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From the field to the microscope: funerary archaeoentomology workflow

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Abstract. Funerary archaeoentomology is a recent bioarchaeological discipline. It shares the same bulk of knowledge of forensic entomology, which is the science of analysing entomological evidence (insects and other arthropods) as an aid to legal investigations. In archaeo-funerary contexts insects can provide information to describe the funerary practices but also to understand the taphonomic processes involving human and other animal bodies. The discipline was founded by Jean-Bernard Huchet in 1996. In this paper a workflow in seven main steps - documentation, collection on site, insect-sediment separation, specimens cleaning, mounting, labelling and identification - is suggested in order to maximize the information that can be derived from insect samples collected from archaeo-funerary sites.

Key words: insects, entomology, specimen preparation, funerary archaeology, protocol

Introduction

Insects represent about the 75% of the known animals with more than 1,300,000 described species. Due to their high number, worldwide distribution, high rate of reproduction and elevate adaptability insects are quite common in all the environments of our planet. Insects are also associated with anthropic environments where they can found microhabitats similar to their natural habitats and benefit from their facultative or obligate association with humans (King, 2014).

In 1978, Kenward highlighted the importance of the study of insects from archaeological contexts where they can provide additional information to better reconstruct past environmental and climatic conditions, landscape usage and cultural practices (Kenward, 1978).

Later, in 1996 the French researcher Jean-Bernard Huchet defined a new discipline – funerary archaeoentomology (Huchet, 1996). This discipline focus on insects and other arthropods from funerary contexts using the forensic entomology approach. As highlighted by Giordani et al. (2018) and Vanin (2023), the two disciplines have different aims, but they share the same techniques and methodologies.

It is important pointing out that the presence of the insect necrofauna in an archaeological context depends on two distinct modes of colonization – the pre- and the post- depositional phases (Vanin & Huchet, 2017). In addition, it is also strongly affected by the taphonomy processes affecting the insects *per se* (e.g.: beetles are more resistant than flies) (Panagiotakopulu, 2004), by the different vagility of the species and also by other phenomena affecting the context such as landfill, water percolation and the chemical transformation of the substratum. For these reasons the entomofauna collected after years or centuries from a context does not correspond to the initial fauna present on the body/ies. It is also worth mentioning that the excavation and the collection methods may introduce important biases especially when only a part of the specimens is collected due to their size or other

characteristic (color, shape, shining, location, etc.).

Despite the increasing interest in funerary archaeoentomology, as demonstrated by the specific literature (for a summary see: Tuccia et al., 2022; Magni et al., 2023; Vanin, 2023), there is still a lack of a formal workflow, best practices and standards to be used to maximize the information that insects and other arthropods could provide in such a context. This lack of protocols and guidelines can be due to the recent constitution of funerary archaeoentomology as a discipline and because of the high variety of contexts in which it can be used: coffins, burials, crypts, churches, etc. The aim of this paper is to provide a general workflow to properly document, collect and study the entomological evidence from an archaeo-funerary context.

Taking inspiration from the forensic field and from protocols commonly used in archaeology and in entomology the workflow here suggested is composed by seven steps: documentation, collection on site, insect-sediment separation, specimens cleaning, mounting, labelling and identification (Fig. 1). A last additional step "interpretation" will be presented and discussed in a further paper.

Documentation

The first step of any scientific investigation is the correct documentation of the context, the detailed description of the collection site and of the methods used in the field and in the lab work. This will help other colleagues facing the same conditions or addressing the same questions in different sites. In addition, the correct reporting of any information useful to relate the collected material with the context, the body/ies remains and any other element present *in loco* is vital to properly interpret the acquired data. Documentation can be done by sketching, photographing, videotaping but also by simply reporting and describing the site object of the archaeological investigation. Some forms (e.g.: Cecchi et al.,2022, the Italian version of this form is available at the link https://www. simlaweb.it/procedure-sopralluogo/), useful for documenting the location of insects from human and animal cadavers, have been designed by forensic entomologists, pathologists and anthropologists and, *mutatis mutandis,* they can also be used to documents entomological findings from a funerary archaeological context, especially when dealing with single bodies from confined spaces (e.g.: coffins) (Amendt et al., 2007; Cecchi et al., 2022).

