

ORIGINAL ARTICLE

Successional patterns of the insect fauna on a pig carcass in southern Italy and the role of *Crematogaster scutellaris* (Hymenoptera, Formicidae) as a carrion invaderTeresa BONACCI¹, Tullia ZETTO BRANDMAYR¹, Pietro BRANDMAYR¹, Vannio VERCILLO² and Francesco PORCELLI³¹Dipartimento di Ecologia, Università della Calabria, Arcavacata di Rende (CS), ²Azienda Sanitaria Provinciale Sezione di Medicina Legale, Cosenza, and ³DiBCA sez Entomologia e Zoologia Università di Bari, Bari, Italy**Abstract**

The sarcosaprophagous fauna plays a key role in organic matter decomposition. Moreover, the biological, ecological and behavioral specificities of the taxa are useful to reconstruct the decay history of a corpse or carcass, often back to the lethal event. Here we report the seasonal succession of the insect fauna on a pig carcass exposed in a rural area in Calabria (southern Italy) during summer 2007 and 2008. The aim is to identify and qualitatively assess the major taxa of forensic importance in this region. The principal fly invaders were *Lucilia caesar* (L.), *L. sericata* (Meigen, 1826), *Chrysomya albiceps* (Wiedemann, 1819), *Sarcophaga* (Meigen, 1826) spp. and *Amobia* (Robineau-Desvoidy, 1830) spp., *Musca domestica* (L.) and *Muscina stabulans* (Fallen, 1817). The primary beetle species collected in summer belonged to Dermestidae, *Dermestes maculatus* (De Geer, 1774) and Cleridae, *Necrobia rufipes* (De Geer, 1775). This paper also examined the ecological role of ants in the insect succession and describes the evidence of skin injuries directly inflicted by the acrobat ant *Crematogaster (Acrocoelia) scutellaris* (Olivier, 1791) (Hymenoptera Formicidae) while feeding on pig carrion. Ants belonging to two other species were also collected: *Camponotus aethiops* (Latreille, 1798) and *Tetramorium semilaeve* (André, 1881). Ants can invade carcasses and corpses directly, disrupting blowfly egg laying or preying on their larvae. Our data on the carrion faunal composition and role of ants as invaders should be useful for further forensic cases in Calabria (southern Italy). This is among the few reports of ants as forensically relevant species.

Key words: acrobat ant, forensic entomological evidence, Imenoptera Formicidae, insect succession, skin lesion, *Sus scrofa*.

INTRODUCTION

Insect successions are very useful for estimating the *post-mortem interval* (PMI) and thus it is interesting to study the order of appearance of insect taxa on a corpse (Catts & Goff 1992; Anderson 1996; Benecke 2001). The entomological evidence related to decomposition stages of a corpse provide useful data in forensic case work (Nurteva 1977; Benecke 2001; Byrd & Castner 2009). The recognition of the species involved, the pattern and time

of arrival of adult insects on the corpse and the knowledge of their developmental rates can give an indication of the time of death. In general, data obtained from non-human carcasses are used to estimate the PMI and to apply the results to investigations involving human remains. There are two main methods for estimating the PMI using information on the insects that invade a corpse. The first method is development-based, in which the developmental stage of the flies on the corpse is used to indicate the time since death (Catts 1990). The other method uses succession-based studies to obtain information on the successional pattern of the carrion insect fauna (Schoenly *et al.* 1996). The taxa attracted to a carcass usually change in a predictable pattern as decomposition progresses through the different stages

