



Characterisation of the entomo-fauna from the mummies of Castelsardo (Sardinia, Italy) and considerations about the minimum number of individuals (MNI) in funerary archaeoentomology

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Abstract

During renovations within the crypt of the Sant'Antonio Abate Cathedral in Castelsardo (Sardinia, Italy), an ossuary and several mummies were discovered. Entomological remains were collected in two different surveys: one performed by archaeologists in 2011 and a second one conducted by an entomologist in 2018. In 2018, Giordani and colleagues published a paper presenting the results of the entomo-fauna analysis of samples collected during the first survey. The present paper presents the results of the second survey. Thousands of specimens have been newly collected and analyzed, including insects belonging to Coleoptera, Lepidoptera, Hymenoptera, Siphonaptera and Diptera, as well as arthropods belonging to Arachnida and Malacostraca. The results here presented confirm the Giordani's conclusion but also provide new information about the presence of potential vectors of diseases in the Castelsardo population in the end of the 18th—beginning nineteenth century.

Keywords Taphonomy · Decomposition · Mummification · Insects · Diptera · Coleoptera · Sardinia

Introduction

Necrophagous insects are specialized in consuming decaying organic matter: dead animals including humans. This trophic group follows a specific pattern of cadaver colonization depending on the biology and feeding preferences of each species (Gaudry 2010; Merrit and De Jong 2016; Smith 1986), thus forming distinct waves of body colonization. Founded at the end of the nineteenth century, forensic

entomology analyzes necrophagous insects collected from cadavers and/or crime scenes to provide useful information to support forensic investigations. Information such as the time of colonization corresponding to the minimum Post Mortem Interval (min PMI) (Higley and Haskell 2001), concealment and/or the postmortem transfer of the body from the primary to a secondary crime scene (Amendt et al. 2007) can be fundamental in leading to the resolution of a case.

More recently, a new bio-archaeological discipline—funerary archaeoentomology—has emerged from the entomo-forensic background. In 1996, Jean-Bernard Huchet officially defined it as the study of insects associated with ancient human remains, such as natural-anthropogenic mummies or graves (Huchet 1996). This discipline provides valuable information not only about thanatology but also about funerary practices (Huchet and Greenberg 2010), the season of death, social habits, and hygiene and health conditions of past populations (Tuccia et al. 2022; Vanin 2023; Vanin and Huchet 2017).

Given that insects' development is related to specific temperature ranges and habitats (Byrd and Butler 1998; Higley and Haskell 2001; Raspi and Antonelli 1995), the presence of certain species in archaeological sites may also help in the paleoecological and paleoclimatic reconstruction of past

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environments, and sometimes even to detect biodiversity changes throughout centuries (Giordani et al. 2018; Morrow et al. 2016; Nystrom et al. 2005; Tuccia et al. 2022).

Insects are also widely acknowledged as vectors of many disease agents, and they may preserve pathogens for a long time (Bain 2004). Pathogen analyses, primarily based on their DNA, can be used to reconstruct the health condition or the hygiene level of a population. At times, this becomes the sole scientific approach to establish the cause of death during significant historical events (Raoult et al. 2006; Barbieri et al. 2021). It is worth mentioning that this practice is commonly used currently in suspicious cases of human and animal diseases (eg. epidemic arbovirosis).

In archaeological contexts or old forensic cases, cadavers can undergo skeletonization or mummification depending on environmental conditions. However, traces of insects' colonization can still be detected due to the unique alterations left by insect activity on the body (Viero et al. 2019) and the presence of Coleoptera fragments, Diptera puparia, Lepidoptera exuviae and cocoons (Giordani et al. 2018; Pradelli et al. 2019; Vanin and Huchet 2017). The presence of fragments of the above mentioned taxa arises from the exceptionally hard exoskeleton of Coleoptera, providing significant resistance to decay, and the external chitinous structure of dipterous puparia, which tend to be recorded in substantial numbers in protected environments, such as coffins, tombs, and crypts (Huchet 2013; Merritt et al. 2007; Panagiotakopulu 2001, 2004; Panagiotakopulu and Buckland 2012; Pradelli et al. 2019; Vanin and Huchet 2017; Vanin et al. 2021).

During renovations within the crypt of the Sant'Antonio Abate Cathedral in Castelsardo (Sardinia, Italy), an ossuary and several natural mummies were discovered. Archaeoentomological analysis was performed to reconstruct the depositional patterns of the corpses inside the crypt (Giordani et al. 2018) as a part of a multidisciplinary investigation of the site

that included classic archaeology, anthropology, paleopathology and other disciplines (DeLaconi et al. 2014; Mazzarello et al. 2014). Indeed, only a multidisciplinary and integrated approach can provide the correct elements to reconstruct the whole scenario. Entomological samples were collected in two different surveys, one performed by archaeologists in 2011, and a second one conducted by an entomologist (SV) in 2018. The initial entomo-fauna analysis, based on the sampling performed by archaeologists, was published by Giordani and colleagues, and yielded interesting results (Giordani et al. 2018). In that context, only a few specimens were sampled and analyzed. Despite the extraordinary finding of a species, *Phormia regina* Meigen, 1826 (Diptera, Calliphoridae), no longer reported from Sardinia, the list of insects of funerary archaeological interest appeared to be incomplete. Therefore, a second sampling from the mummies was conducted in 2018 to address this limitation.