The reporting of the mesh size used in the *in loco* sieving activity is important to understand if an involuntary exclusion of the entomofauna was performed during the excavation and/or during the first triage procedures. The exposure and the accessibility of the site and of the excavated material to meteorological phenomena but also to the free access to animals, both vertebrates and invertebrates, is also important to be reported in order to avoid wrong interpretations about the presence or the absence of certain species. For example, only the knowledge that a crypt, where some human mummies were stored, was also regularly inhabited by cats allowed a correct interpretation of

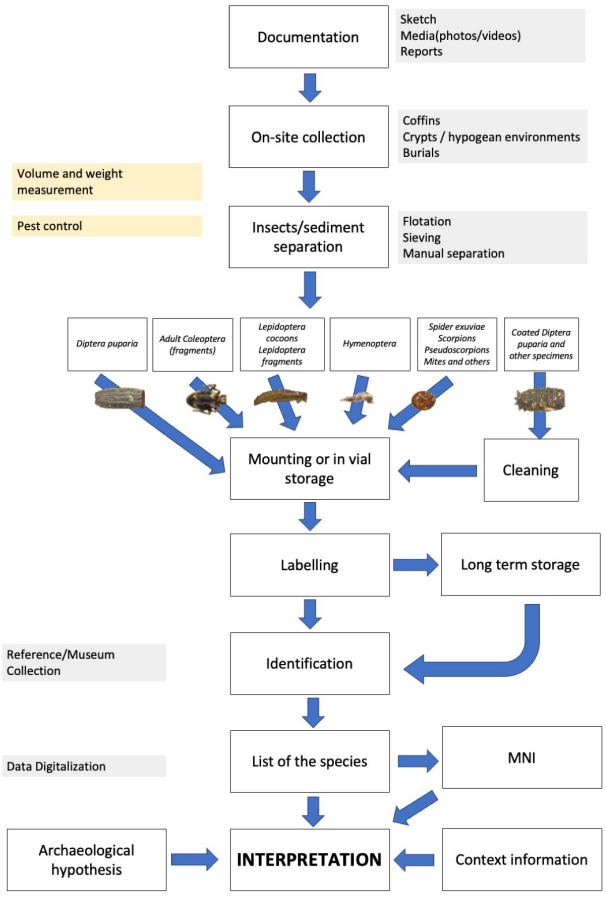

Figure 1. Schematic representation of the suggested workflow to maximize the information derived from the insect collection from an archaeo-funerary context.

the presence of some fleas on the clothes of the human bodies (Vanin, unpublished). The volume and the weight of the soil/sediment collected have to be recorded before the insect extraction. This information will be important for further analyses and interpretations.

On site insect collection

The onsite collection is, in most of the cases, the activity reserved to the archaeologists but, it implies specific attention and knowledge to collect the tiny and fragile insects. Huchet (2014) suggests paying caution to identify the exact location of the insect remains. To this end, the implementation of an internal grid (squares of 10 cm wide) is of great value in providing the exact location of each entomological sample, especially when working on sarcophagi.

Due to their fragile nature insects can be easily removed using brushes and small shovels, however, to avoid selection biases in several cases the usage of a low power vacuum can be the best collection method. It allows a general collection of the specimens and, if the air flux is not too turbulent, a first separation of the small stones and it can guarantee a good preservation of the specimens. The usage of this tool on the clothes of mummified bodies or on the inner part of the coffin can provide a complete sampling also of the small specimens such as the Coleoptera in the family Latridiidae, fleas (Siphonaptera) and mites (Acarina). To avoid a direct contact between the nozzle of the vacuum and the clothes or the body skin, a rigid mesh of 0.5 – 1.0 cm mesh can be put in front of the nozzle. This method is often used by researchers working on the mummies' clothes to remove any exogenous material. This "waste material" can be collected, stored and later sent to other specialists (e.g.: botanists, palynologists, entomologist, etc.) for further analyses.