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(Smith 1986). Several sarcosaprophagous animal taxa are involved in this process within a specialized ecological succession (Necrophagous, Saprophagous, Predators, Parasites and Omnivorous species) (Smith 1986; Campobasso *et al.* 2001; Byrd & Castner 2009). During the colonization process, insects and other invertebrates feed on carrion in a successional manner and there is extensive damage to tissues and organs of the corpse or carcass as a result of animal feeding (Rossi *et al.* 1994; Byard *et al.* 2002). Sometimes animal feeding results in injuries similar to inflicted wounds, modifying or destroying clues to the cause of death (Tsokos & Schulz 1999; Byard *et al.* 2002; Byard 2005). Hymenopteran (ant) workers can both assist in PMI estimation (Goff & Win 1997) and produce confusing variables (Louw & Van der Linde 1993; Anderson 1996; Knight 1996) when they act as sarcosaprophagous (*Acromyrmex* spp.) (Mayr, 1865) insects (Cornaby 1974). The genus *Crematogaster* Lund (Myrmicinae) includes many species worldwide (Bolton 1995). *Crematogaster scutellaris* is very common in the Mediterranean area and nests in a wide variety of trees. Most *Crematogaster* species that visit trees feed on insects (eggs, larvae and pupae) in addition to nectar and honeydew (Grandi 1951; Cavallo & Delrio 1975; Redolfi *et al.* 1999; Richard *et al.* 2001). Many authors have reported that *C. scutellaris* workers are active in preying on various insect pests (Richard *et al.* 2001; Schatz & Hossaert-McKey 2003; Schatz *et al.* 2003; Radeghieri 2004; Ranganathan & Borges 2009) and fig wasps (Schatz *et al.* 2003). Here we report the case of skin feeding by *Crematogaster scutellaris* (Hymenoptera: Formicidae) on a pig carcass and discuss the impact of this ant species on forensic entomological evidence during colonization by insect invaders. Studies on the entomological structure of the sarcosaprophagous community and the succession and phenology of forensic species in Calabria (southern Italy) could be of great practical importance. In fact, there is a substantial lack of knowledge about the composition and seasonal dynamics of the sarcosaprophagous fauna in this geographical area. It is important to note that forensic entomology is gaining acceptance in many countries and has great potential for applications in legal investigations of human cases.

MATERIALS AND METHODS

Animal model and experimental procedure

One 25 kg female piglet (*Sus scrofa* (L.)) was killed by an intra-cardiac injection of KCl (with veterinary assistance) and exposed at midday (at 11:50 A.M.) on 10 July 2007, within 40 min after death, in a rural habitat near Cosenza (Calabria, southern Italy). The carcass was

placed on the ground in a mixed wood of *Quercus pubescens* (Willd., 1805), *Pyrus* sp. (L.) and *Olea europaea* (L.) trees. The site coordinates are 39°21'25.71"N and 16°13'42.87"E; site elevation is about 220 m a.s.l. A metal screen was placed on the carcass to exclude large sarcosaprophagous animals. Two digital data loggers were placed nearby (30 cm) and on the carcass surface to record environmental and carcass temperatures. Insects were collected from the carcass (at 1 h intervals each day, from 8.00 to 20.00 h), by direct capture or with entomological nets. An assessment was also made of the relative abundance of taxa based on visual observations and collected specimens. Maggots and pupae were collected daily directly from the carcass. The ant activity was observed and recorded at 30-min intervals each day (from 8.00 to 20.00 h). The mean number of ant workers on the carcass was estimated with the help of a square transect (5 cm wide). The number of maggots on the carcass was estimated with help of a square transect (10 cm wide). The experiment lasted until complete carcass consumption and thereafter we examined the soil to collect entomological material from the surrounding area, i.e. directly under the remains of carcass and in its proximity. Faunal data were combined to define the successional pattern for the corpse. To confirm the observations, we carried out an experiment on the skin feeding preference of *Crematogaster scutellaris* at the same place, starting on 30 July 2008. Three almost complete piglet skins (c. 60 × 40 cm) were placed on the ground at 1 m, 2.5 m and 3 m from the original carcass placement site. The skins remained in the field for five days.

