ARCHAEOENTOMOLOGY IN THE CENTRAL SAHARA:

RESEARCH INTO THE TAKARKORI ARCHAEOLOGICAL SITE

JENNIFER PRADELLI

A thesis submitted to the University of Huddersfield in partial fulfilment of the requirements for the degree of Doctor of Philosophy

---

Department of Biological and Geographical Sciences
School of Applied Sciences
University of Huddersfield

December 2020

To Mum

COPYRIGHT STATEMENT

i. The author of this thesis (including any appendices and/ or schedules to this thesis) owns any copyright in it (the "Copyright") and s/he has given The University of Huddersfield the right to use such Copyright for any administrative, promotional, educational and/or teaching purposes.

ii. Copies of this thesis, either in full or in extracts, may be made only in accordance with the regulations of the University Library. Details of these regulations may be obtained from the Librarian. This page must form part of any such copies made.

iii. The ownership of any patents, designs, trademarks and any and all other intellectual property rights except for the Copyright (the "Intellectual Property Rights") and any reproductions of copyright works, for example, graphs and tables ("Reproductions"), which may be described in this thesis, may not be owned by the author and may be owned by third parties. Such Intellectual Property Rights and Reproductions cannot and must not be made available for use without permission of the owner(s) of the relevant Intellectual Property Rights and/or Reproductions.

TABLE OF CONTENTS

ACKNOWLEDGEMENTS 7

LIST OF RESEARCH OUTPUTS 9

ABSTRACT 10

LIST OF TABLES 12

LIST OF FIGURES 14

LIST OF ACRONYMS AND ABBREVIATIONS 17

1 INTRODUCTION 18

1.1 THE INSECTS' "EMPIRE" 18

1.2 A JOURNEY INTO THE PAST: THE INSECT EVIDENCE 19

1.3 ARCHAEOENTOMOLOGY IN NORTH AFRICA 20

1.4 THE CENTRAL SAHARA 21

1.5 OBJECTIVES OF THE THESIS 23

1.6 STRUCTURE OF THE THESIS 26

2 MATERIAL AND METHODS 27

2.1 TAKARKORI: sample origin and storage 27

2.2 COLLECTION OF ENTOMOLOGICAL SAMPLES IN SOIL 34

2.3 TECHNICAL NOTE: cleaning techniques 36

2.3.1 Introduction 36

2.3.2 TEST 1: Materials and Methods 37

2.3.3 TEST 2: Materials and Methods 39

2.3.4 TEST 1: Results and Discussion 40

2.3.5 TEST 2: Results and Discussion 43

2.3.6 Conclusion 48

2.4 MICROSCOPY AND MICROPHOTOGRAPHY 49

2.5 MORPHOLOGICAL IDENTIFICATION OF INSECTS 49

2.6 SYNCHROTRON RADIATION APPLIED TO INSECT IDENTIFICATION 51

2.6.1 Introduction 51

2.6.2 Elettra Synchrotron Trieste 54

2.6.3 SYRMEP beamline 56
2.6.4 TomoLab 57
2.6.5 The Takarkori sample 58
2.7 MOLECULAR IDENTIFICATION OF INSECTS 59
2.7.1 Ancient DNA 62
2.7.2 The Takarkori samples 63
3 HOLOCENE CLIMATE CHANGES: the entomological evidence 65
3.1 RECONSTRUCTION OF PAST ENVIRONMENTS 65
3.2 INSECT FAUNA AT TAKARKORI: results 66
3.3 DISCUSSION 71
3.3.1 ORTHOPTERA 71
3.3.2 LEPIDOPTERA 76
3.3.3 ISOPTERA 78
3.3.4 HYMENOPTERA 81
3.3.5 TAKARKORI ENVIRONMENTAL RECONSTRUCTION 86
3.4 CONCLUSION: THE ENTOMOLOGICAL EVEDENCE 88
4 SYNANTHROPIC, HUMAN AND ANIMAL PARASITES, FOOD PEST: evidence of human presence and activities 90
4.1 SYNANTHROPIC INSECTS 90
4.2 DIPTERA 91
4.2.1 Infamous dipterous families 92
4.2.2 PIOPHILIDAE 93
4.2.3 SPHAEROCERIDAE 94
4.2.4 CALLIPHORIDAE 95
4.2.5 SARCOPHAGIDAE 97
4.2.6 MUSCIDAE 98
4.2.6.1 *Musca domestica* 99
4.2.7 OESTRIDAE 101
4.2.7.1 *Oestrus*, Linnaeus, 1758 106
4.3 PHTHIRAPTERA: the human lice 110
4.4 COLEOPTERA: food pests 113

4.4.1 Stored-product insects 114
4.4.2 TROGIDAE 115
4.4.3 TENEBRIONIDAE 116
4.4.4 PTINIDAE 117
4.4.5 DERMESTIDAE 118
4.4.5.1 *Dermestes maculatus*, De Geer, 1774 119
4.4.6 CURCULIONIDAE 120
4.5 CONCLUSION 121
5 FINAL CONCLUSIONS **124**
5.1 FURTHER RESEARCH 129
5.2 SCIENTIFIC OUTPUTS 129
REFERENCES **131**

ACKNOWLEDGEMENTS

I would like to first say a very big thank you to Leverhulme Trust Doctoral Scholarship programme for funding my doctoral degree.

I would like to express my sincere gratitude to my advisor and my mentor Prof Stefano Vanin for the continuous support during my PhD and related research, for his patience, motivation, and immense knowledge. His guidance helped me not only in developing skills of research but also in becoming a better person outside the academic field.

Besides my advisor, my sincere acknowledgements go to the members of my thesis committee: Prof Martin B. Richards and Dr Gareth Parkes for accepting the role of supervisor and co-supervisor for the last year of my PhD. I also sincerely thank them, along with Dr Ceiridwen Edwards and Dr Jarek Byrk, for being my progression viva examiners and for giving me insightful comments that strongly helped me to improve my research.

My sincere thanks go also to Prof Savino di Lernia (Sapienza University of Rome) and Prof Anna Maria Mercuri (University of Modena and Reggio Emilia) for being my international advisors, suggesting the Takarkori material and for hosting me in their facilities, providing me essential material for my research and advising me since the early days of the work. Thanks also to Dr Lucia Mancini (Senior Beamline Scientist in the SYRMEP group at the Elettra - Synchrotron Trieste Laboratory) for giving me the opportunity to perform SR micro-CT analysis and to Dr Alessio Veneziano for helping me in the visual reconstruction of data.

I am also very grateful to the team of technicians, with a special acknowledgement to Jim, Felix, and Ibrahim for being always kind and helpful.

In addition, I am thankful to all the experts from other academic institutions that helped to identify insect remains and whom it has been a pleasure working and established a solid professional network with, Dr Alberto Zilli, Dr Fabrizio Rigato, Dr Giulio Gardini, and Davide Dal Pos.

Last in the list but first in my heart, I want to thank all my loved ones.

My warmest and heartfelt acknowledgements are for my family, Mum and Dad who always believed in me and still support me in every moment of my life. A special thanks also to my sister Katia, her husband Ciro and my nephew Stefano for the unconditional love and support. I will always be grateful to my dear cousin Jossa (Gill) who has been a sister to me.

I thank my special friends Giorgia, Fabiola, and Katharina, together with I lived the life-changing experience of a PhD. Without your friendship, I would not be the person I am now.

A sincere thanks to my friend and housemate Nicolò for not leaving me alone during my darkest times. I will never be grateful enough.

A special thanks also to Rohan, Pierre and Manon for being a great company.

A thought also goes to Lorenzo, my fashion soulmate, who with the smile on his lips encouraged me emotionally.

A special thanks goes to Noemi, Ottavia, Anka, and Beatrice, my childhood friends that will always be there for me, even when we are scattered around the world.

A heartfelt thanks is to my friends Lidia, Luca, Espo, and Mauri for never stopping to be so close to me, despite distant friendship, and for always waiting for my return home with enthusiasm.

Thanks also to my Volleyball friends Taylor, Amir, Nicholas and many others for being a hilarious and supporting company.

LIST OF RESEARCH OUTPUTS

In partial fulfilment of the requirements for the degree of Doctor of Philosophy, I contributed either as first author or co-author to publish the following peer-reviewed papers whose outcomes are not included in the PhD thesis here presented:

2019 Mukherjee S., Singh P., Tuccia F., **Pradelli J.**, Giordani G., Vanin S. DNA characterization from gut content of larvae of *Megaselia scalaris* (Diptera, Phoridae). *Science and Justice* 59(6): 654-659 doi: 10.1016/j.scijus.2019.06.006.

2019 **Pradelli J.**, Rossetti C., Tuccia F., Giordani G., Licata M., Birkhoff J.M., Verzeletti A., Vanin S. Environmental necrophagous fauna selection in a funerary hypogeal context: The putridarium of the Franciscan monastery of Azzio (northern Italy). *Journal of Archaeological Science: Reports* 24: 683-692 https://doi.org/10.1016/j.jasrep.2019.02.028.

ABSTRACT

The transition from hunting-gathering to food production shows distinct and different pathways worldwide. Evidence from the Central Sahara suggests original trajectories to the onset of early herding, largely related to fluctuating climate and environmental conditions. Thanks to the outstanding well-preserved stratigraphy which covers from Late Acacus to Late Pastoral period (approximately 10,200 to 4,600 cal years BP), Takarkori archaeological site, located in southwest Libya, excavated by the Libyan Department of Antiquities and by Sapienza University of Rome, is an enormous pool of early to middle Holocene information. This study intends to present the first archaeoentomological analysis performed in North Africa becoming a reference for future works. Methodological issues, such as the collection and recovery methods, cleaning techniques, and identification process, experienced during the analysis of ancient insect samples are discussed. Solutions and innovative approaches, such as the new application of the synchrotron radiation to a dry insect sample, have been explored and presented highlighting the great potential in the field. Insects, such as migratory locusts, tiger moth, and termites, allowed the reconstruction of Holocene paleoclimate confirming the progressive desertification. The recovery of the human head louse, *Pediculus humanus*, and the sheep nasal botfly, *Oestrus ovis*, not only confirms the re-colonisation of the area by hunters and gatherers but also gives information about their hygiene level and the health state of their animals. In addition, the discovery of these obligate parasites brings the opportunity to better understand their co-evolution with their host. Takarkori is also the most ancient settlement with traces of the housefly, *Musca domestica*, placing 3,000 years earlier its appearance in close relationship with humans. Besides, flies help in interpreting the past population habits suggesting a tendency to process meat and consume meals without properly dispose of food waste. Several stored product pests, such as *Sitophilus granarius* and *Dermestes maculatus*, indicate other human activities, such as the presence of seed storages and human crafted by-products like desiccated meat or fur/wool production at the site.

The work presented identifies Archaeoentomology as a powerful and effective tool to be used in archaeological context for the interpretation of the past.

LIST OF TABLES

Table 1: Cultural Phases in Takarkori.....30

Table 2: Selected samples with co-ordinates of their location and total weights of soil which has been analysed. 33

Table 3: Cleaning methods selected.....37

Table 4: Quantifications of samples cleaned immediately after adults' emergence (CNTRL = control; NaOH = sodium hydroxide; SON= sonication; GAA= glacial acetic acid; H2O = warm water and soap; ZAN= hydrochloric acid/sodium bicarbonate solutions; BL= bleach; ✓ positive results for the expected fragment, ✗ negative results for the expected fragment).....44

Table 5: Quantifications of samples placed in the mixture for a week and then cleaned (CNTRL = control; NaOH = sodium hydroxide; SON= sonication; GAA= glacial acetic acid; H2O = water/soap; ZAN= hydrochloric acid/sodium bicarbonate solutions; BL= bleach; ✓ positive results for the expected fragment, ✗ negative results for the expected fragment).....46

Table 6: Light source parameters. 56

Table 7: X-rays at the sample. 57

Table 8: Up to date progress of DNA barcoding library (BOLD*) of insects. 59

Table 9: List of common molecular targets used in entomology (modified from Tuccia, 2020).....61

Table 10: List of species from Takarkori and their abundance (*= 1, **= 2-10, ***= <100, ****=>100; LA = Late Acacus, EP = Early Pastoral, MP = Middle Pastoral, LP = Late Pastoral). 67

Table 11: Abundance of each ant in each chronological phase (*= 1, **= 2-10, ***= <100, ****=>100; LA = Late Acacus, EP = Early Pastoral, MP = Middle Pastoral, LP = Late Pastoral). 83

Table 12: Taxonomic classification of oestrid flies and their preferred anatomical site of infestation (modified from Colwell et al., 2006). 102

Table 13: Oestrids and their hosts (modified from Colwell et al., 2006).....103

Table 14: List of natural hosts and distribution of each species inside *Oestrus* genus (modified from Colwell et al., 2006). *Cosmopolitan in domesticated livestock, absent in wild hosts in Sub-Saharan Africa. 106

Table 15: Summary of the entomological contributions in the understanding of Takarkori settlement. 125

LIST OF FIGURES

Figure 1: The Tassili-n'Ajjer, the Hoggar, and the Tadrart Acacus massifs in the red circle (Biagetti and di Lernia, 2013). 23

Figure 2: Tadrart Acacus and its caves and rock-shelters. The archaeological site of Takarkori is indicated with the red arrow (Biagetti and di Lernia, 2013). 24

Figure 3: Takarkori rock-shelter and the 30-meter-high cliff (http://www.palinopaleobot.unimore.it). 28

Figure 4: Representation of the four sectors excavated in Takarkori rock-shelter (Cremaschi et al., 2014). 29

Figure 5: Example of Takarkori soil sediment (sample n.1 LA1, layer L355, square R16) divided into bags after using three different size meshes during excavation. The samples have been stored at the University of Modena and Reggio Emilia. 29

Figure 6: Visual representation of samples location collected from the Northern Sector and in the Main Sector (LP= Late Pastoral, MP= Middle Pastoral, EP= Early Pastoral, LA= Late Acacus). 32

Figure 7: Before and after pictures: A-B Water and soap solution, C-D Glacial Acetic Acid, E-F Sonication; G-H Sodium hydroxide 10 % solution, I-J Hydrochloric acid/sodium bicarbonates solutions, K-L Sodium hypochlorite 1-5% solution. Scale bars: 500 μm. 41

Figure 8: Agarose gel visualizing the PCR products. On the right the positive/negative controls. (CNTRL = control; NaOH = sodium hydroxide; SON= sonication; GAA= glacial acetic acid; H2O = warm water and soap; ZAN= hydrochloric acid/sodium bicarbonate solutions). 45

Figure 9: Agarose gel visualizing the PCR products obtained from samples immersed for 5 and 10 minutes in different sodium hydroxide solutions (saturated, 10%, and 1%), including positive and negative controls. 48

Figure 10: Fly life cycle. 52

Figure 11: Elettra Synchrotron Trieste research center in Basovizza (Trieste, Italy) (https://www.wayforlight.eu, photo credit: Gabriele Crozzoli). 54
Figure 12: Beamlines currently active at ELETTRA (https://www.elettra.trieste.it). 56
Figure 13: X-ray micro-CT scanner inside TomoLab (https://www.elettra.trieste.it)....58
Figure 14: Pedipalps of pseudo-scorpions. Scale bar 500 µm. 66
Figure 15: Life cycle of caeliferans (modified from Latchininsky, 2013). 72
Figure 16: Migratory locust in solitary form, from Defaut (2013). 74
Figure 17: Fragments of hind legs of *Locusta migratoria* collected at Takarkori, scale bar 2 mm. 75
Figure 18: Abundance of migratory locust fragments in each chronological phase (LA = Late Acacus, EP = Early Pastoral, MP = Middle Pastoral, LP = Late Pastoral). 76
Figure 19: Takarkori Articiinae, A dorsal view, B ventral view, scale bar 500 µm. 78
Figure 20: Termite fragments; A latero-dorsal view, B latero-ventral view of a worker; C dorsal view, D ventral view of a termite head; E dorsal view, F ventral view of a different termite head; G dorsal view, H ventral view of a soldier. Scale bars 500 µm. 80
Figure 21: Dorsal and ventral view of ant heads collected at Takarkori. A *Lasius* sp., B Gen. sp., C *Messor* sp., D *Pheidole* sp. major worker, E *Brachyponera sennaarensis*. Scale bars 500 µm. 83
Figure 22: Male specimen of Bradynobaenidae (Apterogyninae) collected at Takarkori. Scale bar 500 µm. 86
Figure 23: Visual representation of the locust disappearance during the chronological sequence at the Takarkori site caused by environmental changes. Green star marks the 8.2K event, yellow start marks the desertification process (LA = Late Acacus, EP = Early Pastoral, MP = Middle Pastoral, LP = Late Pastoral). 89
Figure 24: Piophilidae specimens collected at Takarkori. A-B ventral view and close up of posterior spiracles of species 1; C-D ventral view and close up of posterior spiracles of species 2. Scale bar 500 µm. 94