Insect traces and body alterations

As summarized by Viero et al. (2019) insect feeding, pupating and nesting activities can affect not only the deposition/burial site and the clothes but also the body tissues. Holes, tunnels, cracks and other skin and bone alterations must be documented in order to provide a whole scenario of the insect activities on the body.

Pest Control

Particular attention must be paid at this stage to the presence of potential "museophagous" living specimens on the collected samples (Vanin et al., 2021) such as carpet beetles in the genera *Anthrenus* and *Attagenus* (Dermestidae) that can destroy the collected insects and other arthropod fragments. For this reason, freezing the samples after collection for at least 3 days at -20°C can remove any pest that could potentially affect/destroy the samples. The storage of the samples in modified atmosphere containers can also stop the development of the pests but this treatment is more expensive and time consuming than the simple sample freezing. Insecticides can also be used but they require specialized personnel (Chiappini et al., 2001), and they could affect further analyses of the specimens.

Separation of the insects from the sediment

This procedure can be performed in the excavation site or in the lab. It is particularly important in burials where insect remains are enclosed into the soil making their detection and isolation quite difficult. In these cases, especially when big volumes must be analysed, flotation is the most appropriate technique for insect's sampling. Flotation can be done by immersion of the sediments in a big volume of water and after stirring the floating elements are collected with a sieve, dried and then observed under a stereomicroscope. To improve the rate of extraction a flotation using water and paraffin (kerosene) was suggested by Coope & Osborne (1968) in a paper on the insects from a Roman well.

The advantage of this technique is that it preferentially concentrates insect remains over the plants and the mineral matrix of the sample. Standard sieve size of 300-250μm will allow the collection of the majority of the fragments also of a small size. The float is washed in a mild detergent to remove the paraffin, and then transferred to a beaker of either distilled water or 70% alcohol that increases the preservation time of the specimens and it reduces the surface tension when

sorting. The disadvantage of this technique – paraffin floatation and alcohol storage – is that it cannot be used if anything in the sample is to be used for radiocarbon dating.

In general, it is worth mentioning that only the collection of a big quantity of sediments will allow a good entomological sampling reducing, in this way, the collection biases. A minimum of five kilos of sediment is the amount suggested by several archaeoentomologists, however how much sediment can be available depends on the context. A good practice is to define, during the excavation/collection planning, the amount of sediment to be stored for the entomological analysis but also its origin (e.g: bottom of the coffin, clothes, pelvis area, head, etc.).

Specimens cleaning

Insect specimens, depending on the extraction method used for their collection are often coated by external substances, like dust, decomposed fluids, dirt, fibres and soil debris which might cover and hide the diagnostic characters (Fig. 2) making difficult, if not impossible, their correct identification. For this reason, the cleaning of the specimens is requested to make visible the diagnostic features and so doing, achieving the lowest level of identification (order, family, tribe, genus, species).

In principle, in order to be correctly identified, specimens have to preserve all the distinctive features after the cleaning treatment. Therefore, avoiding any damage to the sample is a priority. In practice, all methods and techniques affect the state of preservation of specimens, both molecularly and morphologically, although the extent of these effects can vary significantly based on the amount of time each sample is processed.

Figure 2. Dipteran puparium coated by decomposition fluids (scale bar: 1mm).

Pradelli et al. (2021) tested the efficiency of different cleaning methods on Diptera puparia from archaeological and forensic contexts. In their work Pradelli and co-authors (2021) tested the following methods derived from the entomological literature: Warm Water and Soap solution, Sonication, Glacial Acetic Acid, Sodium Hydroxide solution (NaOH), Hydrochloric Acid/Sodium Bicarbonate and Sodium Hypochlorite (NaOCl) (Gurney et al., 1964; Gifawesen et al., 1975; Zangheri 1981; Ronderos et al., 2000; Sukontason et al. 2007; Stueben & Linsenmair, 2008; Giordani et al., 2018). Pradelli et al. (2021) demonstrated that all the six methods successfully cleaned the puparia. However, if morphological and molecular analyses are considered together, the best methods, with positive results, is the warm water/soap, sonication and sodium hydroxide solutions.