The animal was killed using experimental procedures in accordance with the Guide for Care and Use of Experimental Animals issued by the European Communities Council Directive of 24 November 1986 (86/609/EEC). Efforts were made to minimize suffering and to minimize the number of specimens used.

RESULTS

Carcass decomposition stages

In summer 2007, we identified four decomposition stages (fresh, bloated, decay and dry) and the decay of tissues and organs was considerably accelerated. The mean environmental temperature was $T_{\text{mean}} = 27.65 \pm 5.58^{\circ}\text{C}$. The complete process of decomposition lasted 25 days.

Insect succession

In total, 2026 individuals (adults) were collected and they belong to 14 families: Calliphoridae, Sarcophagidae, Muscidae, Piophilidae, Platystomatidae,

Dermestidae, Cleridae, Histeridae, Staphylinidae, Silphidae, Carabidae, Braconidae, Vespidae and Formicidae. The fauna was classified according to the ecological categories of Smith (1986), as reported in Figure 1.

Insects were collected every day from the fresh to dry stage of decomposition. Diptera and Coleoptera were the two most important orders in the faunal composition. In the fresh stage of carcass decomposition, *Lucilia sericata*, *L. caesar*, *Chrysomya albiceps*, *Musca domestica*, *Muscina stabulans* and several Sarcophagidae were collected, while *Calliphora* (Robineau-Desvoidy, 1830) spp. were not detected in this season (Fig. 1) (Bonacci *et al.* 2010). Adults of Calliphoridae were observed massively laying eggs 24 h after carcass placement. The first instar fly larvae were observed 45 h after carcass placement. Large numbers of third instar larvae were observed and specimens were collected after four days (Fig. 2). The first fly pupae were collected nine days after carcass placement. After 27 days, large numbers of calliphorid puparia (in total $n = 3586$) were collected from the soil under the carcass: empty puparia (75.15%), entire puparia (19.91%) and parasitized puparia (4.94%). In the dry stage, the most abundant species were *Dermestes maculatus* (Coleoptera, Dermestidae) and *Necrobium rufipes* (Coleoptera, Cleridae). Adults of both species were found seven days after carcass placement, while the *Dermestes* larvae were abundant after 20 days in pieces of skin and bones and until complete decay of the pig. Predators of flies, such as Staphylinidae, Silphidae, Histeridae, Vespidae and Formicidae, joined the sequence in response to the availability of large numbers of fly eggs, larvae and adults.

Ant activity

Fifty minutes after carcass placement, about 30 workers of *Tetramorium semilaeve* were observed on the eye rims of the pig. This ant species was active on the carcass for approximately one day. One hour and a half after carcass placement, about 60 workers of *Crematogaster scutellaris* (Fig. 3a,b) were observed feeding on the eyes of the pig (Fig. 3c). About four hours later, about 230 workers were observed feeding on the skin, around and inside the mouth, the eyes, the vagina and the anus of the piglet (Fig. 3d). Twelve hours after carcass placement, a total of about 260 ant workers was observed. Nine serpiginous skin injuries (Fig. 3e) appeared on the abdomen, tongue and external genitalia, around the eyes, anus and tail base about six hours after carcass placement. These injuries were 1–4 cm long. *Crematogaster scutellaris* workers preyed on eggs laid by *Lucilia* (Robineau-Desvoidy, 1830) spp. and *Ch. albiceps* during the flies' first oviposition wave. The injuries were

clearly related to the activity of food gathering by workers of *C. scutellaris*. Workers probably collect keratin, as shown by Byrd (2005) for other ant species. The ant post-mortem injuries look like ante-mortem wounds and thus can be misinterpreted (Byrd 2005; Campobasso *et al.* 2008; Byrd & Castner 2009). A third species of ant, *Camponotus aethiops* was also observed on the carcass. A mean number of 34 workers was active on the carcass from the third day of piglet placement (Fig. 4) until the twelfth day. This ant species was found preying on maggots on the carcass. As observed for *T. semilaeve*, no injuries were observed in relation to the activity of *C. aethiops*.