In this paper, results drawn from entomological material collected during the second survey are reported and discussed. Additionally, a comparison between the two surveys is provided, critically examining the Minimum Number of Individuals (MNI), a common quantification unit in archaeozoological analyses. The Castelsardo case is a good example of how the sampling method can dramatically influence the calculation of the MNI.

Historical context

The Sant'Antonio Abate Cathedral is a Romanic church in Castelsardo, a town located on the Asinara Gulf on the Northern coast of Sardinia, Italy (Fig. 1).

The church was elevated to the status of a cathedral in 1503 AD and underwent a complete reconstruction led by Bishop Juan Sanna Porcu between 1587 and 1599.

In 2011, as part of the "Restauro della Cattedrale e delle cripte sottostanti" project (Restoration of the Cathedral and the



Fig. 1 Mediterranean, Italian and Sardinia Map. The black dot shows the location of Castelsardo. (maps used for the plate modified from: https://d-maps.com/carte.php?num_car=3130&lang=en; [\[maps.com/carte.php?num_car=4828&lang=en\]\(https://d-maps.com/carte.php?num_car=4828&lang=en\) and \[https://d-maps.com/carte.php?num_car=22838&lang=en\]\(https://d-maps.com/carte.php?num_car=22838&lang=en\)\)](https://d-</p>
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underlying crypts), excavation of the crypts was conducted. This excavation revealed human remains, including bones and several partially mummified bodies, scattered within a section known as "room 3". To establish the sequence of deposition of these bodies, a multidisciplinary approach involving archaeological, geological, entomological, and historical analyses was employed (Delaconi et al. 2014; Mazzarello et al. 2014). The entomological analysis focused primarily on two individuals, referred to as "Bob and Mary," believed to be the most recent interments.

These two bodies, displaying partial mummification and partial skeletonization, were found at the top of the excavation near the entrance of the crypt. All burials within the Cathedral were dated to have occurred between the beginning and the end of the eighteenth century. Following this period, Napoleon's Saint Cloud Edict prohibited burials within cities, villages, and buildings.

Material and methods

During the second survey in 2018, an entomologist (SV) conducted insect collections from the same human individuals analyzed in the first survey, as well as from their clothing. The collection process utilized tweezers, paintbrushes, and an electric suction system. The collection and the study of the archaeological material from the Castelsardo crypts was authorized by the Ufficio Culturale — Diocesi di Tempio Ampurias in March 2011.

Before microscopic observation, the collected specimens underwent various cleaning methods, including sonication and a warm water and soap solution, to eliminate external substances that obscured the diagnostic features necessary for identification (Pradelli et al. 2021; Vanin et al. 2023). Subsequently, the samples were identified using specific keys (Giordani et al. 2019a; Skidmore 1985; Smith 1986) and were compared with an existing reference collection of identified specimens. Furthermore, certain specimens were identified by specialized taxonomists (Lepidoptera; Siphonaptera; Hymenoptera Formicidae; Diptera Dolichopodidae; Coleoptera Histeridae, Tenebrionidae, Curculionidae; Pseudoscorpionida; as detailed in the Acknowledgments section.

Microphotographs were captured using a Keyence VHX-S90BE digital microscope, outfitted with Keyence VH-Z250R and VH-Z20R lenses and VHX-2000 Ver.2.2.3.2 software (Keyence).

Results and discussion

Dry fragmented insects belonging to the orders Coleoptera, Siphonaptera, Diptera, Lepidoptera and Hymenoptera were identified among the collected material (Table 1). In addition, other arthropods such as Arachnida and Malacostraca were also found (Table 2).

Coleoptera

Among Coleoptera, specimens belonging to the family Histeridae, Dermestidae, Ptinidae, Cleridae, Tenebrionidae and Curculionidae were collected (Fig. 2, 3 and 4).

Only a single species of Cleridae was identified: *Necrobia violacea*, commonly referred to as the black-legged ham beetle. This beetle exhibits a dark blue coloration, including its legs and features black antennae (Fig. 2A,B). It is known to infest carrion during the advanced stages of decomposition and is frequently found in Italian archaeological burial sites (Mattia et al. 2022; Vanin et al. 2020).

Ptinidae (Fig. 2C-F), which are commonly known as spider beetles and death-watch beetles, have recently undergone a hierarchical re-classification (Marshall 2018). Two previously distinct families, Ptinidae and Anobiidae, are now considered a single family. During the 2018 survey, one species from the subfamily Anobiinae was collected: *Anobium punctatum*, commonly known as the common furniture beetle or common house borer. Additionally, a species from the genus *Ptinus* Linnaeus, 1766 was also identified from the remains.