Specimen mounting and long-term preservation

After separation from the substratum and after removal of any coating material, specimens are ready for their identification that is generally based on diagnostic morphological features. Some authors identify the specimens without mounting them, but just observing the specimens/fragments placed in a Petri plate or on a glass slide. This approach allows the possibility to observe the specimens from all the positions but due to the fragility of the specimens it could affect their integrity. In addition, this approach does not allow an easy comparison of the specimens with already identified material and it requires particular attention and experience when handling, with thin paint brushes or tweezers, the specimens.

The alternative is to mount the specimens or the fragments sticking them on entomological cards using a hydro-soluble glue (this kind of glues can be purchased from specialized shops, a traditional and effective recipe is reported in Zangheri, 1981) (Figg. 3, 4). This kind of preparation has the only disadvantage that ventral diagnostic features can be observed

only removing the specimens form the card – this can be bypassed mounting some specimens ventrally. The method has several advantages in term of handing, storing, comparing, observing and also protecting the specimens from accidental mechanical stresses. After been fixed on the entomological cards the specimens are pinned, added of the information label and eventually of the identification label, as reported in the next paragraph, and finally stored in standard entomological boxes (Fig. 4).

Figure 3. *Ptinus fur* specimen (Coleoptera, Ptinidae) glued on an entomological card. This species is very often found in archaeological contexts.

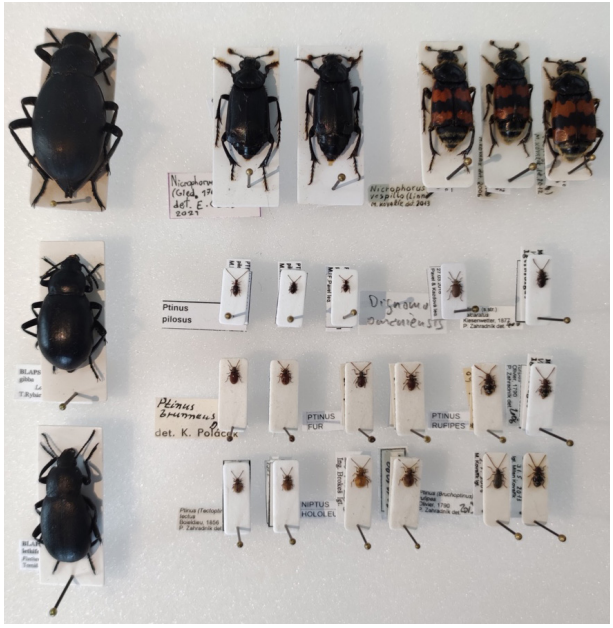

Figure 4. Example of entomological collection with mounted specimens of Coleoptera. This kind of collections are used to compare the specimens or the fragments of specimens collected from the archaeological contexts.

Large amount of specimens cannot be mounted, a fraction of them can be pinned or glued on entomological cards, the other stored in plastic/glass vials in dry conditions or immersed in 70% Ethanol. Particular attention must be paid when dry specimens are transferred in an aqueous solution because of the fact that distortions may happen.

Specimen labelling

A correct labeling of each vial or each mounted specimen is fundamental to avoid any mixing of the material. This is a common practice in entomology, but it is also a common practice in any other discipline dealing with samples, and it is also a standard practice in archaeology.

The following kinds of information must be reported in the label(s) associated with the insect: location of the collection site (geographic information, e.g.: Italy, Umbria, PG, Cerreto di Spoleto) – latitude and longitude can be also reported (e.g.: 42°48'55"N 12°54'53"E); source of the sample and US (e.g.: mummified body); historical period (e.g.: XVII century); collection date (e.g.: 25.vii.2022 or just "July 2022"); name of the collector with the prefix Leg. (Leg. = Legit = collected by) (Figg. 5, 6).