Crematogaster scutellaris and *T. semilaeve* left the carcass after, respectively, one and two days from the piglet's death, once the bloated stage had started. *Camponotus aethiops* was active on the carcass up to the dry stage. In summer 2008, *Crematogaster scutellaris* workers invaded the pigskins about 30 min after their placement. About 90 *Crematogaster scutellaris* workers were observed feeding on the skins after 40 min, while about 270 workers were observed feeding after 1 h and 45 min. Thirty hours later, about 40 ant workers were still observed feeding on the skins. About six hours after the workers invaded the pigskins, seven serpiginous injuries appeared on the skins.

DISCUSSION

From the point of view of faunal composition, the insect succession in this study was defined by Diptera and Coleoptera. As also observed in other studies, Diptera Calliphoridae was massively collected during the initial stages of decomposition and Coleoptera in the decay and dry stages. In other investigations in Calabria, Bonacci *et al.* (2009) found that *Calliphora vicina* and *C. vomitoria* were associated with the autumn and winter seasons, whereas *Lucilia* spp. appeared in all seasons and *Chrysomya albiceps* appeared in spring, summer and autumn seasons. The lack of *Calliphora* spp. in Calabria in summer is a very important ecological datum. In our summer investigation, Calliphoridae species appeared as primary invaders when the decomposition process was shortened. Predators of maggots, such as Staphylinidae, Silphidae, Histeridae, Vespidae and Formicidae, joined the sequence in response to the availability of large numbers of fly eggs, larvae and adults (from the bloated to dry stage). The progressive carrion decomposition made it more favorable for species attracted by high decay levels (dry stage), such as *Necrobium rufipes* and *Dermestes maculatus*. Two of the ant species observed during this study, namely *T. semilaeve* and *C. scutellaris*, colonized the just-dead body