Gibbium psylloides is a wingless species, it is cosmopolitan, favouring dry environments, typically develops on plant-based material mostly wood, often found in houses and archaeological contexts. While most species in this family are wood-boring, a few are detritivores, scavengers, or pests of stored products (Hinton 1945).

Curculionidae (Fig. 2G,H), commonly known as weevils (Lawrence and Newton 1982), encompass a wide variety of sizes and shapes and are distributed worldwide. Their notable elongated snouts are adapted for penetrating plants for feeding or preparing a hole for egg-laying (Crowson 1981). The majority of weevil species inhabit plant material, with some specialized in consuming fungi and decaying plant matter (Marshall 2018). Curculionidae are also recognized as significant pests of food, fibers and wood. Among the wood-boring weevils, *Pentarthrum huttoni* Wollaston, 1854 has been identified in coffins within the crypt of St. Michael's Church in Vienna, Austria. The extensive tunnelling by larvae and surface damage by adult weevils led to the degradation of historically important wooden coffins from the eighteenth century (Halmschlager et al. 2007). The species collected in Castelsardo, *Amaurorhinus sardous*, is native to Sardinia and represents the first recorded instance of its presence in cultural heritage materials. Another species from the same genus, *Amaurorhinus bewickianus* (Wollaston, 1860), has been reported in the wooden choir of Santa Maria di Castello Church in Genova (Liotta 2015). Nevertheless, due to the fact that *Amaurorhinus* species can colonize wood of varying ages, it is difficult to form hypotheses about their timing of arrival on the coffins of Bob and Mary or on any wood present in the crypt.

Table 1 Insects collected during the second entomological survey in 2018 from the partially mummified remains of “Bob and Mary” buried in the crypt of the church of Castelsardo (Sardinia, Italy)

Order	Family	Species	
Coleoptera	Histeridae	<i>Saprinus subnitescens</i> Bickhardt, 1909	
		<i>Gnathoncus communis</i> (Marseul, 1862)	
		<i>Gnathoncus rotundatus</i> (Kugelann, 1792)	
	Dermestidae	<i>Dermestes</i> sp.	
		<i>Anthrenus</i> cf. <i>caucasicus</i>	
	Ptinidae	<i>Anobium punctatum</i> De Geer, 1774	
		<i>Ptinus</i> sp.	
		<i>Gibbium psylloides</i> Czenpiński, 1778	
	Siphonaptera	Cleridae	<i>Necrobia violacea</i> (Linnaeus, 1758)
		Tenebrionidae	<i>Akis bacarozzo</i> (Schrank 1786)
<i>Blaps mucronate</i> Latreille, 1804			
<i>Alphitobius diaperinus</i> Panzer 1797			
Curculionidae		<i>Amaurorhinus sardous</i> Folwaczny, 1972	
Ceratophyllidae		<i>Nosopsyllus</i> cf. <i>fasciatus</i> (Bosc d'Antic, 1800)	
Pulicidae		<i>Echidnophaga</i> cf. <i>murina</i> (Tiraboschi, 1903)	
		<i>Pulex irritans</i> Linnaeus, 1758	
Diptera		Leptosyllidae	<i>Leptosylla segnis</i> (Schonherr, 1811)
		Dolichopodidae	<i>Medetera</i> sp.
	Phoridae	Gen. sp.	
	Fanniidae	<i>Fannia</i> sp.	
	Muscidae	<i>Hydrotaea capensis</i> (Wiedemann, 1818)	
		<i>Musca domestica</i> Linnaeus, 1758	
	Calliphoridae	<i>Calliphora vicina</i> Robineau-Desvoidy, 1830	
		<i>Phormia regina</i> Meigen, 1826	
		<i>Chrysomya albiceps</i> (Wiedemann, 1819)	
	Lepidoptera	Sarcophagidae	<i>Sarcophaga</i> spp.
Pyralidae and Tineidae		Gen. spp.	
Hymenoptera	Braconidae	subfamily Braconinae	
	Formicidae	<i>Messor ibericus</i> Santschi, 1931	
		<i>Tetramorium meridionale</i> Emery, 1870	

Specimens from the genera *Anthrenus* Geoffroy, 1762 and *Dermestes* in the family Dermestidae have been identified from the remains of “Bob and Mary” (Fig. 3A-D). The family Dermestidae comprises various species such as skin or hide beetles, carpet beetles and larder beetles. Adult beetles in this family possess elytra covered with hairs and distinct bare clubbed antennae. Many of them feed on animal-derived products, including leather, fur, hair, skin, wool and even dairy items (Merritt and De Jong 2016). Because of their feeding habits, they are frequently employed in taxidermy and by natural history museums for cleaning animal skeletons (Pahl 2020) and also in forensic contexts for cleaning human bones (Charabidze et al. 2014). However, they can pose significant threats to museums by causing damage to entire insect and osteological/anthropological collections (Vanin et al. 2021). Species belonging to the genus *Dermestes* Linnaeus, 1758 are found in various habitats where human or animal cadavers or other food sources such as dried

meat are present (Peacock 1993). They generally appear later in the decomposition process, particularly when the cadaver starts to desiccate (Grassberger and Frank 2004).