Figure 25: Sphaeroceridae specimen collected at Takarkori site; A-B dorsal and ventral view of the posterior region, C close up of posterior spiracles, D close up of one posterior spiracle. Scale bar 100 μm. 95
Figure 26: Fragment of Calliphoridae puparium. A-B dorsal and ventral view; C-D close up of posterior spiracles; E close up on speculation; F close up on anal plate. Scale bar 100 μm. 97
Figure 27: Fragment of Sarcophagidae puparium. A posterior region, ventral plate; B posterior spiracles. Scale bar 500 μm. 98
Figure 28: Fragment of *Musca domestica* puparium. A-B dorsal and ventral view; C-D close up to posterior spiracles. Scale bar 100 μm. 101
Figure 29: Life cycle of Oestrinae subfamily (modified from Colwell et al., 2006). 104
Figure 30: Dorsal, lateral, and ventral view of *Oestrus ovis* specimen. Scale bar 100 μm. 108
Figure 31: 3D reconstruction of the pharate. Lateral view. Internal organs are highlighted in blue in the bottom image. 109
Figure 32: Fragments of head louse collected at Takarkori. A dorsal view, B ventral view. Scale bar 500 μm. 113
Figure 33: Trogid beetles. A dorsal view, B ventral view. Scale bar 500 μm. 116
Figure 34: Biggest fragment of Tenebrionidae recovered at Takarkori. A dorsal view, B ventral view. Scale bar 500 μm. 117
Figure 35: Two specimens of *Mezium* sp. Scale bar 500 μm. 118
Figure 36: Best preserved dermestid of Takarkori. A dorsal view, B ventral view. Scale bar 500 μm. 119
Figure 37: *Sitophilus granarius* specimen recovered from Takarkori. A dorsal view, B ventral view. Scale bar 500 μm. 121

LIST OF ACRONYMS AND ABBREVIATIONS

aDNA	ancient DNA
BC	Before Christ
BCE	Before Common Era
BOLD	Barcoding of Life Database
BP	Before Present
bp	base pair
CT	Computer Tomography
cal	calibrated
DNA	Deoxyribonucleic Acid
EP	Early Pastoral
EtOH	ethanol
FLEA	Forensic Laboratory of Entomology and Archaeology
HW	Hot Water
KA	*Kilo-Annum*
LA	Late Acacus
LP	Late Pastoral
MP	Middle Pastoral
mPMI	minimum PMI
NCBI	National Center for Biotechnology Information
NHM	Natural History Museum
SEM	Scanning Electron Microscope
SR	Synchrotron Radiation
uncal	uncalibrated
yr	years

1 INTRODUCTION

1.1 THE INSECTS' "EMPIRE"

Despite their small sizes, insects are the most successful animals in the World. The insect class have evolved on Earth around 480 million years ago, during the Ordovician of the Palaeozoic Era, probably at the same time as terrestrial plants. Following the numerous global climate fluctuations, they underwent two major radiations, during the Carboniferous (360-300 million years ago) and the Permian (300-250 million years ago). Their evolution faced a great downturn when the largest mass extinction of the planet hit just before the Triassic (250-200 million years ago) (Rasnitsyn and Quicke, 2007). Despite this, survivors managed to flourish again during the Jurassic period between 200 and 145 million years ago and essentially persisted to this day. Many modern taxa that we are familiar with, such as Carabidae (ground beetles), Staphylinidae (rove beetles), and Buprestidae (jewel beetles), developed during the Cretaceous (145-66 million years ago) and early phases of the Cenozoic (66 million years ago) (Grimaldi and Engel, 2005). Nowadays, over one million species have been described worldwide, but the exact number of species is still unknown. The global estimate of insect diversity varies approximately between six and ten million species. However, richness is not their only extraordinary feature: their bewildering adaptability allowed them to exploit all kinds of natural and anthropogenic ecosystems, both terrestrial and aquatic environments (Gullan and Cranston, 2014).

Insects were already widespread and well established around the entire planet long before genus *Homo* evolved, about 2.5 million years ago. It was unavoidable for us to start a

coexistence with these ever-present creatures. Our desire to understand them arose once we became aware of how essential they are and how deeply they affect our lives, our crops and our domestic animals. In fact, the first evidence of human interest in insects is found in prehistoric times (Rasnitsyn and Quicke, 2007). A rock-depiction of a bee dated back to 13,000 BC has been discovered in a cave in Valencia, Spain. However, the systematic scientific study of insects, called entomology, began only during the 16th century (Saltini, 1984). Entomology covers the total range of biological disciplines, from evolution to ecology, from physiology to genetics and it can be applied to several other scientific disciplines, such as agriculture, pest control, waste management and nutrition (Sarpong *et al*., 2019; Setti *et al*., 2019). In the last decades, entomology also stepped back into the limelight to the general public thanks to its novel application in forensic investigation. In this thesis, another original application of entomology which is recently gaining attention will be covered: archaeoentomology.

1.2 A JOURNEY INTO THE PAST: THE INSECT EVIDENCE

Archaeoentomology is defined as the study of insects found in archaeological sites (Buckland and Wagner, 2001). It differs from the closely related discipline Palaeoentomology because it focuses specifically on insects recovered from past anthropic settlements. Entomological fragments due to their chitinous exoskeletons can be preserved for several millennia (Erickson, 1988). Insects are sensitive biological indicators and they can be used to describe environmental contexts, especially if stenotopic species, which have a restricted range of habitats, are considered (Bain, 1999). However, depending on the preservation status of the samples, interpretation can be difficult. Particularly, identification at species level could be trivial when diagnostic characteristics are missing or are damaged. Until the end of the 20th century, insect remains in geological and archaeological soil samples were not deemed important, as their applications were underrated (Bain, 2001). Moreover, the belief that insects were constantly evolving, avoiding comparison with the present fauna, slowed down the exploitation of the subject (Elias, 2009; Dussault *et al*., 2014). Studies have proved that insects did not undergo significant speciation during the

Quaternary period allowing evaluations by comparison with modern specimens/species (Bain, 1999).

There are many different topics of research in archaeoentomology: insect biogeography, environmental ecology, funerary archaeoentomology and public health are just some examples (Bain, 1999). The comparison between a past biome and the present-day biome might allow for the detection of changes in the composition of the fauna. For instance, insects may have been introduced in a new environment by humans through trade routes, intentionally or unintentionally (Carrott and Kenward, 2001). On the other hand, some species may not be present anymore in certain regions for various reasons, such as climate change or habitat changes (Giordani *et al*., 2018). These reconstructions are also called paleo-economic re-enactments and they are useful to understand past populations and their economies (Bain, 1999). Preserved insects may also provide information about ancient medical practises and sanitary and health conditions of past populations (Huchet and Greenberg, 2010; Pradelli *et al*., 2019). During archaeoentomological analyses, beetles (Coleoptera), flies (Diptera), bees, ants, wasps (Hymenoptera), moths (Lepidoptera), grasshoppers, locusts, crickets (Orthoptera), fleas (Siphonaptera), lice (Anoplura) and mites (Acarina) are commonly recovered (Bain, 1999).

1.3 ARCHAEOENTOMOLOGY IN NORTH AFRICA

Since Napoleon's campaign in the 18th century, an increasing interest in ancient Egyptian culture spread around Europe leading to the unwrapping and the studying of many human and animal mummies (Audouin, 1835; Blair, 1935). On the wave of this new scientific focus, at the beginning of the 19th century Reverend F. W. Hope, a British naturalist and entomologist, introduced one of the earliest examples of a successful archaeoentomological study. The recovery of beetle remains inside an Egyptian mummified ibis gut (Hope, 1842), despite the scepticism of ornithologists, explained the bird's diet habits (Panagiotakopulu, 2001).

Between the 1960s and 1970s, the first archaeoentomological extensive researches were carried out in the UK (Coope and Osborne, 1967; Buckland *et al*., 1974). Since then,

archaeoentomological investigations have been also conducted in other parts of the world, such as North Atlantic Islands (Buckland *et al*., 1995; Vickers *et al*., 2005; Panagiotakopulu *et al*., 2007; Buckland *et al*., 2009), Northern Europe (Hellqvist and Lemdahl, 1996; Nielsen *et al*., 2000; Ponel *et al*., 2000), North America (Bain, 1998; Bain and Prévost, 2010), South America (Huchet and Greenberg, 2010; Giordani *et al*., 2020), the Mediterranean Basin and the Near East (Panagiotakopulu, 1999, 2001, 2004b; Kislev *et al*., 2007; Panagiotakopulu and Buckland, 2009).

Although the archaeoentomological analysis is gaining recognition, it is still not commonly applied during archaeological excavations. Lots of key areas around the world have yet to be analysed from an entomological point of view: North Africa is one of them. Very little has been published about insects recovered from North African archaeological sites (Panagiotakopulu, 2001; Huchet, 2010; Panagiotakopulu *et al*., 2010; Henríquez-Valido *et al*., 2020). Most of the entomological studies focused on specimens collected from the Egyptian pharaonic period (Audouin, 1835; Blaisdell, 1927; Blair, 1935; Alfieri, 1956; Chaddick and Filce Leek, 1972; Burleigh and Southgate, 1975). Entomological data from other northern African countries, like Morocco, Algeria, Tunisia, and Libya are rare. The little information published refers to lake flies (Chironomidae), solitary carpenter bees belonging to *Xylocopa* Latreille, 1802 genus and tenebrionid beetles (Naegele, 1960; Anketell and Ghellali, 1984; Hunt *et al*., 1987; Ramdani *et al*., 2001; Eggermont *et al*., 2008; Ahmed *et al*., 2018).

1.4 THE CENTRAL SAHARA

Central Saharan archaeology is intertwined with the elaborate colonial and postcolonial history of the area. Early interests in the archaeological artefacts of the region can be found in medieval Arabic texts (Fenwick, 2012). However, it is during the 18th and early 19th centuries that French and British explorers started focusing on the discovery and collection of artefacts for museum purposes (Lorcin, 2007). During the colonial period, roman archaeology was the priority (Munzi, 2004; Mattingly, 2011), but after the independence of Libya (1951), Tunisia (1957), and Algeria (1962) the archaeological agendas changed,

becoming more inclusive of different historical periods and heritages (Oulebsir, 2004). From the 1970s to 1980s further outbreaks of interest supported by international conspicuous investments lead to pivotal archaeological excavations, awareness for site preservation and the creation of new museums to encourage heritage tourism (Fenwick, 2012). These developments have prompted research that focuses on Saharan prehistory.

During the majority of Pleistocene, the Central Sahara was uninhabited due to severe climatic conditions. Its re-colonisation started around 11,500 years ago at the beginning of the Holocene with the amelioration of the climate (Cremaschi and di Lernia, 1998; di Lernia, 1999b). Africa underwent a high rainfall period called the African Humid Period (AHP) between 11,300 and 6,000 cal yr BP. The landscape was dominated by continuous grasslands, such as Cyperaceae and Poaceae, which are monocotyledonous flowering plants typical of savannah (Trevisan Grandi *et al*., 1993; Cremaschi, 1996). This kind of environment might have facilitated the exploitation of the new resources by hunter-gatherers, who probably reflect a complex intermingling of different peri-Saharan cultures (di Lernia, 1996). Caves and rock-shelters typical of the region allowed occupation, leading to the introduction of pastoralism as the main economic system (Barich, 1987). The Saharan Pastoral-Neolithic period is divided into a series of sub-phases, distinguishable thanks to the changes in the material used to produce pottery and to the increasing dependence on animal husbandry (di Lernia and Cremaschi, 1996).

A hyper-arid condition started to spread rapidly around 5,900 years ago leading to what is presently the world's largest hot desert, the Sahara Desert, which is still expanding its boundaries. The onset of a new extreme climate forced the arising populations to adapt to drier environments in order to survive (Cremaschi and di Lernia, 1995). About 3,000 years ago the setting up of irrigated agriculture bypassed the problem of the desertification with the introduction of oasis farming (Cremaschi, 1996). The Garamantian civilisation was based on those new inventions and flourished until 1,500 years ago (Liverani, 2000; Mattingly and Wilson, 2003; Liverani, 2004; Mori, 2013). Nowadays, their Berber descendants, such as the Tuareg tribe, still inhabit those areas.

1.5 OBJECTIVES OF THE THESIS

Between the 1950s and early 1990s, ground-breaking archaeological expeditions were carried out in the massif of the Tassili-n'Ajjer, the Hoggar, and the Tadrart Acacus (Fig. 1), where rock-shelters and caves have turned out to be a potentially enormous source of early Holocene information (Biagetti and di Lernia, 2013).

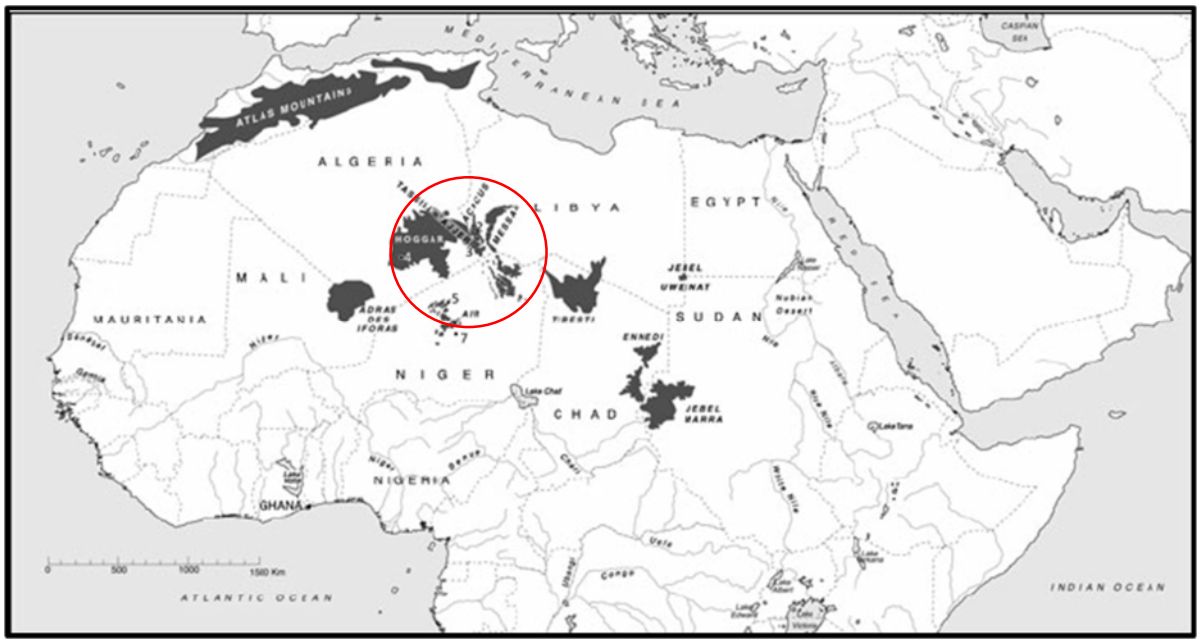

Figure 1: The Tassili-n'Ajjer, the Hoggar, and the Tadrart Acacus massifs in the red circle (Biagetti and di Lernia, 2013).

In particular, those studies revealed the importance of the Tadrart Acacus and its rock-shelters, which contained outstandingly well-preserved stratigraphy ranging from about 11,500 to 2,000 years ago (Fig. 2) (Barich, 1987).