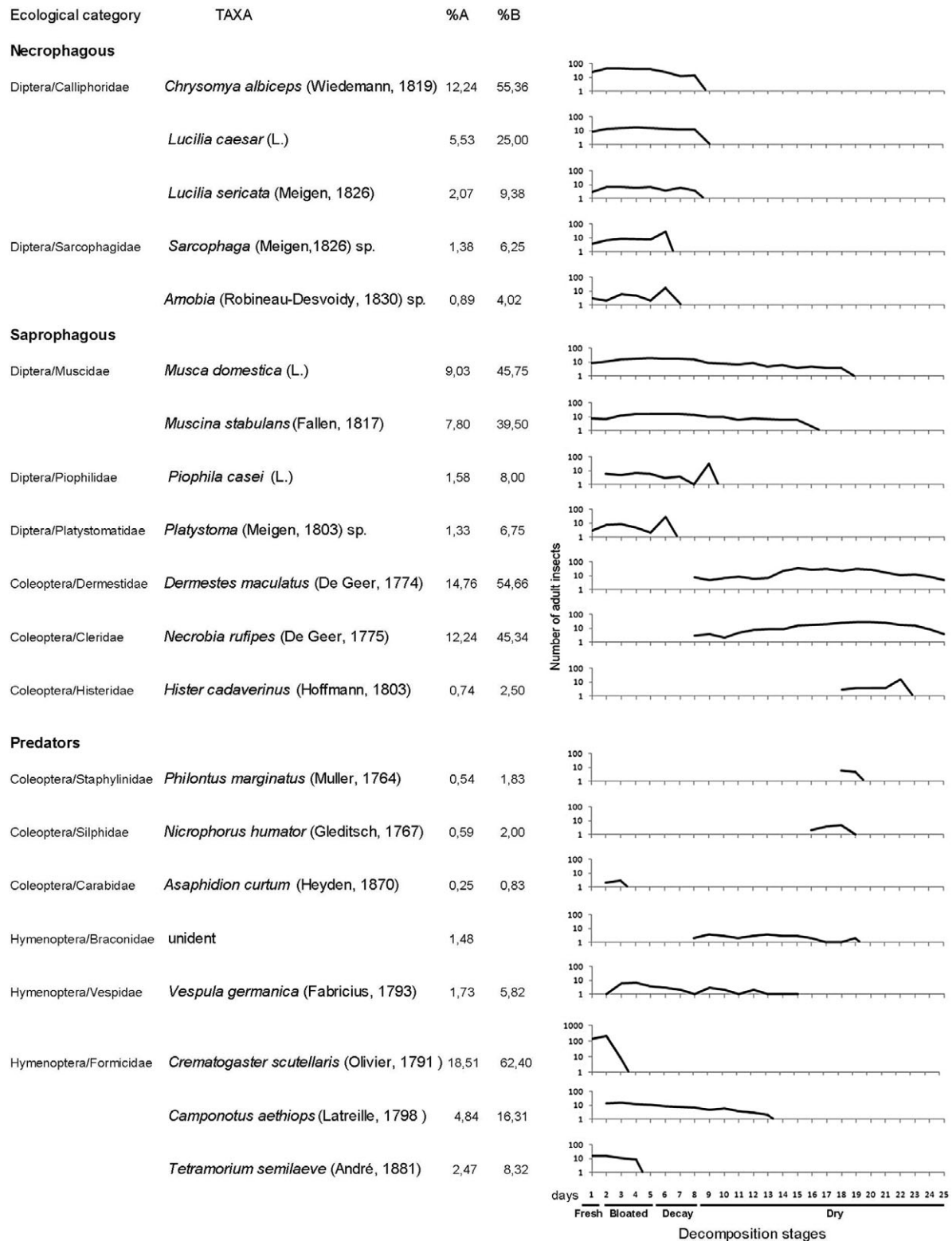


Figure 1 Daily fluctuations of adult insects on a pig carcass in summer 2007. %A shows the relative abundance of the taxa expressed as percentage of the total insects collected. %B shows the relative abundance of taxa expressed as percentage within the respective Ecological Category (Necrophagous, Saprophagous Diptera and Saprophagous Coleoptera, Predators and Parasitoids). The insects were collected on carcass every day by direct capture and with entomological nets.