Tenebrionidae (Fig. 4A-H) are commonly referred to as darkling beetles. This family has a global distribution and exhibits considerable variability in size and color. Many species are adapted to arid environments. In Castelsardo, three species of Tenebrionidae were collected and identified: *Blaps mucronata* (Fig. 4A-D), *Akis bacarozzo* (Fig. 4E,F) and *Alphitobius diaperinus* (Fig. 4G,H). The genus *Blaps* Fabricius 1775 is known to favor enclosed anthropic environments, while *A. diaperinus*, commonly known as the lesser mealworm, is a pest of stored food grains and products like flour. In Southern Italy, it has been found on a human cadaver in an advanced state of decay during the summer period (Vanin, unpublished data).

Three distinct species within the Histeridae family were also collected: *Gnathoncus rotundatus*, *Gnathoncus*

Table 2 Other arthropods collected during the second entomological survey in 2018 from the partially mummified remains of “Bob and Mary” buried in the crypt of the church of Castelsardo (Sardinia, Italy)

Class	Order	Family	Species
Aracnida	Pseudoscorpionida	Cheiridiidae	<i>Cheridium museorum</i> (Leach, 1817)
		Chernetidae	<i>Pselaphocheernes laceratosus</i> (L. Koch, 1873) <i>Allocheernes powelli</i> (Kew, 1916) <i>Allocheernes wideri phaleratus</i> (Simon, 1879)
	Araneae	Gen. sp.	
	Acarina	Gen. sp.	
Malacostraca	Isopoda	Porcellionidae	Gen. sp.

communis and *Saprinus subnitescens*. Histeridae are recognized as predators, as they frequently prey on the eggs, larvae and adults of other insects. They can also be found on dung, animal cadavers and decomposing vegetation (Kulshrestha and Satpathy 2001; Merritt and De Jong 2016; Vienna 1980). Most of these species prefer arid, decaying habitats, with cadaver-feeding species mainly targeting dipteran and dermestid larvae. Adult histerids lay their eggs on corpses in later stages of decay. Their prevalence is typically higher during spring and summer. These species have previously been collected in Italy from human or animal cadavers (Vanin, unpublished data).

Siphonaptera

Siphonaptera, commonly known as fleas, are hematophagous ectoparasites of mammals and birds (Whitaker 2007). Like other bloodsucking insects, they play a significant role in disease agent transmission. Four different flea species were isolated from the bodies of “Bob and Mary”: *Nosopsyllus* cf. *fasciatus* (northern rat flea), *Echidnophaga murina*, *Leptopsylla segnis* (European mouse flea) and *Pulex irritans* (human flea).

Nosopsyllus cf. *fasciatus* is a flea measuring 3–4 mm that may serve as a minor vector for the black plague etiological

Fig. 2 A–B Cleridae, *Necrobia violacea*; C–D Ptinidae, *Ptinus* sp.; E–F Ptinidae, *Anobium punctatum*; G–H *Amaurorhinus sardous* (scale bar: 500 µm)

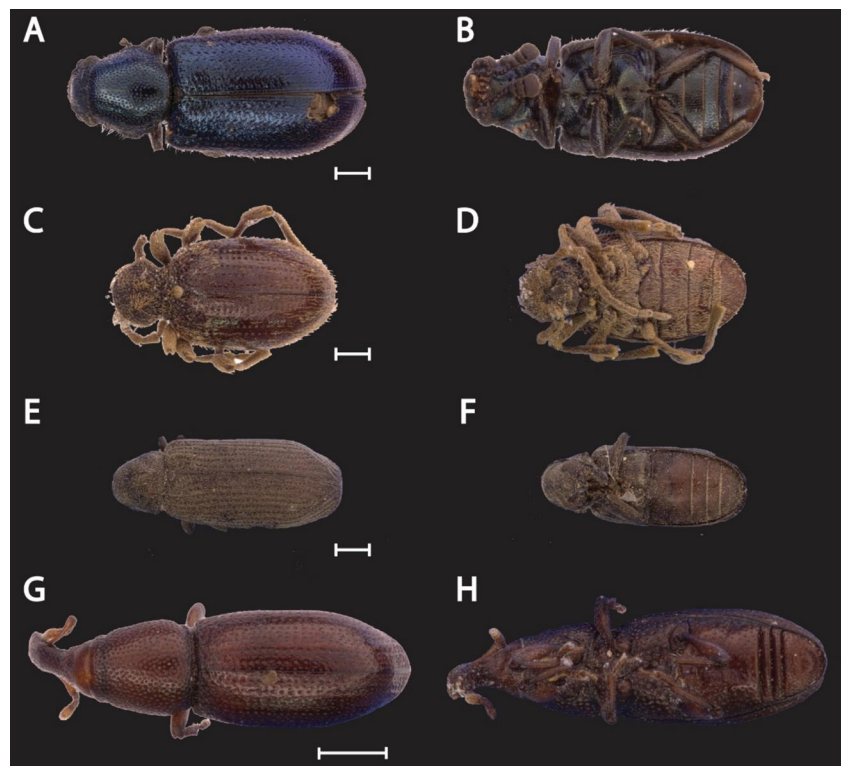


Fig. 3 Dermestidae. **A–B** *Anthrenus* cf. *caucasicus*, **C–D** *Dermestes* sp. (scale bar: 500 μ m)