Identification

Morphological identification needs to be performed by specialists: as listed at the beginning of the introduction insects are more than 1,300,000 species and some of them are morphologically similar. In addition, the insects collected from (funerary) archaeological contexts are very often fragmented or affected

Italy – Umbria – PG Cerreto di Spoleto, Borgo Cerreto 42°48'55"N 12°54'53"E 25.vii.2022 Leg. S.Vanin	 Mummified body XVII century Male BC12

Figure 6. Specimens of Coleoptera mounted on an entomological card, pinned with all the information and identification labels.

Figure 6. Specimens of coleoptera mounted on an entomological card, pinned with all the information and identification labels.

by the taphonomic processes (Figg 7, 8). Identification in these cases is particularly difficult and the comparison with previous identified specimens is highly recommended. An identification label is added to the specimens (Fig. 9). All the observations of the entomological material are performed using a stereomicroscope but for particularly small specimens, mounted on microscope slide (e.g.: mites) a compound microscope has to be used.

Figure 7. Beetle fragments (Ptinidae, Cleridae, Histeridae, Staphylinidae and Cryptophagidae) and Pseudoscorpion pedipalps collected from an archeo-funerary site.

Figure 8. Crashed puparia of Calliphoridae and Muscidae species collected from an archaeo-funerary site.

In Archaeological studies, different measures to quantify the collected specimens (bones, snails, pottery fragments, etc) have been proposed (Casteel, 1977; Casteel, 1978; Chase & Hagaman, 1987; Banning, 2000). Some of them are simple enumeration of a particular sample, such as NISP (Number of Identified Specimens), weight or mass, MNE (Minimum Number of Elements), and MAU (Minimum Animal Units), while others involve a more complicated calculation to estimate different parameters. However, the most common and widespread unit of quantification currently in use is MNI (Minimum Number of Individuals).

In archaeoentomology, the measure of abundance is widespread (e.g.: Huchet, 2014; Forbes et al., 2015;

Nitidula carnaria (Schaller, 1783) Det. S. Vanin 2022
Nitidula flavomaculata Rossi, 1790 Det. S.Vanin 2022

Figure 9. Example of two identification labels. The name of the species, the authority with the description year and the name of the person that identified the specimens are reported. The presence or the absence of the brackets on the authority's name depends on the systematic history of the taxon (see International Code of Zoological nomenclature: https://www.iczn.org/the-code/the-code-online/). Not- specialists must refer to the specific taxonomic databases (e.g.: www.faunaeur.org).

Smith, 2018; Henríquez-Valido et al., 2020), even though MNI was specifically developed for vertebrate remains, such as cattle bones, fish bones, and human remains (Banning, 2000). The MNI is the smallest number of individuals representing the archaeological assemblage, but not the individuals who originally contributed to it (Banning, 2000) for this reason a lot of attention must be paid when the MNI has to be interpreted. As previously mentioned archaeoentomologists must be aware of the collection bias that can strongly affect the number and the quality of the extracted specimens.

Conclusions

The present work suggests a first workflow to maximize the information that can be derived from insect collected from archaeo-funerary sites. Despite being the first protocol, it derives from the protocols usually used in archaeology and forensic entomology and it has been tested – and amended – by the authors based on a long experience (see Giordani et al., 2020; Tuccia et al., 2022). Seven steps are suggested: documentation, collection on site, insect-sediment separation, specimens cleaning, mounting, labelling and identification. Particular attention must be paid on the understanding that the entomofauna collected from a site des not correspond to the original one, however the application of a correct method can significantly reduce the collection and information bias. This work is the first of a series of papers aims to standardize the procedures carried out from the excavation site to the interpretation of the entomological evidence from archaeo-funerary projects.

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