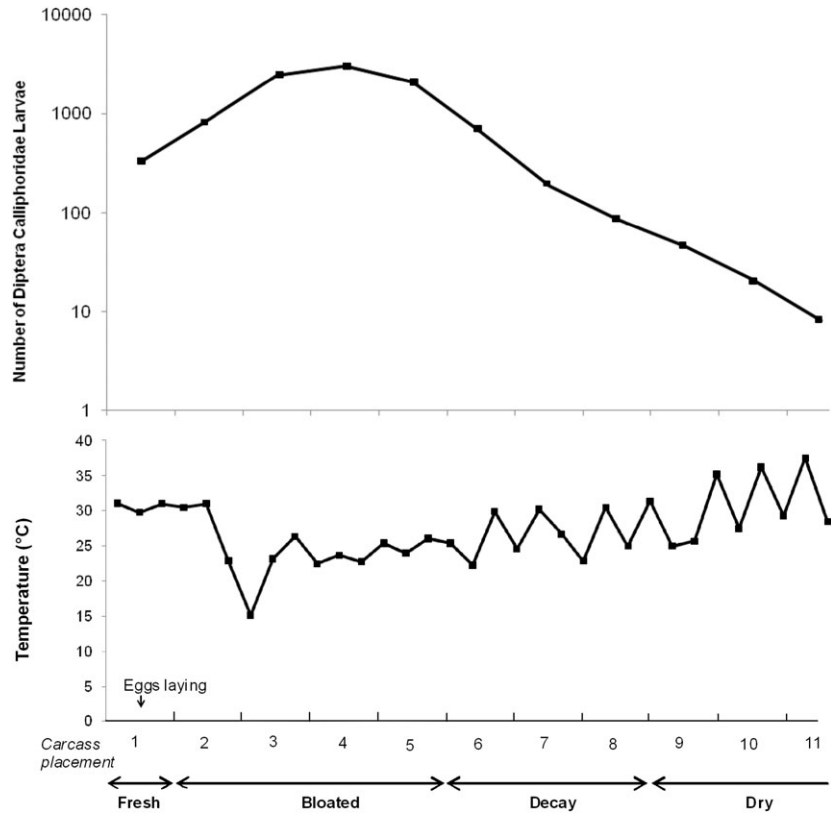


Figure 2 Relative abundance of Diptera Calliphoridae larvae on the pig carcass in comparison with the environmental temperature (collected near the carcass) and decomposition stages. The number of maggots on the carcass was estimated during daily observations. The larvae were collected on carcass by direct capture within of a square transect (10 cm wide).

but soon left it. *Tetramorium semilaeve* appeared to collect only ocular secretions and no injuries related to it were observed.

The predatory activity of *Crematogaster scutellaris* workers postponed the appearance of the fly egg masses, which were recorded about 27 h after carcass placement. In both series of observations, the ant workers abandoned the dead pig or its skins after about 48 h. As observed for *Tetramorium semilaeve*, none of the observed injuries were related to the activity of *Camponotus aethiops*.

The expected waves of sarcosaprophagous insects were observed during the 12 days of carcass decomposition despite the ant activities. Comparison of the ant worker dynamics on the carcass revealed that *T. semilaeve* was the first to invade the pig body lasting for one day, while *C. scutellaris* remained for two days. *Camponotus aethiops* was found on the carcass for a long time, from 72 h to 12 days after carcass placement. Thus, although *C. aethiops* explored the carcass for a long time, it was missing from the just-dead body.

According to the mean worker occurrence, the presence of *T. semilaeve* on the carcass could not be recognized once the workers had gone. Moreover, the collection of lachrymal secretion by this ant could be

related to T°C, RH%, wind and other very local factors. Therefore, we consider the presence of *T. semilaeve* as occasional. The presence of *C. aethiops* was only indirectly related to the tissue degradation process, as it is a maggot predator. Hence, *C. aethiops* does not leave signs of its presence, although the maggot reduction can be easily related to the ant's presence on the carcass. Indeed, *C. scutellaris* behaves as an active sarcosaprophagous insect involved in carcass body degradation during the fresh stage. Moreover, the occurrence of *C. scutellaris* can be referred to a particular phase and time of body degradation, and the damage can occur in a very short period of time. The injuries caused by *C. scutellaris* give a cue to detect ant-related Diptera egg laying disruption, as reported by Byrd and Castner (2009) for *Crematogaster lineolata* which was observed to feed on fly eggs and larvae. *C. scutellaris* is a tree-nesting species involved in homopteran tending, usually found in agricultural ecosystems and often in relation to scale insect or aphid infestation. It is more rarely found in houses or urban areas.

The results of our study in Calabria (southern Italy) indicate that both seasonal and environmental factors are very important in the process of carcass colonization, as reported in previous investigations in the same