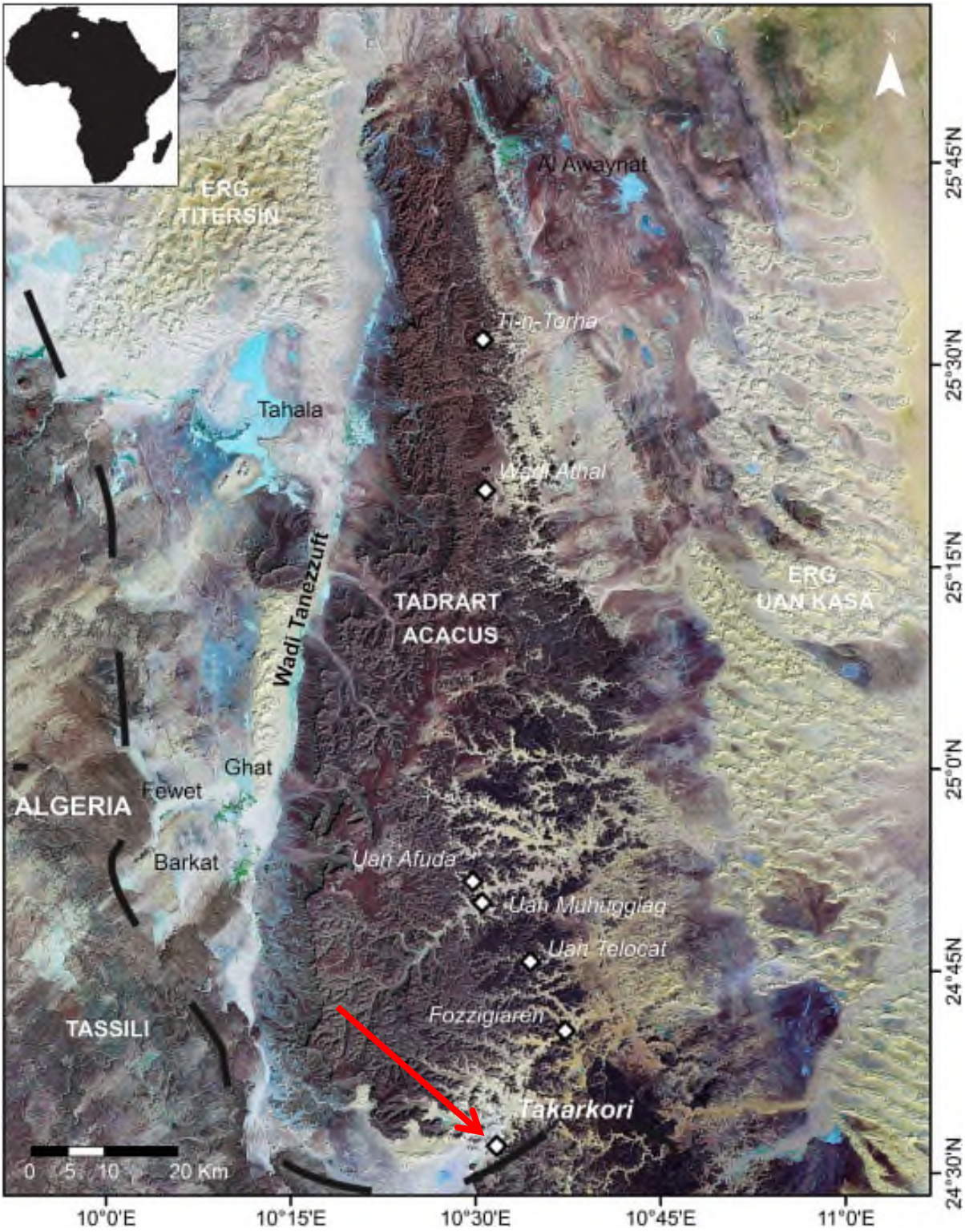

Figure 2: Tadrart Acacus and its caves and rock-shelters. The archaeological site of Takarkori is indicated with the red arrow (Biagetti and di Lernia, 2013).

This thesis reports the first archaeoentomological investigations on sediments from the Takarkori rock-shelter (Fig. 2). For the first time in the region, the amazing state of insect

preservation allowed the reconstruction of the past environment over about 10,000 years ago in detail. Moreover, insights on human adaptation and past population habits will be covered in this study using synanthropic insects. Archaeobotanical research has been extensively recorded on the same site by Mercuri and colleagues (Mercuri *et al*., 2018), allowing a comparison.

The aims of this study are:

i) **to optimise insect cleaning techniques to aid identification.**

Several methods have been described in the past to clean insects, designed mainly to prepare them for exhibition in private and museum collection or for microscopical observation. Their applicability to samples collected from archaeological contexts has never been tested before.

ii) **to evaluate the potential of synchrotron radiation micro-CT scan technique to aid identification of immature stages from archaeological contexts.**

This technique has been solely used to analyse insects preserved in amber. The taphonomic processes under which archaeological specimens went, commonly modify and/or remove their diagnostic features. Specifically, the identification of immature stages is exacerbated by the lack of identification keys. No attempt has been conducted to analyse dry specimens from archaeological contexts before.

iii) **to evaluate the potential of insects to reconstruct specific past environments.**

The paleo-reconstruction of past climate and habitats through insect has been extensively applied to glacial environments. The good preservation of insects in permafrost allowed researchers to trace in detail the Pleistocene glacier fluctuations. However, is it possible to apply the same method to a completely different environment and climate change, such as desertification in North Africa? Despite the differing conservational issues

and the contrast in insect faunas, there are positive expectations in the Takarkori case. The outstanding quantity of entomological evidence covering the entire Holocene chronological sequence is promising.

iv) **to ascertain the presence of humans based on insects strictly related to them, from species-specific parasites to synanthropic species, and to confirm the co-presence of livestock at the site.**

In the past, synanthropic insects have been successfully used as indicators of population health, hygienic condition, and food storage habits. However, it is not common to recover synanthropic insect, especially when Early and Middle Holocene periods are considered. Thanks to the enormous quantity of material available from Takarkori, there are great expectations to statistically find synanthropic insects in it.

1.6 STRUCTURE OF THE THESIS

In Chapter 2, a detailed description of the archaeological site and the material analysed are presented. Explanations of specific problems and solutions encountered during the archaeoentomological analysis are discussed. In addition, an overview of the methods used to carry out all the experiments is clarified.

Chapter 3 presents the paleo-reconstruction of the Early and Middle Holocene environment through insects.

Chapter 4 presents the human activities at the site from an entomological point of view.

Chapter 5 discusses the final conclusion in relation to the aims given in the paragraph above.

2 MATERIAL AND METHODS

2.1 TAKARKORI: SAMPLE ORIGIN AND STORAGE

Takarkori is a large rock-shelter opening on the left side of a valley that divides the Libyan Tadrart Acacus from the Algerian Tadrart (El-Ghali, 2005). In North African Arabic, valleys are called wadi (وَادِي) and they usually refer to dry *alluvium*, typically triangular-shaped unconsolidated deposits formed in mountainous areas and commonly found in arid or semi-arid environments. In fact, the Takarkori rock-shelter is near a fluvial system east of the Algerian Tassili, probably active until the desertification started during the Middle Holocene (Cremaschi *et al*., 2014). Usually, rock-shelters in Central Sahara are formed due to the presence of harder *stratum* such as rocks of different origins that survived weathering erosion throughout millennia. Takarkori is not an exception and it is above a small and well-defined sandstone terrace, 100 metres elevated from the plain floor.

It is sheltered on the east side by a 30-meter-high cliff (Fig. 3) (Cremaschi *et al*., 2014), which has protected its most recessed area allowing extraordinary preservation of artefacts.

Figure 3: Takarkori rock-shelter and the 30-meter-high cliff (http://www.palinopaleo-bot.unimore.it).

Its excavation was carried out over different years and in different seasons (2003, 2004 and 2006) during the "Archaeological Mission in the Sahara" of Sapienza University of Rome directed by Professor Savino di Lernia. A total of 143 m^2 out of the whole surface of the terrace has been excavated. Four areas have been outlined (Fig. 4): the Main Sector (117 m^2), the Northern Sector, the T1 Sector, and the Western Sector (Biagetti and di Lernia, 2013).

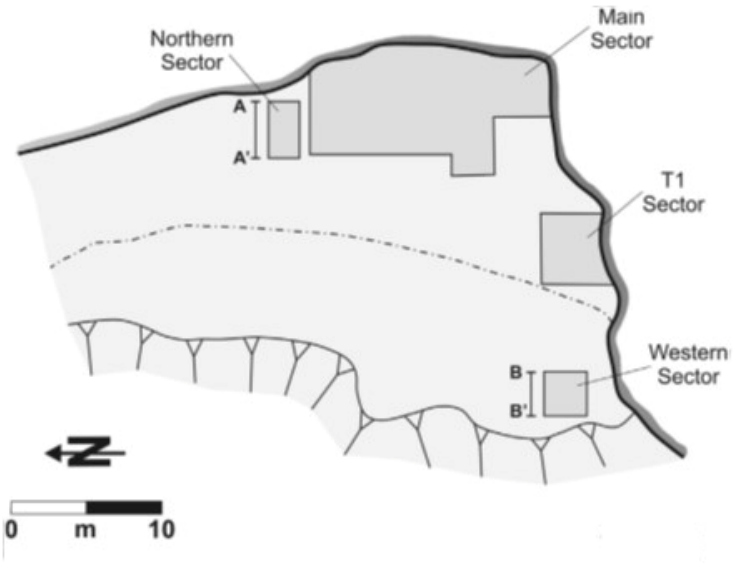

Figure 4: Representation of the four sectors excavated in Takarkori rock-shelter (Cremaschi et al., 2014).

The sediments from all sectors were periodically collected and dry-sieved with different meshes (10.2 and 0.5 mm) (Fig. 5) (Biagetti and di Lernia, 2013). The Takarkori site is divided into eight cultural phases listed in Table 1 (Biagetti and di Lernia, 2013).

Figure 5: Example of Takarkori soil sediment (sample n.1 LA1, layer L355, square R16) divided into bags after using three different size meshes during excavation. The samples have been stored at the University of Modena and Reggio Emilia.

Table 1: Cultural Phases in Takarkori.

CULTURAL PHASES	uncal BP	cal BCE	cal KA
Late Acacus (LA) 1	8.900-8.500	8.250-7.500	10,2-9,4
LA 2	8.500-7.900	7.600-6.650	9,5-8,6
LA 3	7.900-7.400	7.050-6.100	9,0-8,0
1st Ancient Holocene crisis	7.500-7.200	6.350-6.050	8,3-8,0
Early Pastoral (EP) 1	7.400-6.900	6.400-5.700	8,3-7,6
EP2	6.900-6.400	5.900-5.300	7,8-7,2
1st Middle Holocene crisis	6.400-6.100	5.450-5.150	7,4-7,1
Middle Pastoral (MP) 1	6.100-5.500	5.200-4.250	7,1-6,2
MP2	5.500-5.000	4.450-3.700	6,4-5,6
2nd Middle Holocene crisis	5.100-4.800	3.950-3.650	5,9-5,6
Late Pastoral (LP) 1	5.000-4.000	3.950-2.350	5,9-4,3

Since the last expedition in 2006, unfortunately, the area has been inaccessible due to political reasons. Notwithstanding this, researchers have been able to keep archaeological studies of the Takarkori site alive, thanks to the development of novel techniques and innovative analyses on previously collected sediments and artefacts. Archives and collections have never been as important as in this historical moment.

Thankfully, several soil samples collected from Takarkori, have been stored at the Laboratory of Palynology and Palaeobotany of the University of Modena and Reggio Emilia directed by Professor Anna Maria Mercuri. The samples are rich in botanical residues and they were transferred to Modena to be studied from an archaeobotanical point of view. The material covers almost the entire chronological sequence of the Takarkori site, from the Late Acacus to the Late Pastoral period. Due to the dry nature of the soil, the material has been stored in a ventilated storeroom to prevent excessive humidity and to protect them from any sort of damage and contamination. Each sample has been labelled with a unique code that consists of a series number followed by the square of the excavated area and the layer where it comes from.

Figure 6 shows the exact location where the samples currently held at the University of Modena and Reggio Emilia have been collected during Takarkori past excavations. The biggest and the most ancient excavated areas of the rock-shelter, respectively the Main

sectors and the Northern sectors are highlighted in beige dotted squares. For each excavated square, different coloured symbols indicate the position of recovered soil samples belonging to distinct periods. On each symbol, the serial number which identifies univocally the stratigraphic layer of the sample.

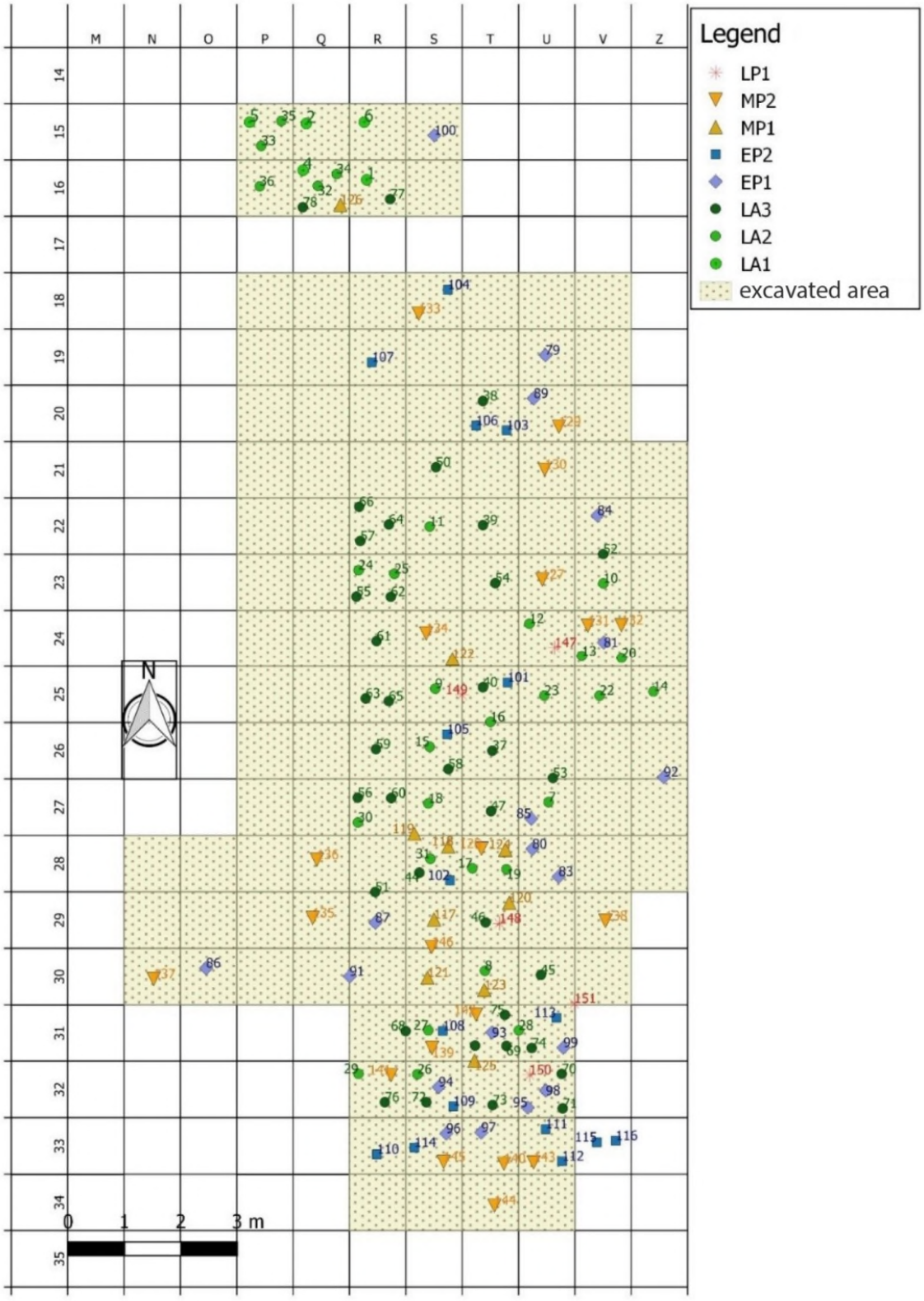

Figure 6: Visual representation of samples location collected from the Northern Sector and in the Main Sector (LP= Late Pastoral, MP= Middle Pastoral, EP= Early Pastoral, LA= Late Acacus).

Thanks to the presence of this exhaustive archive in Modena, in addition to archaeobotanical analyses, it was possible to study the Takarkori site from an archaeoentomological point of view. Fifty-seven soil samples were randomly selected for analysis. The list of samples studied with the weight analysed for each, expressed in grams, is listed below (Tab. 2).

Table 2: Selected samples with co-ordinates of their location and total weights of soil which has been analysed.

Sample N.	Chronology	Layer	Square	Weight of soil analysed (g)
1	LA1	L355	R16	1471.51
4	LA1	L394	Q16	817.32
5	LA1	L401	P15	582.56
6	LA1	L409	R15	1807.93
32	LA1	L374	Q16	507.51
34	LA1	L382	Q16	1008.97
35	LA1	L383	P15	858.32
36	LA1	L384	P16	1318.9
16	LA2	L220	T25-26	455.47
17	LA2	L224	T28	283.11
18	LA2	L225	S27	266.18
20	LA2	L228	V24	500.95
22	LA2	L240	V25	518.21
25	LA2	L275	R23	413.63
28	LA2	L302	T-U31	321.90
30	LA2	L314	\	341.13
46	LA3	L103	T29	591.57
47	LA3	L103	T27	152.18
50	LA3	L130	S21	133.85
56	LA3	L195	R27	433.88
57	LA3	L196	R22	273.27
58	LA3	L213	S26	569.28
61	LA3	L249	R24	143.14
69	LA3	L289	T31	208.39
78	LA3	L369	Q16	380.69
80	EP1	L38	U28	196.17
86	EP1	L38	V22	185.88
87	EP1	L82	R29	253.67
89	EP1	L93	U20	215.72

94	EP1	L268	S32	324.41
95	EP1	L286	U32	293.17
97	EP1	L290 contatto L276	T33	449.94
99	EP1	L291	U31	370.74
100	EP1	L336	S15	198.11
103	EP2	L66	T20	268.34
105	EP2	L66	S26	269.41
107	EP2	L94	R19	538.51
109	EP2	L245	S32	247.60
111	EP2	L245	U33	338.02
114	EP2	L271	S33	263.30
117	MP1	L42	S29	141.07
119	MP1	L45	S 27-28	160.09
121	MP1	L56	S30	174.5
122	MP1	L108	livello cineroso	410.59
123	MP1	L132	T30	471.18
124	MP1	L132	T28 lente	30.79
125	MP1	L263	T32-33	268.45
126	MP1	L358	Q16	292.00
127	MP2	L25	U23	138.85
129	MP2	L25	U20	54.90
128	MP2	L25	T28 lente	173.11
136	MP2	L76	Q28	424.06
140	MP2	L191	T33	236.66
144	MP2	L226	T34	333.84
147	LP1	L6	U24	1778.51
148	LP1	L14	T29	497.50
149	LP1	L19	S-T25	217.26

2.2 COLLECTION OF ENTOMOLOGICAL SAMPLES IN SOIL

The most extensively used method among archaeobotanists and archaeoentomologists to recover organic residues from sediments is currently paraffin flotation (Buckland, 1976; Panagiotakopulu, 2000). Paraffin, also known as kerosene, is a hydrocarbon complex derived from petroleum (Lam *et al*., 2012). Insect cuticles attract paraffin due to their wettability because it is less dense than water (Kenward, 1974). The hydrophobic nature of paraffin makes the coated insects float on the surface when immersed in water

(Rousseau, 2011). The first application of this method for palaeoentomological analysis was performed by Coope and Osborne in 1967. Since then, it appears to have been used systematically as a standard method by numerous researchers in this field. Throughout the decades, some improvements have been adopted to increase the recovery rate and to decrease the mechanical damage to the entomological sample (Kenward *et al*., 1980). Once insect fragments are collected and carefully rinsed from the paraffin, they are usually preserved in alcohol (Rousseau, 2011). Although the method speeds the recovery process greatly, it cannot be applied in all environments and to all soils (Kenward and Large, 1998). While waterlogged lands lend themselves well to this kind of treatment, dry sediments risks to be compromised by it (Panagiotakopulu, 2000). Furthermore, as stated by Rousseau (2011), the paraffin flotation efficiency to collect insects is around 85%, which can be considered insufficient for specific studies focused on the presence or absence of certain taxa.

Meticulous evaluation of the Takarkori soil revealed paraffin flotation inappropriate for several reasons. Firstly, the sand-size and dryness of the samples that allowed incredible organic material preservation could have been destabilised by water. Secondly, some of the soils selected for the study were excavated from fire pits. Additional precautions have to be considered when charred materials are involved due to their brittleness. At last, the alleged loss of information caused by the method itself was considered substantial and not suitable for the Takarkori case, in which even the recovery of a single fragment would have been important.

An average between 20-100% of soil for each sample selected have been examined under a binocular stereoscopic microscope at the Laboratory of Palynology and Palaeobotany of the University of Modena and Reggio Emilia. Insect fragments were collected manually with tweezers and fine paintbrushes to prevent additional damages of the specimens. During the collection, insects from different meshes were kept separated and divided into taxonomical orders. All the entomological material has been stored dry in plastic vials labelled with the same unique code previously described. Then, the material has been

transferred at FLEA (Forensic Laboratory of Entomology and Archaeology) at the University of Huddersfield, for further entomological analysis.

2.3 TECHNICAL NOTE: CLEANING TECHNIQUES

Identification of insect fragments may prove difficult and time-consuming, especially when archaeological specimens are considered. External substances further negatively affect their state of preservation, covering and obscuring diagnostic features. Although Takarkori specimens resulted in an extraordinary good preservation pattern, a great number required cleaning procedures before identification.

2.3.1 Introduction

During the past decades, several methods and techniques of insect cleaning, designed especially for adult beetles belonging to museum collections or for immature stages prepared for SEM (Scanning Electron Microscopy) observation, have been described (Nelson, 1949; Keirans *et al*., 1976; Corwin *et al*., 1979; Jenkins, 1991; Harrison, 2012; Schneeberg *et al*., 2017). In literature, cleaning techniques are categorised into two main groups: methods based on mechanical removal of the dirt particles, and methods based on a soaking system using different solvents (Harrison, 2012). The selection of which method is the most suitable for a specific specimen is strictly related to the state of insects' conservation (how fragile the specimen is, the developmental stage considered, how old the sample is, etc.) and to the chemical and physical nature of the substance deemed to be covering it. In principle, to be correctly identified, specimens have to preserve all the distinctive features after the cleaning treatment. Avoiding any damage to the sample is, therefore, 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. Thus, it is important to balance the efficiency in processing entomological samples. In addition, because of the more and more common application of DNA techniques for species

identification, cleaning processes should not interfere with the DNA extractability and integrity (Amendt *et al.*, 2010).

In order to better understand which cleaning technique was the most suitable to be used specifically on Takarkori specimens, six chemical-physical methods have been tested. Two different experiments have been performed. The first one aimed to evaluate the efficiency of each method in removing external substances, improving the visual assessment of diagnostic features. The second one was designed to investigate the compatibility of each cleaning technique with potential molecular identification. Procedure guidelines are presented, and tooltips for each method are listed.

2.3.2 TEST 1: Materials and Methods

Six methods were selected from the literature according to their ability to dissolve or remove specific substances (Tab. 3). Costs and availability of solutions were considered to select methods affordable by an entomological laboratory. Diptera puparia from forensic and different archaeological contexts covered by various substances were selected for their high availability at the laboratory (FLEA). After a preliminary qualitative evaluation under a microscope, the most adequate method according to the substances present was applied to the puparia.

Microphotographs of before and after treatments were taken using a Keyence VHX-S90BE digital microscope, equipped with Keyence VH-Z250R and VH-Z20R lens and VHX-2000 Ver.2.2.3.2 software (Keyence).

Table 3: Cleaning methods selected.

METHOD	SUITABLE FOR	REFERENCES
WARM WATER AND SOAP SOLUTION	✓ Fibres ✓ Dust ✓ Sludge	(Giordani *et al.*, 2018)
SONICATION	✓ Dross ✓ Soil debris ✓ Sand ✓ Botanical residues	(Ronderos *et al.*, 2000)

GLACIAL ACETIC ACID	✓ Inorganic crystals	(Zangheri, 1981)
SODIUM HYDROXIDE SOLUTION	✓ Putrefactive liquids ✓ Any organic matter	(Gurney *et al*., 1964) (Sukontason *et al*., 2007)
HYDROCHLORIC ACID/SODIUM BICARBONATE	✓ Oily substances ✓ Grease	(Zangheri, 1981)
SODIUM HYPOCHLORITE	✓ Organic matter ✓ Bacteria ✓ Mould/Fungi	(Gifawesen *et al*., 1975) (Stueben and Linsenmair, 2008)

a) WARM WATER AND SOAP SOLUTION METHOD

Puparia were soaked in a solution of warm water (~ 60 °C) and commercial dish soap (depending on the brand of dish soap, component percentages might vary: sodium linear dodecylbenzene sulfonate, sodium lauryl alcohol triethoxy sulfate, lauric/myristic monoethanolamide, hydrotrope mixture, magnesium sulfate, colourant, petrolatum, perfume, ethyl alcohol 95%, deionized water) for approximately 10-30 minutes depending on the substances attached on their surface, and then they were wiped with paintbrushes. The processed samples were then rinsed with deionized water and air-dried.

b) SONICATION

Puparia were placed separately inside vials filled with deionized water and then individually sonicated between 5 and 15 seconds, depending on the preservation status, using a sonicator bath (QH. Kerry Ultrasonic Limited, f = 50 Hz). The samples were rinsed with clean deionized water and air-dried.

c) GLACIAL ACETIC ACID

Puparia were gently wiped with a paintbrush soaked in glacial acetic acid or immersed in the acid for 5 minutes. They were then rinsed several times with deionized water, to stop the chemical reactions, and air-dried after.

d) SODIUM HYDROXIDE SOLUTION

Puparia were immersed in sodium hydroxide (NaOH) 10% solution either for 5 or for 10 minutes. The solution was prepared by adding sodium hydroxide solid crystal to water.

The samples were then washed gently in running deionized water to stop the chemical reaction, and air-dried.

e) HYDROCHLORIC ACID/SODIUM BICARBONATE

This method combines several different solutions in a pre-set order. Puparia were first immersed in distilled water for 24 hours, and then placed in a clean vial with hydrochloric acid for 10 minutes and soaked in a saturated solution of sodium bicarbonate for 15 minutes immediately afterwards. Finally, puparia were wiped with paintbrushes. The samples were washed with deionized water and air-dried, before being microscopically observed.

f) SODIUM HYPOCHLORITE

Puparia were soaked for 5 and 10 minutes in a 5% solution of sodium hypochlorite. The specimens were washed under deionized running water and air-dried before identification.

2.3.3 TEST 2: Materials and Methods

All the molecular analyses were performed on modern puparia of *Lucilia sericata* (Meigen, 1826) obtained from a breeding colony at FLEA. Puparia were subjected to two different treatments prior to DNA extraction. The first batch of puparia underwent previously described cleaning procedures immediately after the adults' emergence and straight after DNA extractions had been performed (as a control, three puparia without any cleaning treatment were selected). The second batch, after adults' emergence, was placed in small pierced plastic boxes containing a mixture of decontaminated horse blood, cat food, and ground soil, mimicking the conditions of thanatocoenosis and taphocoenoesis. The containers were closed and stored inside the laboratory at room temperature. Then, the six cleaning techniques were applied to the puparia after seven days of incubation inside the mixture (as a control, six puparia were selected, three not placed in the mixture and not cleaned with any methods and three placed in the mixture but not cleaned with any techniques). Besides, further sodium hydroxide concentrations (saturated and 1%) were tested. All DNA extractions were performed in triplicate using the QIAamp DNA Investigator Kit (QIAGEN, Redwood City, CA, USA). The manufacturer's protocol was

followed, apart from the additional use of Proteinase K (100 μg/ml) from PROMEGA (Madison, Wisconsin, USA). Elution was performed with 50 μl of Buffer ATE. Quantification was performed using a Qubit® 3.0 Fluorometer (Thermo Scientific, Waltham, Massachusetts, USA). Universal LCO-1490 Forward primer (5′ GGTCAACAAATCATAAAGATATTGG-3′) and HCO-2198 Reverse primer (5′-TAAACTTCAGGG TGACCAAAAAATCA-3′) were used (Folmer *et al*., 1994) to amplify the mitochondrial COI gene (685 bp long) using Polymerase Chain Reaction (PCR). Master-mix reactions of 20 μl final volume were prepared following the PROMEGA GoTaq® Flexi Polymerase protocol, which included Colourless GoTaq Flexi Buffer (5×), MgCl2 (25 mM), primers (IDT) (10 pmol/μl), Nucleotide Mix (10 mM), GoTaq DNA Polymerase (5 u/μl) and 2-4 μl of DNA template. The amplification programme (initial heat activation step at 95 °C for 10 min, 35 cycles of 95 °C for 1 min, 49.8 °C for 1 min, 72 °C for 1 min, and a final extension step at 72 °C for 10 min) was set up on a BioRad C1000 Thermal Cycler (Bio-Rad Laboratories, Inc.). Standard gel electrophoresis in 1.5% agarose gel stained with Midori Green Advanced DNA Stain (Geneflow, Elmhurst, UK) was used to check each PCR reaction. In case of positive results, 15 microliters of PCR products underwent purification using QIAquick PCR Purification Kit® (QIAGEN) following the manufacturer's instructions. Purified amplicons were sequenced by Eurofins (Eurofins Operon MWG, Ebersberg, Germany) following the standard Sanger method. Sequence comparison with online available sequences was performed using the online system BLASTn®.

2.3.4 TEST 1: Results and Discussion

All the methods selected worked differently. Most of them removed the bulk of external substances after the first cleaning attempt. Before and after pictures are shown in Fig. 7.

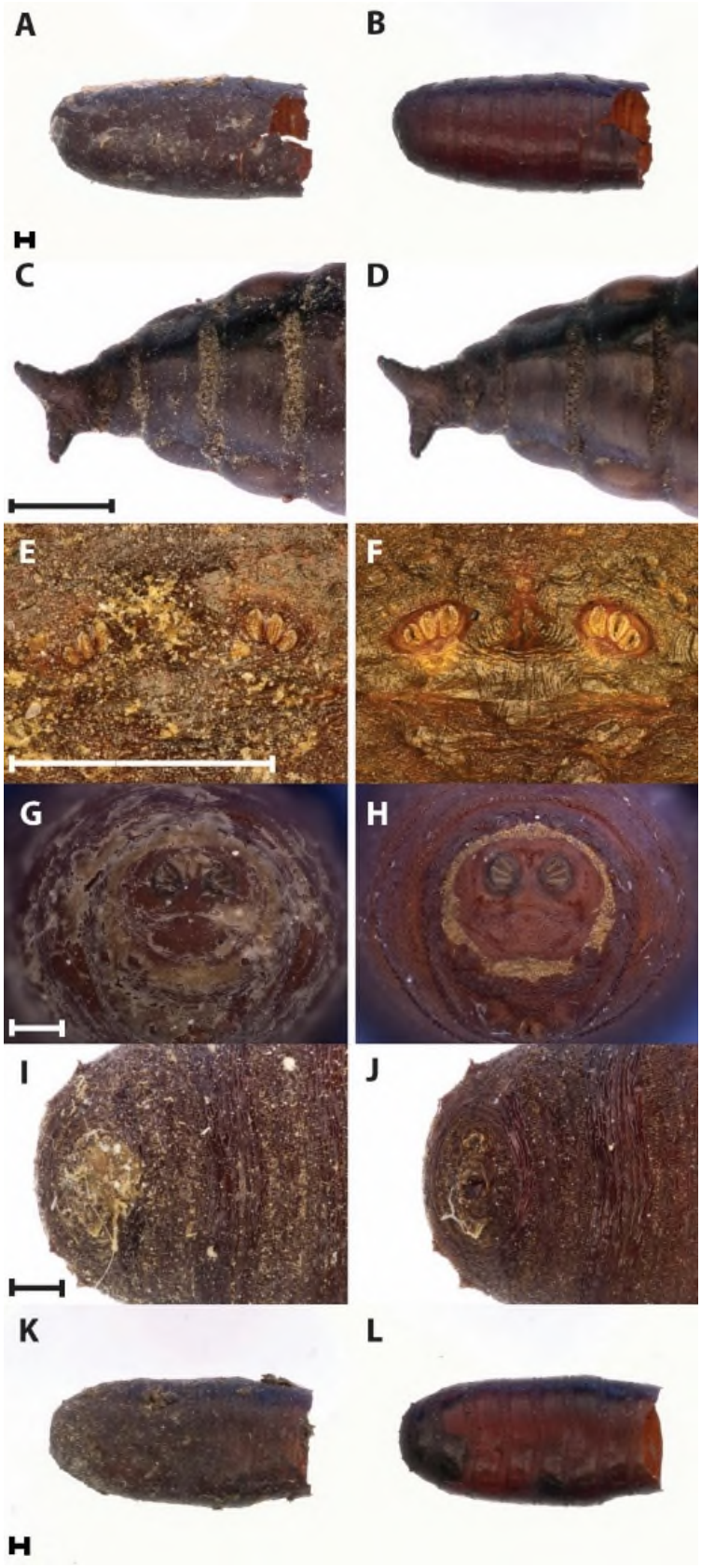

Figure 7: Before and after pictures: A-B Water and soap solution, C-D Glacial Acetic Acid, E-F Sonication; G-H Sodium hydroxide 10 % solution, I-J Hydrochloric acid/sodium bicarbonates solutions, K-L Sodium hypochlorite 1-5% solution. Scale bars: 500 µm.

Despite the excellent visual results, it is worth mentioning that it is not always possible to achieve a totally cleaned puparium surface, due to the nature of the substances covering the puparia, which are a heterogeneous mixture. However, most of the time it is sufficient for identification purposes. Different types of substances can simultaneously cover the external surface of a puparium. Hence, according to the composition of each substance, it may be necessary to use more than one method or to perform the same cleaning method several times to obtain a perfectly clean surface. Even though it may seem reasonable and fair to proceed until reaching the highest level of cleanliness, those multiple and/or combined methods affect the structure of the puparium. The latter is a thin layer of chitin and, according to its conservation status (forensic or archaeological sample), can be particularly fragile. Any damage to the puparia structure, as said before, has to be avoided to prevent loss of information useful for the species identification.
A brief qualitative analysis and tooltips for each method are listed below.

a) WARM WATER AND SOAP SOLUTION METHOD

It is the most affordable and most effective method. The permanence of the puparia in warm water and soap can be prolonged as long as the operator is aware of the positive correlation between time and softness. This means that, during the final brushing, the operator needs to pay attention not to crush the puparium, which becomes more fragile.

b) SONICATION

The sonication method is particularly effective on encrusted debris. It works also on desiccated muddy or sludgy material, but, in those cases, it is a time-consuming process. Desiccated material, once rehydrated, usually stains the water inside the vial, not allowing a precise check on the status of the specimens treated.
A multiple and/or prolonged sonication can widen the cracks present naturally on the puparia after the eclosion of the adults. In worst cases, posterior spiracles and anal plate can be ripped out from the puparium by the vibration, with the consequent loss of identification features. It is suggested, especially on archaeological samples, to check carefully the conservation status (presence of cracks on the surface) of each specimen prior

to sonication. The more cracks are present, the less time is needed inside the sonication bath.

c) GLACIAL ACETIC ACID

This method is effective at dissolving inorganic crystals. Commonly used by coleopterists, it was not previously tested on dipterous puparia. Due to its corrosive nature, low quantities and several rinsing passages are suggested. Some archaeological samples have to be evaluated closely before using acetic acid. In specific cases, due to the process of per-mineralisation (fossilisation process, during which mineral deposit creates a cast of the organism), a total or partial substitution of the organic matter can happen to the pupae. In these cases, acetic acid can destroy the sample.

d) SODIUM HYDROXIDE SOLUTION

The solution is very effective on samples covered by organic substances such as putrefactive liquids. This method is also commonly used to diaphanise larvae for slide microscopy.

e) HYDROCHLORIC ACID/SODIUM BICARBONATE

This method was described by Zangheri (1981) to clean Coleoptera from museum collections. It is the most time-consuming method as it involves an initial 24-hour immersion in water. It is also the least effective of all methods, usually leaving a thin residue layer behind. Hence, additional cleaning with one of the other methods is also required.

f) SODIUM HYPOCHLORITE

Bleach is a common solution and easily present inside an entomological laboratory. It is known to disinfect and to react with many natural pigments. However, the solution is not particularly effective as a cleaning solution and, as a minor result, it decolours the specimens.

2.3.5 TEST 2: Results and Discussion

Results of the first group of puparia treated with cleaning procedures immediately after the adults' emergence are presented in Table 4. DNA was positively extracted from the

controls, from all the samples that were immersed in sodium hydroxide 10% solution for 5 and 10 minutes, from sonicated samples, from samples washed with water and soap, and from samples brushed with glacial acetic acid (Fig. 8). However, DNA extraction failed with samples immersed in glacial acetic acid, in bleach for 5 and 10 minutes, and with samples treated with the combination of hydrochloric acid and sodium bicarbonate solutions.

Table 4: Quantifications of samples cleaned immediately after adults' emergence (CNTRL = control; NaOH = sodium hydroxide; SON= sonication; GAA= glacial acetic acid; H2O = warm water and soap; ZAN= hydrochloric acid/sodium bicarbonate solutions; BL= bleach; ✓ positive results for the expected fragment, ✗ negative results for the expected fragment).

SAMPLES	MEAN DNA CONCENTRATION ± SD (ng/µl)	PCR
1 CNTRL	0.332±0.001	✓
2 CNTRL	0.508±0.400	✓
3 CNTRL	0.441±0.011	✓
1 NaOH 10 %, 5'	<0.001	✓
2 NaOH 10 %, 5'	<0.001	✓
3 NaOH 10 %, 5'	<0.001	✓
1 NaOH 10 %, 10'	<0.001	✓
2 NaOH 10 %, 10'	<0.001	✓
3 NaOH 10 %, 10'	<0.001	✓
1 SON	<0.001	✓
2 SON	0.071±0.006	✓
3 SON	0.062±0.005	✓
1 GAA immersed	0.281±0.067	✗
2 GAA immersed	0.479±0.031	✗
3 GAA immersed	0.465±0.003	✗
1 GAA paintbrush	<0.001	✓
2 GAA paintbrush	<0.001	✓
3 GAA paintbrush	<0.001	✓
1 H_2O	<0.001	✓
2 H_2O	<0.001	✓
3 H_2O	<0.001	✓
1 ZAN	<0.001	✗
2 ZAN	<0.001	✗
3 ZAN	<0.001	✗

1 BL 5'	<0.001	✗
2 BL 5'	<0.001	✗
3 BL 5'	<0.001	✗
1 BL 10'	<0.001	✗
2 BL 10'	<0.001	✗
3 BL 10'	<0.001	✗

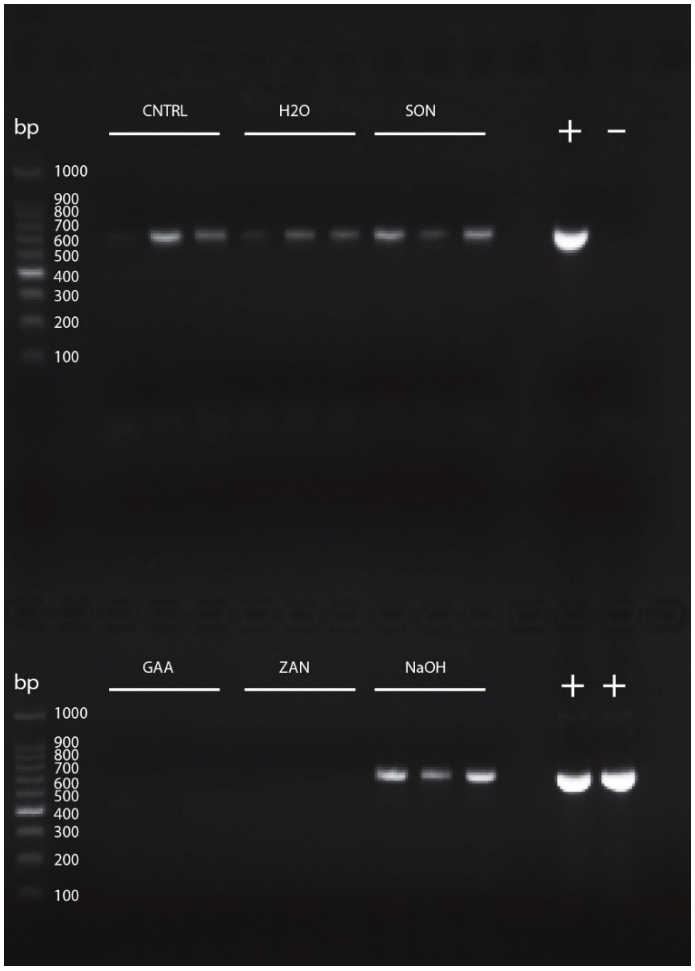

Figure 8: Agarose gel visualizing the PCR products. On the right the positive/negative controls. (CNTRL = control; NaOH = sodium hydroxide; SON= sonication; GAA= glacial acetic acid; H2O = warm water and soap; ZAN= hydrochloric acid/sodium bicarbonate solutions).

Results of the second group of puparia, which were placed in a mixture of decontaminated horse blood, cat food, and ground soil for a week and then cleaned, are presented in Table 5. DNA was extracted from the controls, but the PCR was negative for all the controls except for controls number 2 and 3, which were not cleaned with any methods. PCR was

successful from samples immersed for 5 and 10 minutes in sodium hydroxide solutions (1%, 10% and saturated) (Fig. 9), from sonication, and from the samples brushed with glacial acetic acid. One sample washed in warm water/soap solution, and one sample immersed in glacial acetic acid, also showed positive results. The rest of the puparia cleaned with warm water/soap solution, immersed in glacial acetic acid and bleach, and treated with hydrochloric acid/sodium bicarbonate solutions did not show any positive results.

Table 5: Quantifications of samples placed in the mixture for a week and then cleaned (CNTRL = control; NaOH = sodium hydroxide; SON= sonication; GAA= glacial acetic acid; H2O = water/soap; ZAN= hydrochloric acid/sodium bicarbonate solutions; BL= bleach; ✓ positive results for the expected fragment, ✗ negative results for the expected fragment).

SAMPLES	MEAN DNA CONCENTRATION ± SD (ng/µl)	PCR
1 CNTRL	1.140±0.010	✗
2 CNTRL	0.787±0.082	✓
3 CNTRL	0.687±0.017	✓
1. CNTRL	4.360±0.280	✗
2. CNTRL	4.726±3.002	✗
3. CNTRL	2.666±0.544	✗
1. NaOH 10 %, 5'	<0.001	✓
2. NaOH 10 %, 5'	<0.001	✓
3. NaOH 10 %, 5'	<0.001	✓
1. NaOH 10 %, 10'	<0.001	✓
2. NaOH 10 %, 10'	<0.001	✓
3. NaOH 10 %, 10'	<0.001	✓
1. NaOH 1%, 5'	<0.001	✓
2. NaOH 1%, 5'	<0.001	✓
3. NaOH 1%, 5'	<0.001	✓
1. NaOH 1%, 10'	<0.001	✓
2. NaOH 1%, 10'	<0.001	✓
3. NaOH 1%, 10'	<0.001	✓
1. NaOH sat, 5'	<0.001	✓
2. NaOH sat, 5'	<0.001	✓
3. NaOH sat, 5'	<0.001	✓
1. NaOH sat, 10'	<0.001	✓
2. NaOH sat, 10'	<0.001	✓
3. NaOH sat, 10'	<0.001	✓

1. SON	0.157±0.009	✓
2. SON	<0.001	✓
3. SON	<0.001	✓
1. GAA immersed	0.805±0.007	✓
2. GAA immersed	<0.001	✗
3. GAA immersed	0.229±0.065	✗
1. GAA paintbrush	<0.001	✓
2. GAA paintbrush	<0.001	✓
3. GAA paintbrush	<0.001	✓
1. H_2O	<0.001	✓
2. H_2O	<0.001	✗
3. H_2O	<0.001	✗
1. ZAN	<0.001	✗
2. ZAN	<0.001	✗
3. ZAN	<0.001	✗
1. BL 5'	<0.001	✗
2. BL 5'	<0.001	✗
3. BL 5'	0.224±0.082	✗
1. BL 10'	<0.001	✗
2. BL 10'	<0.001	✗
3. BL 10'	<0.001	✗

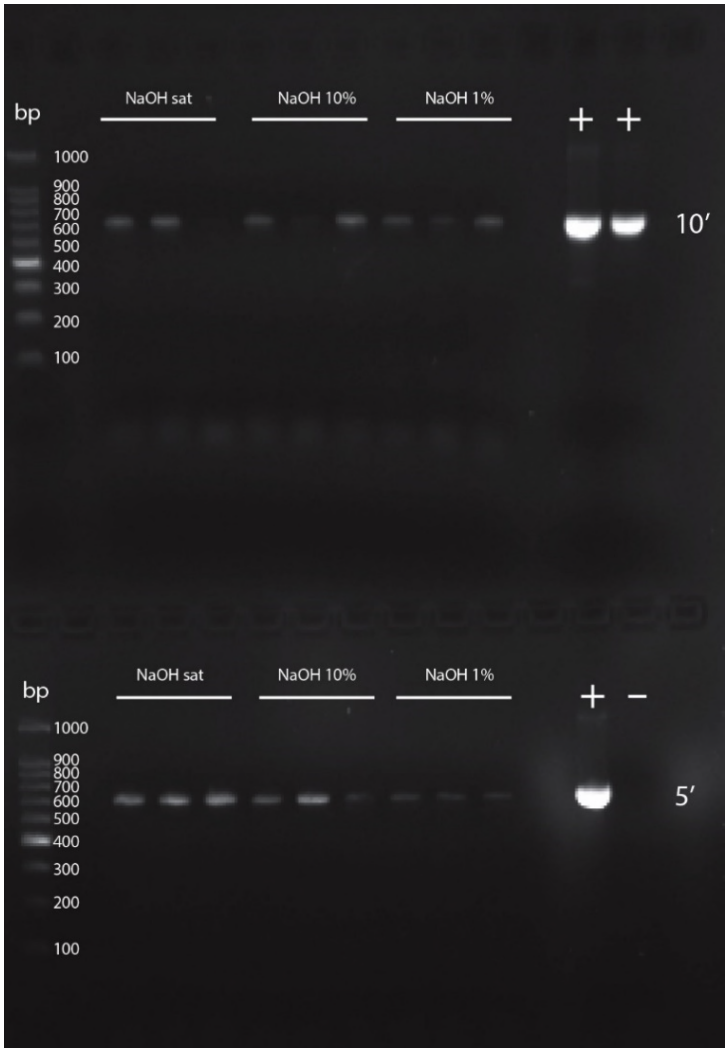

Figure 9: Agarose gel visualizing the PCR products obtained from samples immersed for 5 and 10 minutes in different sodium hydroxide solutions (saturated, 10%, and 1%), including positive and negative controls.

The mean quantifications of both puparia groups were quite low. This was expected due to the low quantities of DNA present in a single puparium. Most of the samples showed quantities below the detectable threshold of the Qubit 3.0 fluorometer, which is 0.001 ng/µl. However, PCR confirmed the amount of DNA extracted was enough for species identification, as all the samples sequenced were identified as *Lucilia sericata*.

2.3.6 Conclusion

All six methods selected successfully cleaned the insects. However, if morphological and molecular analyses are taken into account together, the best methods, with positive results

in both analyses, were the warm water/soap, sonication and sodium hydroxide solutions. Hydrochloric acid/sodium bicarbonate solutions, bleach, and glacial acetic acid immersion are, therefore, not recommended to clean entomological samples if the molecular analysis is required. Furthermore, prolonged and multiple treatments with any of the cleaning methods might result in damage of insect remains and a further loss of DNA.

Following the results of the two tests, the Takarkori material has been treated with warm water/soap, sonication and sodium hydroxide solutions, avoiding when possible hydrochloric acid/sodium bicarbonate solutions, bleach, and glacial acetic acid immersion.

2.4 MICROSCOPY AND MICROPHOTOGRAPHY

Observation for identification purposes of Takarkori material has been conducted using a binocular Leica DM500 microscope (Wetzlar, Germany). Microphotographs of entomological remains were taken using a Keyence VHX-S90BE digital microscope, equipped with a Keyence VH-Z250R and a VH-Z20R lens and the VHX-2000 Ver.2.2.3.2 software (Keyence, Osaka, Japan), as well as a Leica M60 stereomicroscope (Wetzlar, Germany), equipped with a DFC425c digital microscope camera with a c-mount interface and with a 5 Megapixel CCD sensor. Entomological pictorial plates have been assembled using the photo editing software: Adobe Photoshop® and Adobe Illustrator®.

2.5 MORPHOLOGICAL IDENTIFICATION OF INSECTS

The morphological identification of insects is based on the principle that species differ in their phenotypic appearance. At the beginning of the 17th century, the ground-breaking invention of the microscope has led to the description of many species, overcoming the previous limitations related to the small size of peculiar and essential features. A large number of insect types became available for comparison all around the world due to the renewed interest of taxonomists.

Identification, indeed, can be performed by comparison or using dichotomous keys. Traditionally, the latter consist of a sequence of questions about the anatomical (e.g.

presence, shape and colour) structures of insects with already established answers. Starting from the first question, to proceed to the next one, it is necessary to choose one of the options available after a careful examination of the specimens to be identified. In the end, after working down the series of questions, when no further choice is available, the key will terminate with either an order, a genus, a family or a species. Although the final choice would give a name at the specimen analysed, it is important to double-check the identification by verifying the original description of the type for the species involved (Gullan and Cranston, 2014). Theoretically, this method appears to be simple and straightforward. This approach could be applied to all developmental stages, from immature to adult stages. However, several problems can be outlined, and they are all referable to the lack of complete knowledge of the entomological world. First, not all regions of the globe are well documented. Many insects are widespread globally, but many others are adapted to specific micro-environments and are endemic to restricted areas. The absence of extensive insect checklists in some areas can be challenging and sometimes inconclusive. Second, the majority of well-established keys in literature refers to adult insects. Although taxonomists are trying to create new dichotomous keys also for immature stages during the past century, the topic is still quite uncharted (Giordani *et al*., 2019). Third, due to the enormous diversity and species richness of the class Insecta, orders present identification keys when an entomologist took interest in them. Producing a good and viable key involves in-depth and time-consuming observation of anatomy and physiology of the specific group.

Since the beginning of the 20th century, rapid technological improvements in the biological field helped in the discovery of novel approaches to identify insects. Radioactive isotopes, electron microscopy, immunological studies, and paper chromatography are just examples (Foubert Jr and Stier, 1958; Downe, 1961). In the 1980s, the advances in molecular biology have supported the morphological identification process. Nowadays, a combined method which involved morphological and molecular identification is suggested during entomological analyses (Giordani, 2019).

However, despite the numerous novel studies on molecular identification, the morphological approach is still the most reliable method to identify insects especially when the genetic material is limited or degraded (Marchetti *et al.*, 2013).

Takarkori insect fragments have been morphologically identified using the available dichotomous keys present in literature (Skidmore, 1985; Giordani *et al.*, 2019) or by comparison with private and museum collections. External advice was necessary for some specimens. Indeed, identification of Lepidoptera (Alberto Zilli, curator for Macrolepidoptera, Natural History Museum, Department of Life Sciences, London, UK), and Hymenoptera (Formicidae: Fabrizio Rigato, Museo di Storia Naturale, Milan, IT; Aculeata: Davide Dal Pos, Department of Biology, University of Central Florida, Orlando, USA) were performed by taxonomists specialised on those orders. The identification has proved difficult and challenging due to the incomplete nature of the archaeological insect fragments. It was not always possible to achieve identification at the species level. Checklists of Northern Africa are not complete and not always available for consultation. In one case, the morphological identification of a closed puparium belonging to a parasite fly has been attained using monochromatic Synchrotron Radiation (SR) with phase-contrast imaging, an advanced technique which will be explained in the next paragraph 2.6.

2.6 SYNCHROTRON RADIATION APPLIED TO INSECT IDENTIFICATION

2.6.1 Introduction

Diptera remains are commonly recovered in archaeological contexts. Flies are holometabolous insect, which means they undergo a complete metamorphosis. Generally, the life cycle of flies includes egg, larval, pupal and adult stages (Fig. 10). During the pupariation process, the post-feeding larval cuticle goes through a series of chemical and physical changes, with the final formation of a hard case known as "puparium" (Martín-Vega *et al.*, 2016). It acts as a protective case in which the fly transforms and develops

wings. After the adult emergence, the puparium is left empty on the site. Due to its high resistance to decay, puparia can be found in archaeological contexts, where they might be the only traces of insect activity left after centuries or millennia (Vanin and Huchet, 2017; Giordani *et al.*, 2018; Pradelli *et al.*, 2019; Giordani *et al.*, 2020).

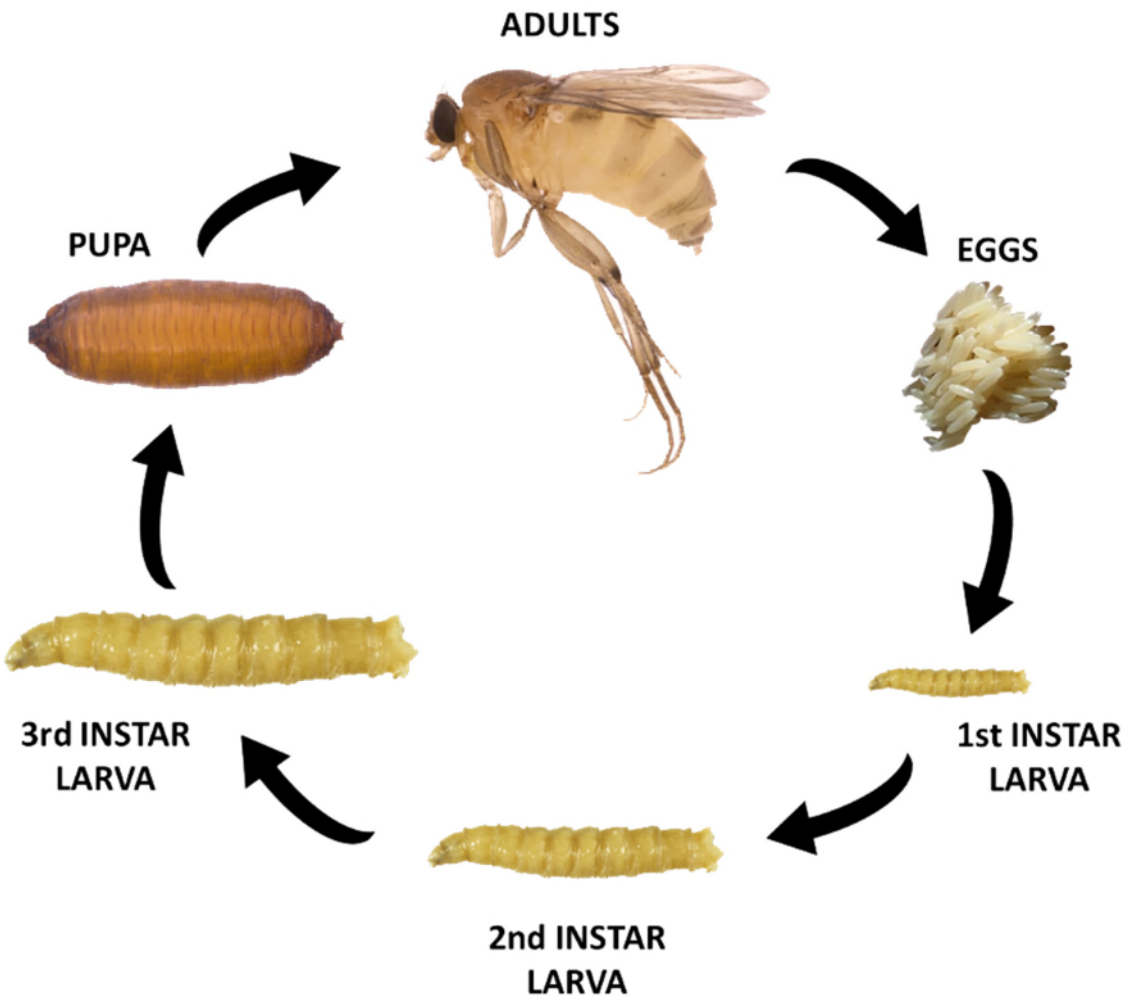

Figure 10: Fly life cycle.

The morphological identification of puparia is challenging due to the presence of a few diagnostic features on their outer surface. Most of the distinctive features are found in the posterior region, such as posterior spiracles and anal plate, and, on the ventral side of abdominal segment number 7, such as the size, shape, and distribution of spiculae (Giordani *et al.*, 2019). It is worth mentioning that oral sclerites can be analysed from a

puparium but often, especially in archaeological contexts, they are no longer present (Amendt *et al*., 2010).

Intrinsic factors and/or external environmental agents can interrupt the metamorphosis process, preventing the fly to emerge from the puparium. In these cases, depending on the stage of development reached by the insect before death, the observation of its hidden remains can help to identify the species. The detection of adult genitalia (a very strong diagnostic character), the shape of the legs and the presence of setae, the larval oral sclerites and the adult head, can be decisive in the success of the identification process. However, it is important, especially when dealing with fragile archaeological specimens, to preserve the integrity of the samples as much as possible. During Takarkori archaeoentomological analysis, a single closed puparium of a parasite fly has been recovered. The ability to observe morphological and anatomical structures, without destroying the evidence relies on new technologies. The standard techniques used to identify insects such as the optical microscopy or the SEM (Scanning Electron Microscopy) are not suitable for the study of a closed puparium. Neither the optical nor the electronic method allows a vision of the inner puparium. Only the dissection and histological analyses of the content have the potential to offer a very high spatial resolution of the internal structure. However, such an approach is extremely invasive and destructive and for this reason not applicable to a unique sample.

X-ray computed microtomography (micro-CT) can facilitate a detailed description of insect anatomy (Hall and Martín-Vega, 2019). This technique has been demonstrated to be useful for species around 1-2 cm but it is not informative enough for small details (Bostock *et al*., 2014) or specimens partially or completely mineralized such as the Takarkori sample.

2.6.2 Elettra Synchrotron Trieste

The Takarkori sample has been analysed at the Elettra Synchrotron Trieste S.C.p.A in Basovizza (Trieste, Italy) (Fig. 11). It is a multidisciplinary international research centre specialised in advanced light sources since 1993.

Figure 11: Elettra Synchrotron Trieste research center in Basovizza (Trieste, Italy) (https://www.wayforlight.eu, photo credit: Gabriele Crozzoli).

The facility is set up in two assets according to the type of light source provided. There is a third-generation electron storage ring called ELETTRA, which generates high-quality synchrotron radiation, and a linear accelerator named FERMI (an acronym for Free-Electron laser Radiation for Multidisciplinary Investigations), which produce a free-electron laser.

Synchrotron radiation is emitted when charged electrons are accelerated at relativistic speed in a curved path by magnetic fields (Kunz, 1974). The ELETTRA storage ring is 260 metres in circumference, and it is equipped with twelve identical groups of magnets. Each group is composed of four different types of magnets:

- bending magnets – to deflect electrons into a circular path
- quadrupoles – to focus the beam
- sextupoles – to compensate non-linear and chromatic effects
- steerer magnets – to stabilise the circular trajectory

This arrangement creates an expanded Chaseman Green magnetic lattice, also known as double ben achromat inside the ring (Wiedemann, 2003). The space in the lattice allows the installation of insertion devices 4.5 metres long, which are the principal source of photons. This configuration is typical of third-generation synchrotrons. Insertion devices can produce linear or circular polarised light depending on their magnetic configuration (electromagnets or permanent magnets) (Kim, 1989). The electron beam can also be adjusted depending on preferences by changing the magnetic field, either by modifying the flow in the coils or by varying the distance between magnet arrays. In 2010, an upgrade of the facility led to the installation of a full-energy injector, allowing users to operate 24 hours per day as the new injector operates in top-up mode. This means that the injector can continuously reinject electrons to keep the circulating current stable and constant. The configuration of the ELETTRA ring is unique and it is the only synchrotron in the world that can operate at two separate electron energies, 2.0 GeV and 2.4 GeV, for extended ultraviolet performances and x-ray emissions respectively. The synchrotron radiation produced by the ring is currently exploited by 28 beamlines (Fig. 12).

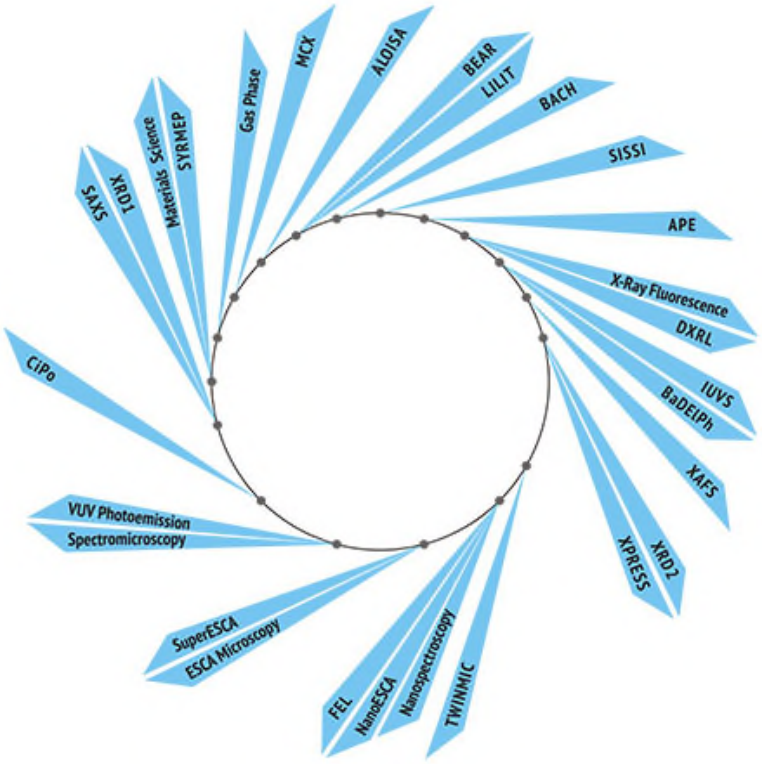

Figure 12: Beamlines currently active at ELETTRA (https://www.elettra.trieste.it).

Each beamline is used separately for different purposes. X-ray beams can be used in a wide range of techniques such as spectroscopy, spectro microscopy, lithography, and infrared microscopy.

2.6.3 SYRMEP beamline

The acronym SYRMEP stands for Synchrotron Radiation for Medical Physics. Its light source is the first bending magnet of section 6 of the ring. Specific light source parameters of SYRMEP are listed in Table 6.

Table 6: Light source parameters.

Type	Bending magnet
Critical Energy (at 2.0 GeV)	3.21 keV
Critical Energy (at 2.4 GeV)	5.59 keV
Source Size (at 2.4 GeV)	sx = 0.197 mm sy = 0.030 mm sy' = 0.013 mrad
Horizontal Beam Divergence	7 mrad

The beamline is located 23 metres from the source, and it can work with a monochromatic or a white-beam configuration (Abrami *et al.*, 2005). Table 7 presents the parameters of X-rays at the sample position.

Table 7: X-rays at the sample.

Energy range	8-35 keV
Energy Resolution	DE/E = $2^{*}10^{-3}$
Photon Flux (at 23 m, 20 keV, 100 mA, 2.4 GeV)	$2.0^{*}10^{8}$ photons/s mm^2
Beam Size	120x4 mm^2

The SYRMEP has been created by Elettra Synchrotron Trieste in collaboration with the University of Trieste and Istituto Nazionale di Fisica Nucleare (INFN "National Institute for Nuclear Physics") to aid and develop novel researches in medical diagnostic radiology, material science and life science. The beamline is particularly successful in these fields due to its monochromatic and laminar-shape beam, which drastically improve the acquisition of clear clinical images and reduce the absorbed dose of x-rays (Longo *et al.*, 2016). Also, its high spatial coherence and the possibility to use a phase-contrast imaging technique overcome the poor absorption typical of many biological samples (Tromba *et al.*, 2010).

2.6.4 TomoLab

The TomoLab is an X-ray micro-CT laboratory based on a cone-beam geometry and it has been designed to complement the SYRMEP beamline (Tuniz *et al.*, 2013). Created by Elettra Synchrotron Trieste in collaboration with the University of Trieste (Department of Civil and Environmental Engineering and degree in Dentistry and Dental Prosthesis) the TomoLab is active since 2006 (Cosmi and Biasi, 2011; Pasqualini *et al.*, 2012). There are two micro-CT scanners inside the laboratory, both with a sealed microfocus X-ray tube (Fig. 13). One has maximum voltage = 150 kV, maximum power = 75 W, minimum focal spot = 5 microns, the second one has maximum voltage = 130kV, maximum power = 39 W, minimum focal spot = 5 microns. A water-cooled CCD camera is installed as a

detector. These instruments can perform phase-contrast micro-CT measurements (limited phase-contrast compared to a synchrotron X-ray beam).

Figure 13: X-ray micro-CT scanner inside TomoLab (https://www.elettra.trieste.it).

2.6.5 The Takarkori sample

The monochromatic Synchrotron Radiation (SR) exploiting phase-contrast imaging of SYRMEP has been used to analyse the Takarkori sample. This technique allowed to visualise the internal structures of the specimen without extracting it from its puparium in a non-destructive way. A multi-resolution approach has been applied to acquire high-quality information. The specimen has been scanned at 5 μm pixel size to have a whole view of it and then at 0.9 μm pixel size to acquire small details. The photon energy used has been set up at 15 keV. The use of SR micro-CT allowed the observation and measurement of the internal structures, not only to study organisation and shape of the internal organs providing the necessary information for the insect development evaluation but as well visualisation of diagnostic feature for the species identification (e.g. genital organs). The technique also gives the possibility to have 3D reconstruction, allowing a morphological comparison with modern samples. Very low artefacts have been produced

by using a monochromatic SR beam compared to conventional cone-beam. The sample has been also analysed using the X-ray micro-CT scan inside TomoLab.

2.7 MOLECULAR IDENTIFICATION OF INSECTS

As stated in paragraph 2.5, during the last four decades, the rapid development of biomolecular techniques has supported the identification of the living creatures of the planet greatly. In 2004, the Barcoding of Life Project has been launched as a global standard aiming to create the largest database for eukaryotes (Hebert *et al.*, 2003). Hebert and colleagues (2003) have selected a mitochondrial region as the best molecular target to differentiate animals. The use of mitochondrial targets is beneficial as they are present in high copy number inside cells and they have a high mutational rate. The best mitochondrial target is COI (subunit I of Cytochrome Oxidase), which codifies a protein fundamental during the respiration process of a cell. Its whole length is 1535 bp, but only 658 bp in 5' end of the gene has been selected to become the barcode region to identify univocally different species. Insects due to their extreme biodiversity and their fundamental role in economical, epidemiological and agricultural fields have drawn the attention of researchers (Jinbo *et al.*, 2011). Currently, the DNA barcoding technique has become a simple, cost-effective, and rapid tool for taxonomists, ecologists, conservation biologists, agriculturists, etc. around the world. However, the method presents a series of problems and controversies (Meier, 2008; Jinbo *et al.*, 2011). Even though part of the scientific community relies exclusively on DNA-base identification, it is essential to reiterate the importance of morphological analysis to corroborate the result (Schindel and Miller, 2005; Hajibabaei *et al.*, 2007). So far, not every species of insects has been univocally identified through the DNA sequence, indeed, the reference DNA database (Barcode of Life Data Systems, known as BOLD, specifically created only to store COI sequencing data) is not complete yet (Tab. 8) (Ratnasingham and Hebert, 2007).

Table 8: Up to date progress of DNA barcoding library (BOLD*) of insects.

ORDER	Number of specimens barcoded	Number of species barcoded

Diplura	82	18
Protura	180	36
Collembola	58 216	1 315
Archaeognatha	416	38
Zygentoma	102	22
Ephemeroptera	22 072	2 155
Odonata	14 873	1 834
Dictyoptera	61	4
Blattodea	6 769	1 226
Isoptera	1 738	589
Mantodea	997	387
Dermaptera	1 026	64
Plecoptera	12 281	1 171
Orthoptera	20 122	2 969
Phasmatodea	1 777	314
Embioptera	175	70
Zoraptera	3	1
Grylloblattidae	10	5
Mantophasmatodea	2	1
Psocodea	18 375	1 166
Thysanoptera	10 422	395
Hemiptera	138 781	12 287
Neuroptera	4 129	523
Megaloptera	866	113
Raphidioptera	99	24
Coleoptera	239 972	33 398
Strepsiptera	400	82
Mecoptera	740	141
Siphonaptera	1 614	183
Diptera	619 739	23 925
Trichoptera	49 608	5 085
Lepidoptera	875 661	72 747
Hymenoptera	283 258	32 239
TOTAL	**2 384 566**	**194 527**

*Data accessed 16 August 2020 https://www.boldsystems.org/index.php.

Although the DNA extraction, amplification and sequencing have been successful for a lot of species, it is not always possible for others. Insects, specifically, can respond differently to this approach. Close related species, which present a low inter-specific divergence, do not differ enough to have a reliable identification (Moritz and Cicero, 2004; Meyer and

Paulay, 2005; Wiemers and Fiedler, 2007; Gilarriortua *et al*., 2015). Besides, when the fragments of DNA are shortened due to several factors, identification is not reliable below a certain threshold. For example, flies belonging to Sarcophagidae family cannot be discriminated using fragments below ~200 bp (Jordaens *et al*., 2013). In these cases, other molecular targets can be used to attempt identification (Tab. 9), but reference sequences are fewer inside databases, e.g. GenBank created by the National Center for Biotechnology Information, also known as NCBI (Benson *et al*., 2015).

Table 9: List of common molecular targets used in entomology (modified from Tuccia, 2020).

MITOCHONDRIAL TARGET	**REFERENCES**
COII	(Sperling *et al*., 1994); (Aly and Wen, 2013); (Boheme *et al*., 2011);
Cytb	(Gilarriortua *et al*., 2013; Gilarriortua *et al*., 2014); (Gilarriortua *et al*., 2015); (Giraldo *et al*., 2011); (Bortolini *et al*., 2018);
ND1	(Giraldo *et al*., 2011);
ND5	(Zaidi and Chen, 2011); (Zehner *et al*., 2004); (Bortolini *et al*., 2018);
28S rDNA	(Gibson *et al*., 2011); (Mcdonagh and Stevens, 2011); (Stevens and Wall, 2001);
16S rDNA	(Guo *et al*., 2014); (Li *et al*., 2010);
NUCLEAR TARGET	**REFERENCE**
CAD	(Gibson *et al*., 2011; Meiklejohn *et al*., 2013);
EF-1α	(Gibson *et al*., 2011); (Mcdonagh and Stevens, 2011); (Bortolini *et al*., 2018);
ITS1	(Zaidi and Chen, 2011);
ITS2	(Gilarriortua *et al*., 2014; Gilarriortua *et al*., 2015); (Zaidi and Chen, 2011); (Yusseff-Vanegas and Agnarsson, 2017);

PER	(Guo *et al*., 2014; Bortolini *et al*., 2018);

The great potential of the molecular identification method has yet to be fully exploited. Numerous and rapid advances in biotechnology are very promising and together with the morphological identification method, molecular identification can be the new key to explore biodiversity (Jinbo *et al*., 2011).

2.7.1 Ancient DNA

The genetic material inside modern specimens is abundant, allowing the molecular identification process to be relatively quick and simple. On the contrary, the retrieval and characterisation of ancient DNA (aDNA) isolated from palaeontological, archaeological, and historical specimens are challenging and very limited. Due to the unique nature of ancient specimens, it is mandatory to use a non-destructive DNA-extraction method. Currently, in literature, several methods have been described and designed to preserve the morphological integrity of specimens (Favret, 2005; Pons *et al*., 2006; Gilbert *et al*., 2007; Rowley *et al*., 2007; Badek *et al*., 2008; Hunter *et al*., 2008; Thomsen *et al*., 2009; Castalanelli *et al*., 2010). Most of the attempts to extract and characterise aDNA from insects has been performed successfully when special conservational pattern occurred to them. So far, insects found inside amber or preserved in permafrost revealed the presence of DNA (Cano *et al*., 1992; Desalle *et al*., 1992; Cano *et al*., 1993; Poinar, 1994; Cano, 1996; Chapco and Litzenberger, 2004; Reiss, 2006; King *et al*., 2009; Heintzman, 2013). However, controversies on the authenticity of some results have been debated within the scientific community (Austin *et al*., 1997). The major issues detected when working with aDNA are:

a) DNA fragmentation and chemical modification
b) PCR inhibitors
c) Contamination
d) Criteria of authenticity

Ancient DNA is highly fragmented (Handt *et al*., 1994; Austin *et al*., 1997; Hofreiter *et al*., 2001; Smith *et al*., 2003; Yang and Watt, 2005; Reiss, 2006; King *et al*., 2009; Fulton and Stiller, 2012; Pedersen *et al*., 2015; Glocke and Meyer, 2017). After the death of an organism, a natural degradation process occurs as all the molecular repair mechanisms end (Hofreiter *et al*., 2001; Reiss, 2006). Also, when cellular structures collapse, DNA is exposed to biotic and abiotic environmental factors, such as exogenous microbial nucleases (Wilson, 1997) and UV light, which can induce further breakdowns of the double helix. The natural degradation process can be slowed down or even interrupted under specific circumstances like rapid desiccation or preservation at low temperatures (King *et al*., 2009). However, despite this, internal chemical reactions can continue destabilising the DNA structure furtherly. Damages caused by oxidation of nitrogenous bases, cross-links, deamination and depurination contribute to DNA decay, fragmenting the molecules until it is impossible to perform any PCR on the material (Reiss, 2006). Such extensive modifications of DNA can prevent amplification and sequencing of the remaining fragments (Heyn *et al*., 2010). Moreover, another problem commonly encountered during the analysis of aDNA is the presence of further inhibitors which can be impurities retrieved during the extraction phase (Wilson, 1997). A not sufficiently purified DNA sample results in a negative PCR. Contamination by modern samples is another additional issue that has to be considered during archaeoentomological analysis (Yang and Watt, 2005). In the rare case that a positive result is obtained, certain criteria have to be in place to prove the authenticity of the discovery (Pääbo *et al*., 1989; Austin *et al*., 1997). Such criteria include correct use of reaction controls, which exclude contamination, a series of replicates with the same result, the possibility to repeat the experiment by another facility, and at last, a worthwhile phylogenetical reconstruction (Handt *et al*., 1994; Hofreiter *et al*., 2001).

2.7.2 The Takarkori samples

The majority of insect fragments collected from the Takarkori archaeological site were not molecularly identified due to their state of preservation. The site is located in the Sahara,

the largest hot desert of the planet. The desertification of the area started around 6,000 cal years BP, which means archaeoentomological samples were exposed to similar existing desert temperatures for at least 4,500 years. The average temperatures in the Sahara can exceed 30 °C during the dry season. Sand and ground temperatures due to the high position of the sun and the common clear sky can be even more extreme, reaching 80 °C or more. High temperatures speed up the degradation process of DNA. Furthermore, the presence of low numbers of insect fragments per family has made an aDNA extraction and characterisation attempt extremely hazardous. The loss of information caused by molecular trials was evaluated and currently, the morphological identification has been considered sufficient. A trial on a selected batch of Ptinidae, spider beetles, as they are the most numerous insect fragments recovered from Takarkori, is planned. A future aDNA extraction cannot be excluded as advances in biomolecular techniques might overcome the previously described issues.

3 HOLOCENE CLIMATE CHANGES: THE ENTOMOLOGICAL EVIDENCE

3.1 RECONSTRUCTION OF PAST ENVIRONMENTS

Several objectives can be investigated during archaeoentomological analyses, but the reconstruction of past environments is the most common among the field. Researchers in palaeoentomology pioneered the use of insects to reconstruct landscapes from different geological eras (Kenward, 1976; Matthews, 1979; Schwert and Ashworth, 1988; Andersen, 1993). The achieved results have prompted applications to more recent ages, marking the outset of environmental reconstruction through insects in archaeology. In the past 40 years, the Quaternary period has been in the spotlight focusing primarily on glacial environments (Coope and Brophy, 1972; Morgan and Morgan, 1980; Schwert *et al*., 1985; Schwert and Ashworth, 1988; Elias, 1994; Coope and Lemdahl, 1996).

Coleoptera is the insect order commonly employed during environmental reconstructions. Beetles are the most diverse group on the planet, and they are known to be sensitive biological indicators (Osborne, 1988). This means that beetles have a quick response to climatic shifts and can reflect temperature gradients (Coope, 1970; Coope and Brophy, 1972; Morgan and Morgan, 1980). Their ability to react to changes makes them the best candidate to reconstruct and understand past climates and habitats. Moreover, another fundamental aspect has to be considered when the Holocene epoch is analysed: the human impact on the landscape. Several studies investigated the ecological impact of human settlements using beetles (Elias, 1985; Morgan *et al*., 1987; Osborne, 1988; Schwert, 1996; Lavoie *et al*., 1997; Bain, 1998) creating references for future works. However, beetles are not the only insects that can be used to reconstruct landscapes. For instance, flies (Pradelli

et al., 2019) and mites (Schelvis, 1997) collected from archaeological sites have also been used in the past. As far as the recovered insect has a specific ecological niche, it is possible to do an accurate reconstruction of the environment. Hence, other groups, such as Lepidoptera, Hymenoptera and Orthoptera cannot be excluded.

3.2 INSECT FAUNA AT TAKARKORI: RESULTS

Fragments of insects belonging to seven different orders were collected from the soil samples analysed (see paragraph 2.1, Table 2): Orthoptera, Coleoptera, Hymenoptera, Isoptera, Diptera, Lepidoptera, and Phthiraptera. Furthermore, fragments of other Arthropoda, such as pseudo-scorpions (Pseudoscorpionida) were found (Fig. 14).

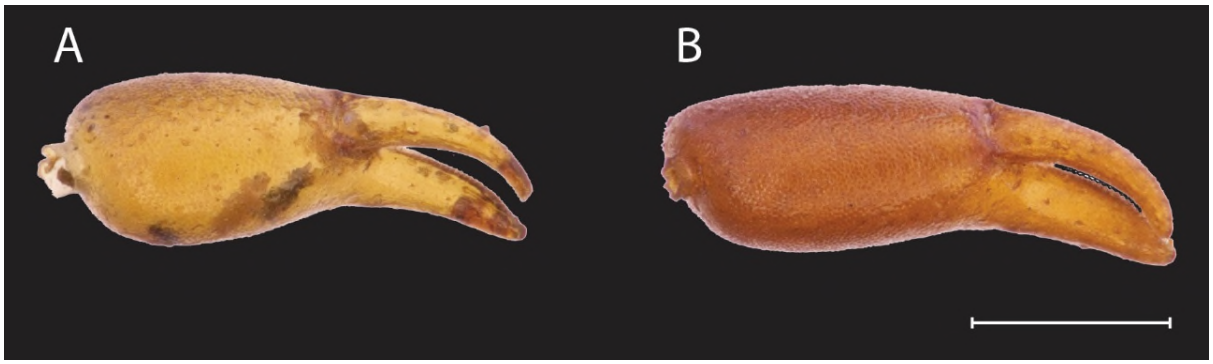

Figure 14: Pedipalps of pseudo-scorpions. Scale bar 500 μm.

Phthiraptera order is represented by an almost complete louse identified as *Pediculus humanus capitis* De Geer, 1778. The identification of the Isoptera order has not been performed due to the lack of specialists, especially when Afro-tropical and Palearctic regions are considered. However, they appear to belong all to the same species. Orthoptera order is represented by *Locusta migratoria* (Linnaeus, 1758). Lepidoptera is represented by a species in the family Erebidae, sub family Arctiinae. Diptera order is represented by species in six different families: Calliphoridae, Sarcophagidae, Sphaeroceridae, Piophilidae, Muscidae, and Oestridae. Four families of Coleoptera have been identified: Dermestidae, Trogidae, Curculionidae and Ptinidae. Hymenoptera order is represented by the super-family Vespoidea, in which Formicidae is composed by three identified at the genus level (*Lasius* sp., *Messor* sp., and *Pheidole* sp.) and one identified specimen at species level

Brachyponera sennaarensis (Mayr, 1862). A single male four-winged specimen belonging to Bradynobaenidae family, Apterogyninae sub-family, has been recovered. The specimen could be identified in the genus *Macroocula* or *Apterogyna*, but it is impossible to distinguish them due to the lack of the head.

The abundance of every taxon in each sample selected throughout the chronological sequence at Takarkori is reported in Table 10.

Table 10: List of species from Takarkori and their abundance (*= 1, **= 2-10, ***= <100, ****=>100; LA = Late Acacus, EP = Early Pastoral, MP = Middle Pastoral, LP = Late Pastoral).

TAXON	LA1								LA2							LA3							
	1	4	5	6	32	34	35	36	16	17	18	20	22	25	28	46	47	50	56	57	58	61	69
ORTHOPTERA	****	****		***	**	***	****	****	****	***	***		*		**	**		**	**	*	****	*	*
COLEOPTERA																							
Ptinidae	**	***	***	***	****	***	****	****	***	***	***			****	***		***	***	**	***	**	****	
Dermestidae			**																				
larvae					***	**	***	**	**	**	*					*			*				
adults					***	**	***	**	*	**					**								
Tenebrionidae					*		**	**															
Curculionidae						*	*																
others	*		**	**	**	**	**	***		**	**			**	**			**	*		**		
HYMENOPTERA																							
Formicidae	**				**	**		***	***	**	**							*	**	**	**		
Pheidole sp.	**																						
Lasius sp.	*				**	**		**			**								*	**			
Messor sp.				**	*	**		**		**	**								**	*	**		
Brachyponera sennaarensis	*				*						*												
Not Formicidae					**	*																	
ISOPTERA	**			**	**	**		**	***		**			**	**	*		**	**		***		*
tunnel	**			**		**				*													
DIPTERA					**		*	**															
Piophilidae	*															*							
Sphaeroceridae	**			*		*	**	***	***	**						*	*	**			**		
Muscidae			**	**	***	***	***	***	**	*	**					*			*	*	***		
Oestridae			*																				
Calliphoridae									*														
Sarcophagidae							**																
LEPIDOPTERA								**											*				
PHTHIRAPTERA	*																						

TAXON	EP1								EP2						MP1						
	80	86	87	89	94	95	97	99	103	105	107	109	111	114	117	119	121	122	123	124	125
ORTHOPTERA	**	**		**	**	**	**		*	**		**	**	*	**	**	**	**	**		**
COLEOPTERA																					
Ptinidae			**	***	**	*	**		***	**	***	**	**	**				*	**	***	**
Dermestidae																					
larvae					*		*							*							
adults			**			*													*		
Tenebrionidae																					
Curculionidae																					
others	**		**	*	**	*	**	*	*	*	*	**	**	**	*	**	*		**		**
HYMENOPTERA																					
Formicidae		**			**	*	***		**	**		**	***	**							**
Pheidole sp.									**			**	*	**			*				
Lasius sp.		*								*		**	*	**							**
Messor sp.					**	*			*	*			**	*							**
Brachyponera sennaarensis		*							*				*	*							
Not Formicidae		*																	*		
ISOPTERA							*							*							
tunnel		*	**		**		***		***	***	**	**		*	**	**	**	**			**
DIPTERA	*																				
Piophilidae																					
Sphaeroceridae							**														
Muscidae				*			*				*	*	**						*		
Oestridae	**	*							*							*					
Calliphoridae																					
Sarcophagidae																					
LEPIDOPTERA																					
PHTHIRAPTERA																					

TAXON	MP2						LP1				
	127	128	129	136	140	144	147	148	149	150	151
ORTHOPTERA		*		*	**	**		*		**	
COLEOPTERA											
Ptinidae	**	**		***	**	***		**		**	
Dermestidae											
larvae		*					*		**		
adults										*	
Tenebrionidae					**						
Curculionidae											
others		**		**	***	**	**	**	*	**	
HYMENOPTERA											
Formicidae		**		**	**	***	**	***			
Pheidole sp.				**	**						
Lasius sp.		**		*	**	**		*			
Messor sp.						*	**	**			
Brachyponera sennaarensis					*	**					
Not Formicidae											
ISOPTERA						**					
tunnel	**	**		**	**	**	**	***	*	**	
DIPTERA											
Piophilidae				*							
Sphaeroceridae							**		**		
Muscidae	*	*				*		**			
Oestridae											
Calliphoridae											
Sarcophagidae											
LEPIDOPTERA											
PHTHIRAPTERA											

3.3 DISCUSSION

Two distinct ecological groups are recognisable: the environmental and the synanthropic fauna. The environmental group could be defined as the set of indigenous insects typical of a specific landscape. In this chapter, description and typical habitats of insects used to reconstruct the past environment at Takarkori are reported. The synanthropic fauna is covered in chapter 4.

3.3.1 ORTHOPTERA

Orthoptera *sensu stricto* encompass grasshoppers, bush crickets, crickets, and locusts. It is one of the oldest orders of insects, present on Earth since the Late Carboniferous (~300 million years ago). Currently, around 25,000 species are known and described (Resh and Cardé, 2009). Orthopterans are usually large insects; the body size ranges from a few millimetres up to 12 centimetres (Béthoux and Nel, 2001). They are widely distributed, and they are present all over the world with an exception for extremely cold environments (Heller *et al*., 1998). They are characterised by the well-developed hind legs used for jumping, a hypognathous head with different chewing mouthparts according to feeding habits, filiform antennae, and two pairs of wings (Resh and Cardé, 2009). They can be diurnal or nocturnal, they can live on leaves (phytophilous), on the ground (geophilous), in caves (cavernicolous) or in symbiosis with ants (myrmecophilous) (Latchininsky, 2013). Orthopterans are among the so-called "economic insect group" since many species are pests of agriculture, horticulture and silviculture (Latchininsky, 2011). However, this is not the only reason this group has drawn human attention: their high protein content and their considerable size serve as a valuable food option for human consumption (Anankware *et al*., 2015). In fact, several cultures, even at the present day, consider grasshoppers a popular food source, commonly available on the market (Mmari *et al*., 2017).

The Orthoptera are classified in Ensifera and Caelifera, two suborders which include respectively 11 and 28 families (Resh and Cardé, 2009).

Suborder Caelifera

Caelifera are also commonly known as short-horned orthopterans and include some of the largest specimens among Orthoptera. There are more than 2,400 genera and 11,000 species arranged in super-families: Acridoidea, Eumastacoidea, Pyrgomorphoidea, Tanaoceroidea, Pneumoroidea, Trigonopterygoidea, Tetrigoidea, and Tridactyloidea (Uvarov, 1944). Most of them are diurnal and primarily tropical. The main source of food for caeliferans is plant material (Farrow and Colless, 1980). None of them is commensal, which means they do not interact with other bigger species to gain benefits. Aposematic colouration is common among this group (Resh and Cardé, 2009). Like all the other orthopterans, caeliferan have an incomplete metamorphosis (Fig. 15). They usually deposit eggs in pods or oothecae inside the ground, but some species can also lay eggs in plant tissues or in cracks between barks (Latchininsky, 2013).

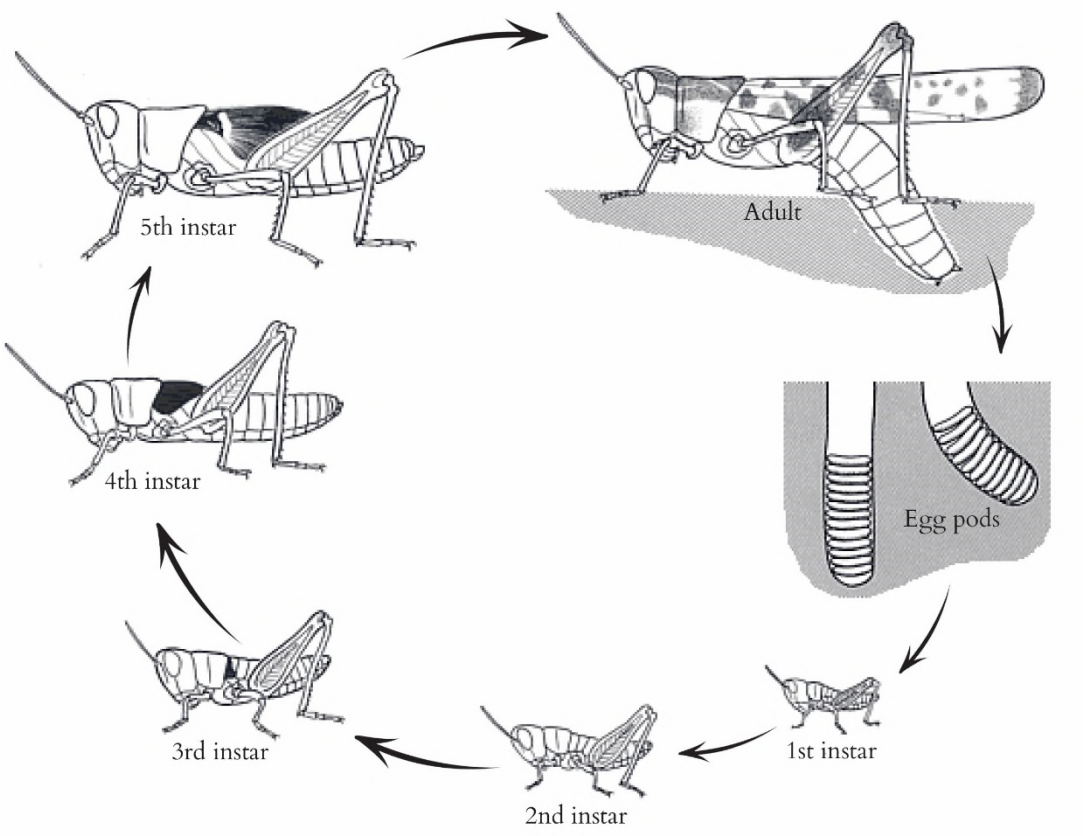

Figure 15: Life cycle of caeliferans (modified from Latchininsky, 2013).

Many species of caeliferans are susceptible to crowding during development (Song, 2011). A visible biotic effect is detectable between solitary and gregarious phases of locusts. They differ not only in morphometrics and colour but also in behaviour (Simpson *et al*., 2006). When locusts develop at low densities, they tend to enter into the solitary phase in which nymphs show a uniform colour and a large-size adult with a very prominent hind femur. Individuals in the solitary phase tend to avoid each other's company and they show a very low vagility (Song, 2011). On the contrary, when favourable climatic conditions allow high survivorship of immature stages and consequently a high density crowed population, locusts enter into the gregarious phase (Resh and Cardé, 2009). Nymphs develop in smaller adults and tend to aggregate in massive extremely mobile swarms. It is during this phase that locusts can cause serious damages to cultivation crops and vegetation (Resh and Cardé, 2009).

Superfamily Acridoidea

Acridoidea is the largest superfamily of Caelifera with 1,700 genera and around 8,000 species worldwide. It encompasses grasshoppers and locusts (Khajehzadeh, 2002). Their size range between 1 cm up to 25 cm (when wings are considered). The group shows pronounced sexual dimorphism, with the male usually being smaller than the female (Chapuis, 2006). The oviposition of eggs is inside pods hidden on grasses, wood, soil and other plant tissues, covered by a foaming protective coating (Defaut, 2013). After the incubation period, a vermiform small larva hatches from the egg and crawls to the surface where it moults into an immature form called nymph which resembles the adult form (Defaut, 2005). Nymphs moult between four and six times according to the species before becoming a full adult. Acridoidea have more than 20 subfamilies of gregarious species which can form migratory swarms (Resh and Cardé, 2009).

Subfamily Acrididae

Acrididae subfamily is also known as the true locust family and it is the largest among the superfamily Acridoidea. The locusts in this group are brown or earth colours, primarily

diurnal. They have a wide distribution, but the majority of them are tropical (Resh and Cardé, 2009). Currently, 1,500 genera have been described. Inside the group, several locusts are famous to be pests such as *Chortoicetes terminifera* (Walker, 1870) or the plague locust *Locusta migratoria*.

Locusta migratoria

The migratory locust is quite a large species (size range between 4,5 cm and 6.5 cm), stubby in appearance (Defaut *et al*., 2012). Body colouration can vary from green to brown during the solitary phase to yellow during the gregarious phase (Fig.16).

Figure 16: Migratory locust in solitary form, from Defaut (2013).

The life span of a migratory locust is approximately 2 months. Females lay eggs every 4 to 6 days, resulting in tropical regions in five or six generations in a single year. Between 50 to 60 eggs are clustered in every pod (Benfekih and Petit, 2010). Usually, oviposition happens after rain because eggs cannot survive in dry conditions. The most favoured oviposition places are moist soils, especially in cultivated areas, silt beds, creek beds or crops (Resh and Cardé, 2009). Depending on the temperature, eggs take about 11-15 days to hatch. Nymphs undergo 5 or 6 moults and usually take 30 days to reach maturity. However, in dry and cold environments the time is considerably longer (Benfekih *et al*.,

2002). Aggregation behaviour is common in this species. It is present in large numbers in Africa, eastern Europe and Asia, where it is considered a major pest of agriculture.

During Takarkori excavations, single femurs, articulated hind legs, and mandibles of *Locusta migratoria* have been collected in large quantities in the Late Acacus early phases (LA1, LA2) (Fig. 17, 18).

Figure 17: Fragments of hind legs of *Locusta migratoria* collected at Takarkori, scale bar 2 mm.

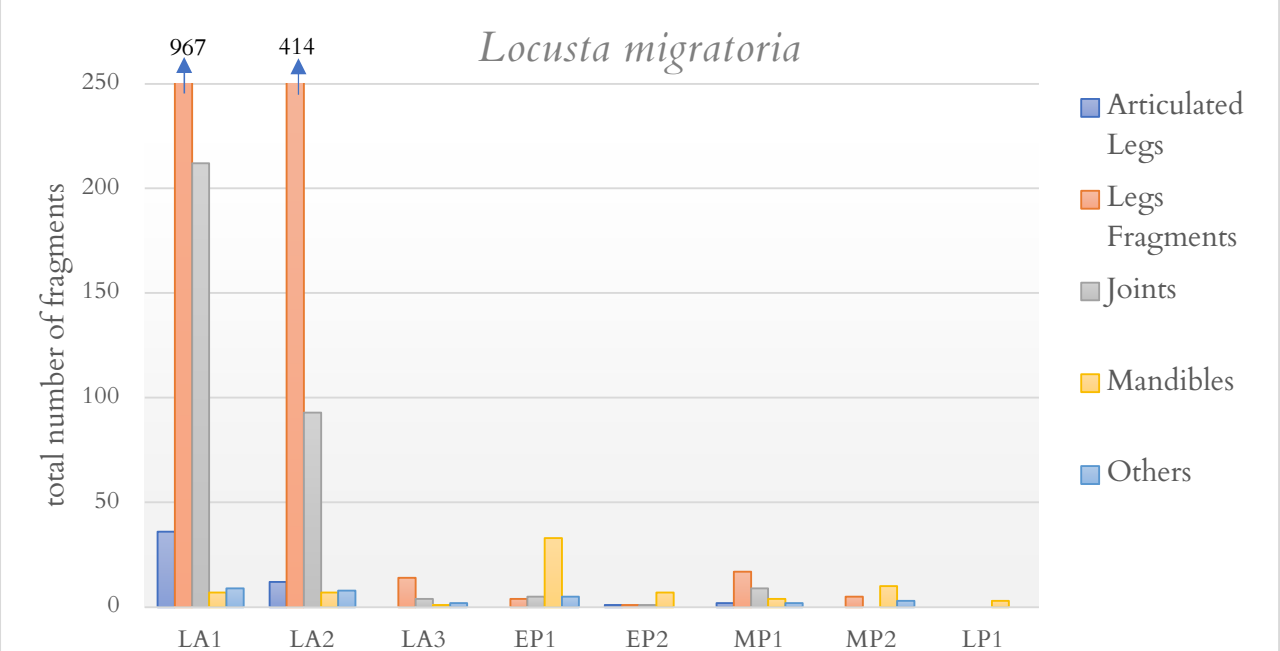

Figure 18: Abundance of migratory locust fragments in each chronological phase (LA = Late Acacus, EP = Early Pastoral, MP = Middle Pastoral, LP = Late Pastoral).

3.3.2 LEPIDOPTERA

Lepidoptera is a monophyletic lineage and it encompasses moths and butterflies. They are characterised by wings with a double layer of scales and a long-coiled proboscis (Resh and Cardé, 2009). They are holometabolous insects, which means they undergo a complete metamorphosis. Currently, around 160,000 species have been described, but recent studies estimate the total number of lepidopterans to exceed 350,000 species (Scoble, 1992). This incredible diversity can be attributed to the radiation associated with flowering plants, which are the main source of food for the group (Renwick and Chew, 1994). The classification of Lepidoptera is complex and arise several debates among researchers. Historically, the group was divided into five suborders, but in recent years a proliferation of superfamily divisions has created some discrepancies (Hajibabaei *et al.*, 2006). Currently, between 45 and 48 superfamilies with around 120 subfamilies are accepted.

Superfamily Noctuoidea

Noctuoidea is the largest superfamily of Lepidoptera including 7,200 genera and almost 60,000 species (Resh and Cardé, 2009). Inside the group, an incredible variety of form,

size, colour, morphology and behaviour can be observed. The most widespread subfamilies are Notodontidae, Lymantriidae, Erebidae, and Noctuidae.

Subfamily Erebidae

The Erebidae is a subfamily that encompasses moths. It is one of the largest and includes well-studies macro moths from several regions of the World (Dowdy and Conner, 2019a; Dowdy and Conner, 2019b). The adult size ranges between 6 mm and 127 mm. The colours range from dark to vivid. Phylogenetic studies debate about the paraphyletic or monophyletic nature of this lineage and still, there is no conclusive answer (Pinheiro and Gaal-Haszler, 2015). At the moment, Erebidae are divided into 18 families: Aganainae, Anobinae, Arctiinae, