Formicidae on corpses in Coimbra with that found in other areas of the Iberian Peninsula (Figure 3) during the same time of year, some differences in the community structure can be observed (Table 3, modified from Arnaldos et al. 2006). The richest community is that found in Murcia (Martínez et al. 2002), whereas the poorest one is the community from Coimbra. These two communities share two species, but the most abundant one in Coimbra (*L. humile*) was rare in Murcia (only one specimen). The communities of Coimbra and Huesca province (Castillo Miralbes 2002) share only one species that is not significant in number in either of the two locations (three specimens in Coimbra and few specimens [sic] in Huesca province). The most abundant species in each of the three Iberian areas are different: in Coimbra, it is an introduced species, considered as a pest; in Murcia, it is a very common, omnivorous, opportunistic species, found several times in relation to corpses; and in Huesca province, a species known by its mutualism with aphids. The fact that the studied community in Coimbra is not very rich can be explained, in addition to the presence of *L. humile* mentioned above, by the settings where the traps were located: an urban park with trees, and a habitat where *L. humile* excludes the dominant species and can only coexist with some inconspicuous species, e.g. those of the genera *Temnothorax* and *Plagiolepis* (Paiva et al. 1998). In addition, it is known that, in general, the richness and abundance of Formicidae are reduced in urban ecosystems (Clark et al. 2008).

To conclude, the communities of sarcosaprophagous Formicidae that are associated with corpses are unique in each of the regions studied so far, providing a potential application in forensic practice, as a geographic indicator.

The data presented here broaden the knowledge of sarcosaprophagous fauna in Portugal and illustrate the inappropriateness of the indiscriminate use of data from the literature for forensic purposes. In addition, our data support the need to enhance knowledge of faunal characteristics at a regional scale, due to its potential application in forensic practice.

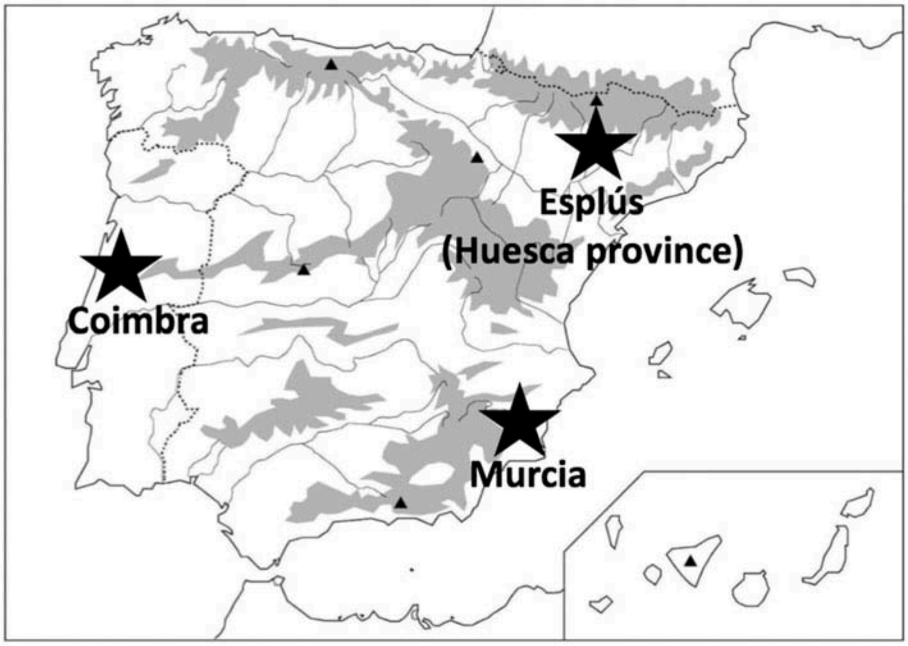

Figure 3. Iberian Peninsula map. The three locations compared in this study are highlighted with a star.

Table 3. Formicidae species present in three different studies in the Iberian Peninsula. The highlighted area indicates the predominant species.

	Coimbra	Esplús (Huesca province)[1]	Murcia[2]
Aphaenogaster iberica Emery 1908			X
Camponotus aethiops (Latreille 1798)		X	
Camponotus sylvaticus (Olivier 1792)			X
Cataglyphis ibericus (Emery 1906)			X
Crematogaster scutellaris (Olivier 1792)		X	
Formica rufibarbis Fabricius 1793		X	
Lasius grandis Forel 1909		X	
Lasius niger (L. 1758)			X
Linepithema humile (Mayr 1868)	X		X
Messor barbarus (L. 1767)			X
Myrmica specioides Bondroit 1918		X	
Pheidole pallidula (Nylander 1849)		X	X
Plagiolepis pygmaea (Latreille 1798)	X		X
Plagiolepis schmitzii Forel 1895			X
Plagiolepis xene Starcke 1936			X
Ponera coarctata (Latreille 1802)	X		
Pyramica membranifera (Emery 1869)			X
Solenopsis Westwood 1840			X
Temnothorax nylanderi (Foerster 1850)	X		
Temnothorax recedens (Nylander 1856)	X		
Tetramorium semilaeve Andre 1883	X	X	

[1]Area of irrigated intensive agriculture and livestock industry with human influence, at about 281 m asl. Traditional collection was used (manual, with entomological net, paintbrushes and forceps).
[2]A periurban site, about 6 km north of Murcia City, located in a natural area of the Experimental Agricultural Field of the Espinardo Campus of Murcia University. Semidesert environment at about 60 m asl. A Schoenly trap was used to collect the specimens.

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Appendix. Studied specimens (workers)

Date	*Linepithema humile*	*Plagiolepis pygmaea*	*Tetramorium semilaeve*	*Ponera coarctata*	*Temnothorax nylanderi*	*Temnothorax recedens*
27.V.2004	4	6				
28.V.2004	22	1				
29.V.2004	49	1			1	
30.V.2004	59	1				
31.V.2004	55	2			1	
01.VI.2004	181	1				
02.VI.2004	62					
03.VI.2004	3					
04.VI.2004	35					
05.VI.2004	11					
06.VI.2004	6					
07.VI.2004	8					
08.VI.2004	3					
09.VI.2004	9					
10.VI.2004	9				1	
11.VI.2004	6		1			
12.VI.2004	19	2				
13.VI.2004	5					
14.VI.2004	12					
15.VI.2004	11	1	1		1	
16.VI.2004	14					
17.VI.2004	5					1
18.VI.2004	6					
19.VI.2004	3					
20.VI.2004	8					
21.VI.2004	12					
22.VI.2004	21					
23.VI.2004	15					
24.VI.2004	19					
25.VI.2004	31		1			
27.VI.2004	45					
29.VI.2004	25					
01.VII.2004	18					
03.VII.2004	11					
05.VII.2004	12					
07.VII.2004	11					
09.VII.2004	8			1		
11.VII.2004	6					
13.VII.2004	7					
15.VII.2004	4					
17.VII.2004	1					
19.VII.2004						
22.VII.2004	2					
25.VII.2004	2					
28.VII.2004	1					
01.VIII.2004	4					
05.VIII.2004	4					
09.VIII.2004						
13.VIII.2004	8					
18.VIII.2004	61					
23.VIII.2004	20					
28.VIII.2004	17					
02.IX.2004	11					
09.IX.2004	13					
16.IX.2004	8					
24.IX.2004	34	1				