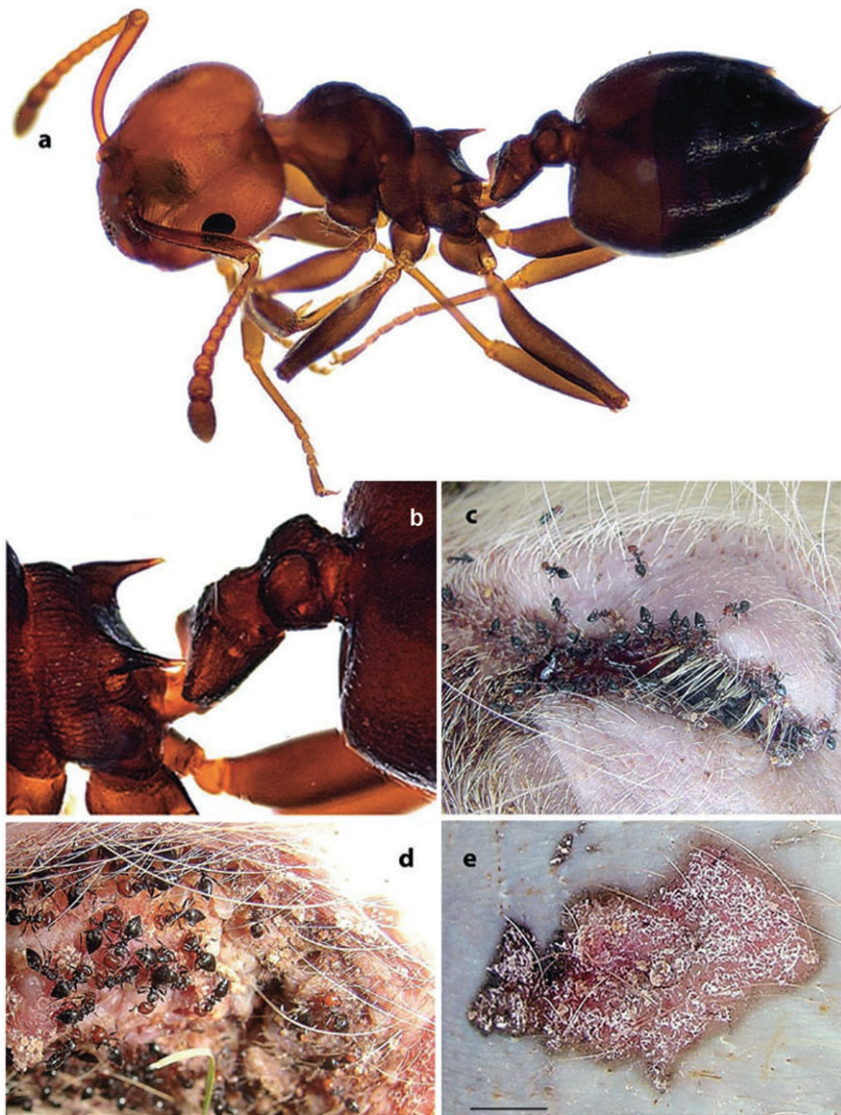


Figure 3 Workers of *Crematogaster scutellaris* (Olivier 1791) and injuries inflicted by them on the pig carcass: (a): worker, side view, alcohol preserved specimen (b): propodeum, petiole and part of the gastrum; (c) ants in and around the eye of *Sus scrofa*; (d); ants around the anus; (e); serpiginous injury on abdominal skin (scale bar, 1 cm).

area by Bonacci *et al.* (2010). The decomposition process in southern Italy varied with the season, being more rapid in summer than in winter (Bonacci *et al.* 2010). These data are in accordance with studies carried out in other geographical areas (Arnolds *et al.* 2001; Tabor *et al.* 2005). To estimate post-mortem intervals (PMI) in Calabria, Calliphoridae primary flies are the most important taxa, and knowledge of the time at which the post-feeding larvae migrate is essential. In summer, this time is 9 days and the species involved are *Lucilia* spp. and *C. albiceps*. These data should be very useful in legal investigations in Calabria. The insect successional pattern is specific to the geographical area and environmental conditions in which a carcass occurs (Payne 1965). Because taxa can

vary among regions, it is important for precise estimation of the PMI to identify the forensically important insects specific to an area (Anderson 2000). In general, our data on the successional pattern on non-human carcasses can be used to estimate the PMI and the results can be applied to investigations involving human remains in Calabria. Another very important result of our investigation is the appearance of *C. scutellaris* as a primary carcass invader. This acrobat ant is an active sarcosaprophagous insect involved in: i) body degradation during the fresh stage, ii) Diptera egg laying disruption and iii) post-mortem injuries. From a medico-legal point of view, we believe that interaction between the forensic entomologist and the pathologist is always advisable to discriminate