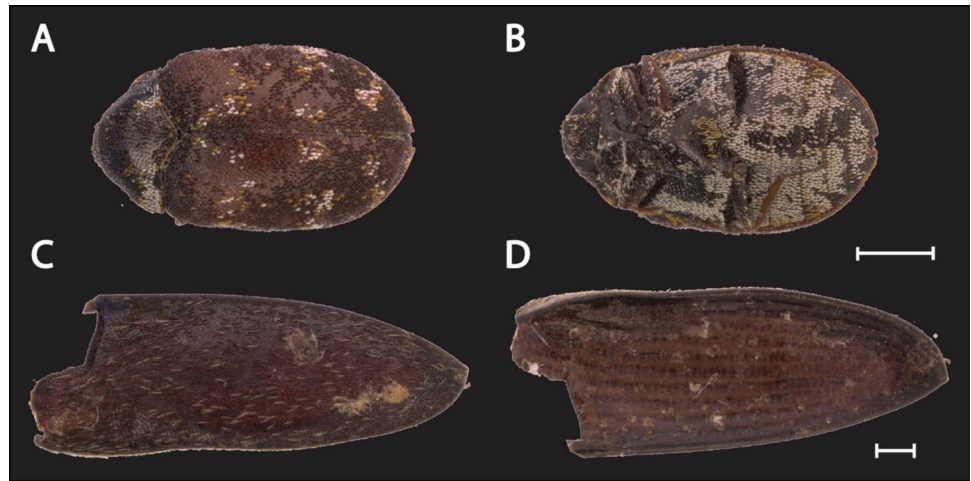
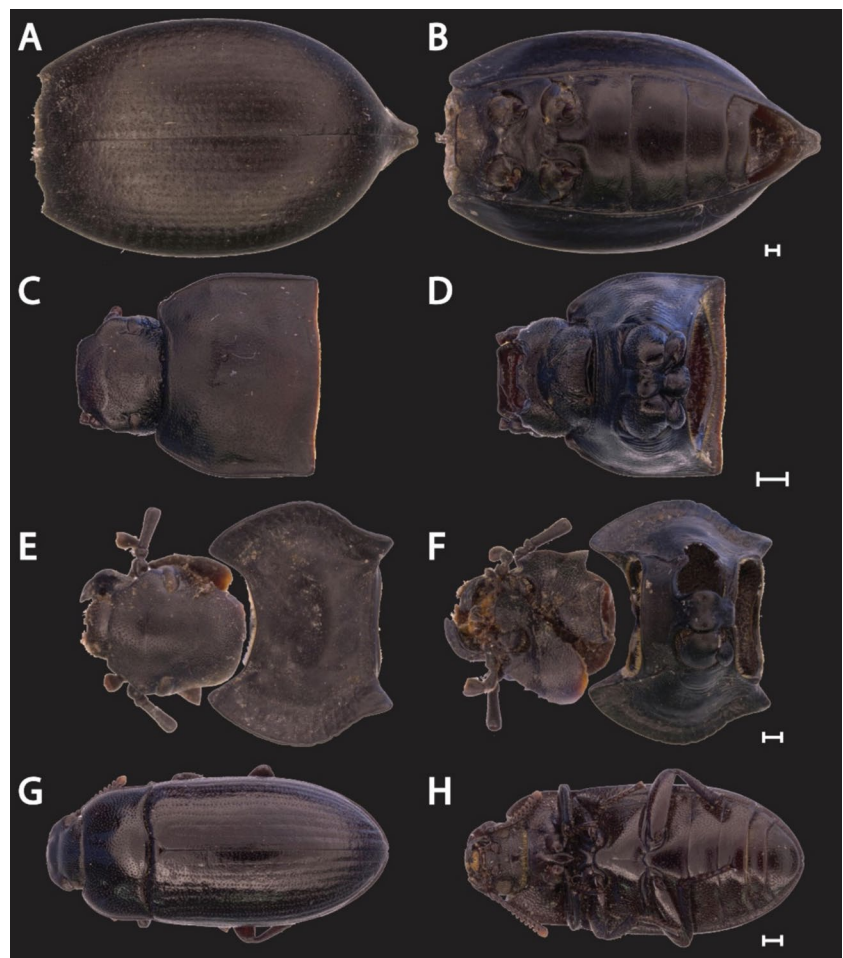


Fig. 4 Tenebrionidae. **A–D** *Blaps mucronata*; **E–F** *Akis bacarozzo*; **G–H** *Alphitobius diaperinus* (scale bar: 500 μ m)



agent (Eskey et al. 1949; Bitam et al. 2010) and is originally from Europe but has since spread worldwide (Chick & Martin 1911). Although it is commonly associated with rats, it is improbable that *E. murina* can transmit Rickettsiae, the causative agents of typhus, to humans (Traub & Wisseman 1978; Mumcuoglu et al. 2001). The mouse flea *L. segnis*

is recognized as a vector for murine typhus pathogen (Azad & Traub 1987). This globally distributed species has previously been reported in Sardinia (Whitaker 2007) (<http://www.faunaitalia.it/checklist/introduction.html>). Humans are generally viewed as secondary hosts for *P. irritans*, while its primary hosts include various animals such as pigs (*Sus*

scrofa Linnaeus, 1758), dogs (*Canis familiaris* Linnaeus, 1758), and cats (*Felis catus* Linnaeus, 1758). This species serves as a vector for several etiological agents of human diseases, including *Yersinia pestis* (Lehmann & Neumann, 1896) van Loghem, 1944, the agent responsible for the black plague, *Rickettsia typhi* (Wolbach & Todd, 1920) Philip, 1943, which causes murine typhus, and *Rickettsia felis* Bouyer et al., 2001 emend. La Scola et al., 2002, the agent linked to flea-borne spotted rickettsiosis (Azad 1990; Azad et al. 1997; Ruiz 2001; Brouqui & Raoult 2006).

The discovery of several fleas on bodies, along with preliminary studies conducted on the sediments of the crypt by the University of Sassari (unpublished), may help explain hypotheses regarding the causes of death for those interred in the crypt. Molecular investigations are planned on the isolated specimens to clarify this point.

Diptera

Fragmented remains of adults belonging to the long-legged flies (Dolichopodidae) were prevalent among the examined materials (Fig. 7).

Dolichopodidae flies have a global distribution and usually prefer shaded environments (Marshall 2012). Adult Dolichopodidae are predators of immature stages of other Diptera as well as springtails (Collembola). The larvae of Dolichopodidae are predatory feeders in soil (Bickel and Dyte 1989). A single species belonging to *Medetera* Fischer von Waldheim, 1819 was found in the Castelsardo crypt. However, due to their poor preservation, it was not feasible to identify them to a lower taxonomic level. Observations conducted by one of the authors (SV) in other Sardinian churches (e.g., Alghero, Sassari) revealed the presence of adults of the same genus. The microclimatic conditions inside the churches, especially during warmer seasons, could be favorable for the survival of *Medetera*.

Puparia belonging to the Calliphoridae (blow flies), Sarcophagidae (flesh flies), Muscidae (house flies), Fanniidae (lesser house flies), and Phoridae (coffin or cuttle flies) were collected and analyzed.

Calliphoridae, along with certain Muscidae and Sarcophagidae, commonly colonize human remains during the early stages of decomposition (Merritt and De Jong 2016). Among Calliphoridae, specimens of *Calliphora vicina*, *Phormia regina* and *Chrysomya albiceps* were isolated from the Castelsardo sample.

Muscidae larvae inhabit diverse habitats, from dung and decaying plant matter to carrion and fungi. Some species are parasites of other insects and animals, while others, like *Musca domestica*, act as vectors for human diseases (e.g., typhoid fever, cholera, dysentery, etc.) (Skidmore 1985). *Hydrotaea capensis* is a synanthropic species of forensic

and archaeological significance. It colonizes exposed bodies during advanced stages of decomposition and is one of the primary colonizers of concealed and buried bodies. This species has been reported from various archaeological contexts associated with mummies and skeletonized bodies (Giordani et al. 2019a; Huchet 2013; Loni et al. 2019; Pradelli et al. 2019; Vanin et al. 2022).

Fanniidae exhibit a wide range of feeding habits: some species are saprophagous, others are coprophagous, some feed on stored products, and a few are mycophagous, often associated with decaying matter or excrements. Immature stages of Fanniidae develop in moist environments (Marshall 2012) and are found on cadavers during the butyric fermentation phase, also known as the body liquefaction stage. *Fannia scalaris* (Fabricius, 1794), commonly known as the latrine fly, and *Fannia canicularis* (Linnaeus, 1761), known as the lesser house fly, are the most common Fanniidae species encountered during forensic entomological investigations (Frost et al. 2009; Grzywacz 2019; Matuszewski et al. 2010; Merritt and De Jong 2016; Reibe-Pal et al. 2008). Moreover, both species have been known to cause myiasis in the urethra and digestive tract of living humans (Aydenizoz and Gokpınar 2020; Perez-Eid and Mouffok 1999).

The Phoridae comprises more than 4,000 described species, with several species still unnamed, making it one of the most morphologically diverse Diptera families. They have adapted to nearly every environment, employing various strategies. Some species in the genera *Megaselia* Rondani, 1856 and *Conicera* Meigen 1830, known as coffin flies, are often found around decaying vegetation or animal matter. They can also be parasitic on different invertebrates. Species from these genera have been observed burrowing from 60 cm to 1.8 m into the soil to reach buried bodies (Disney 2005; Merritt et al. 2007) and are frequently found in enclosed environments in archaeological and forensic contexts. *Megaselia scalaris* (Loew, 1866) and *Conicera tibialis* Schmitz, 1925 are the most prevalent and significant species, especially in older cases and indoor investigations (Bourel et al. 2004; Bugelli et al. 2015; Campobasso et al. 2004; Manlove and Disney 2008; Mukherjee et al. 2019; Reibe and Madea 2010). Other genera that have been recorded on human and animal cadavers include *Phora* Latreille, 1796, *Dohrniphora* Dahl, 1898 and *Triphleba* Rondani, 1856 (Smith 1986; Vanin, data unpublished). However, due to the extensive variety within the Phoridae family, identifying these genera can be challenging. In particular, the immature stages such as larvae and puparia, which are the most commonly recovered insect fragments at crime scenes and archaeological sites (Bugelli et al. 2015; Merritt et al. 2007; Mukherjee et al. 2019; Reibe and Madea 2010), lack proper identification keys and morphological descriptions (Pradelli et al. 2021). This issue extends to other Diptera families as well. Presently, taxonomists and researchers are actively

working to address this gap by describing more immature stages to develop identification keys (Giordani et al. 2019a, 2019b, 2023; Giordani and Vanin 2020).

Lepidoptera

Moth cocoons, potentially belonging to the Tineidae, were abundant in the examined material. Some Tineidae associated with dead bodies or with houses are commonly known as clothes moths. Additionally, the heads of immature moth stages from the Pyralidae were also identified. Both of these moth families are associated with human bodies. Species from the Tineidae and Pyralidae families are known to inhabit cadavers and can contribute to the biodegradation of textiles and other human-made materials (Introna et al. 2021; Kadej et al. 2020; Loni et al. 2019; Matuszewski and Mađra-Bielewicz 2019). These families are commonly involved in colonizing human and animal remains during the dry phases of decomposition and when bodies are mummified or skeletonized.

Hymenoptera

Specimens belonging to the families Braconidae (Fig. 5) and Formicidae (ants) (Fig. 6) were isolated from the Castelsardo samples. Braconidae species are parasitoids of Coleoptera and Lepidoptera larvae. They can also parasitize larvae of Diptera or other wasps (Matthews 1974). Additionally, two species of ants were identified in Castelsardo: *Tetramorium meridionale* (Fig. 7) and *Messor ibericus*. Ants have been observed as predators of dipteran



Fig. 5 Wasp belonging to Braconidae family, subfamily Braconinae (scale bar: 500 μ m)



Fig. 6 Formicidae *Tetramorium meridionale* (scale bar: 500 μ m)

larvae as well as scavengers of cadavers, which can lead to specific alterations in cadaver skin (Bugelli et al. 2015; Campobasso et al. 2009; Merritt and De Jong 2016; Viero et al. 2019).

During the sampling of Bob and Mary's bodies, in addition to insects, other arthropods were also collected as reported in Table 2.

Pseudoscorpionida

Pseudoscorpionida are small arachnids commonly known as false scorpions. Their bodies range from 2 to 12 mm in length, and they have a flat, pear-shaped body with palpal



Fig. 7 Dolichopodidae. *Medetera* sp. (scale bar: 100 μ m)

chelae that resemble those of real scorpions. They are predators of moth and beetle larvae, ants, mites, and small flies. Some species are arboreal, others live in debris and humus, some engage in phoresis with different species, and others inhabit caves and seashores (Del-Claro and Tizo-Pedroso 2009). Pseudoscorpions possess a venom gland in their pedipalps, which they use during predation to immobilize their prey. Currently, around 3,500 species have been described worldwide. *Cheridium museorum* (Fig. 8) is a pseudoscorpion species commonly found in old dusty libraries, where books are not properly handled; they feed on book mites (Cowles 2018). On the other hand, *Pselaphochernes laceratosus* is a phoretic species (Christophoryová and Jablonski 2017; Ricarte et al. 2016). The behavior and habitat of the genus *Allochernes* Beier, 1932 are not yet well-described, but both species collected during the survey have been previously found in caves, forests, and bird nests (Christophoryová et al. 2011a, 2011b; Mahnert 1989).

Acarina

Like pseudoscorpions, Acarina is another taxon within the Arachnida class with more than 50,000 described species worldwide. Highly diverse, they have colonized nearly every habitat on the planet, including aquatic ones (Hoy 2009; Hughes 1959). They are abundant in soil organic matter; some are plant feeders, detritivores, parasites and a few are predators (Pérez-Martínez et al. 2019). Their significance within ecosystems is undeniable, as they are essential decomposers in nature. Many are associated with decaying organic matter and have the potential to serve as medicolegal indicators in forensic investigations, a field referred to as forensic acarology (Kamaruzaman et al. 2018; OConnor 2009; Perotti et al. 2009b). The group of Acarina species of forensic interest comprises phoretic mites from the families Macrochelidae, Parasitidae,

Uropodidae, and Pachylaelapidae (Merritt and De Jong 2016; Perotti et al. 2009a). Furthermore, over the past 50 years, mites frequently recovered from coprolites have also been utilized to interpret archaeozoological contexts, as many species solely survive on specific hosts (plants or animals) within restricted habitats (Baker 2009; Schelvis 1989).

Conclusion

The entomofauna associated with the bodies provides insights into the initial colonization of an open, exposed context, likely during a warm season or in the transition between summer and autumn, as indicated by the presence of species like *Ph. regina*, *Sarcophaga* spp. and *Ch. albiceps*. These three species have a prevalent warmer season phenology in the Italian continental regions with some record also in Autumn. *Chrysomya albiceps* was recorded by Rognes (2011) in Sardinia from July till October.

Conversely, the recovery of *Calliphora vicina* suggests colonization during a cooler season, which might have occurred in both exposed and concealed conditions, such as within the crypt.

Calliphora vicina is reported from Sardinia from May to December, but the summer records correspond to location above 500 m asl (Rognes 2011). The interpretation of the co-presence of these species doesn't exclude the possibility of overlapping periods (potentially autumn) when adults of these species could have laid their eggs on exposed bodies. Moreover, considering the presence of other burials within the same crypt, the potential for cross-contamination cannot be disregarded. This interpretation aligns with the conclusions drawn by Giordani et al. (2018) after analyzing the sample collected in 2011. Among the newly collected entomological material, the discovery of fleas (Siphonaptera) holds significance due to their role as vectors of human disease agents, although

Fig. 8 Pseudoscorpionida *Cheridium museorum* (scale bar: 100 μ m)

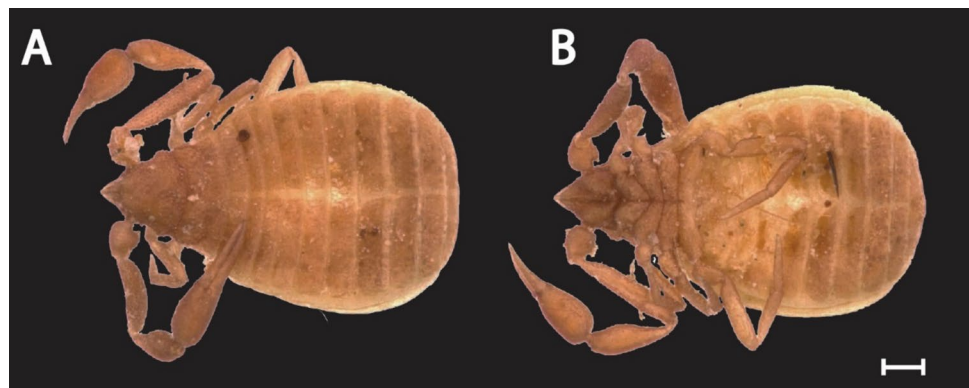


Table 3 Comparison between the MNIs of the 2011 and 2018 survey per taxon

Taxon	MNI (2011)	MNI (2018)
Coleoptera	2	785
Histeridae	1	43
Dermestidae	-	21
Ptinidae	-	151
Cleridae	-	402
Tenebrionidae	1	71
Curculionidae	-	97
Siphonaptera	-	36
Diptera	85	7593
Dolichopodidae (<i>Medetera</i>)	-	219
Phoridae	-	1
Fanniidae (<i>Fannia</i>)	-	5
Muscidae (<i>Hydrotaea</i>)	40	7332
Muscidae (<i>Musca</i>)	-	1
Calliphoridae (<i>Calliphora</i>)	31	19
Calliphoridae (<i>Phormia</i>)	12	2
Calliphoridae (<i>Chrysomya</i>)	-	2
Sarcophagidae	2	12
Lepidoptera	2	342
Tineidae	2	338
Pyralidae	-	4
Hymenoptera	-	56
Formicidae	-	52
Braconida	-	4
Arachnida	-	> 1000
Pseudoscorpionida	-	22
Aranae	-	11
Acarina	-	> 1000

further molecular studies would be required to confirm the transmission of pathogens.

The Coleoptera and other arthropods collected during the second survey provide a more intricate understanding of the unique environment within the crypt. The coleopteran fauna is composed of several synanthropic species associated with underground environments, the presence of wood, organic matter of animal origin, and bird excrement. Some of these species can feed on both stored food products and human or animal cadavers. In an urban environment like Castelsardo in the eighteenth century, these species had a wide variety of potential food sources, and the proximity of the crypt to the houses could facilitate the presence of these taxa on the bodies as well. Ongoing research on each species aims to uncover a deeper comprehension of faunal interactions within this enclosed

synanthropic subterranean microhabitat, where decomposition processes have extended over centuries.

Castelsardo illustrates a significant difference in biodiversity (number of species) and specimen counts between the two collections (2011 and 2018) (Table 3), which can be attributed to the collector's expertise. The initial survey was conducted by archaeologists, whereas the 2018 collection was carried out by a trained entomologist with experience in archaeological sampling. This bias in recovery significantly impacted the MNI calculation. Consequently, comparing sites analyzed by different operators/collectors lacks reliability. However, the interpretation of a single site remains valid and can be considered credible without calculating the MNI.

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Declarations

Competing interest The authors declare no competing interests.

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