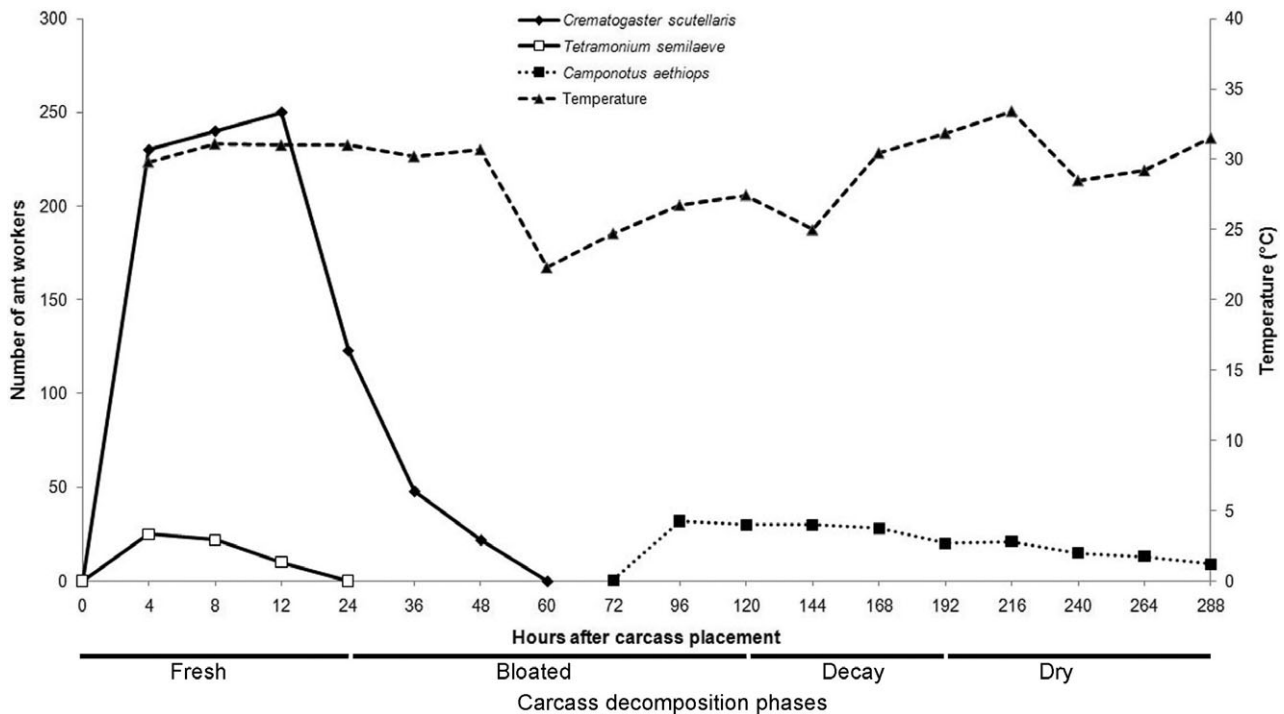


Figure 4 Occurrence of workers of ant species on the pig carcass (from pig carcass placement up to 288 h after) in comparison with the mean environmental temperature (collected near the carcass) and decomposition phases. The number of ants was estimated with the help of a square transect (5 cm wide) every 30 min after carcass placement by direct observations of the individuals on carcass.

post-mortem insect artifacts from ante-mortem and peri-mortem injuries, as reported in Campobasso and Introna (2001). In fact, artifacts produced by ants can be immediately apparent when the ants are identified on the body, but a final diagnosis can only be confirmed at autopsy by gross and microscopic analysis.

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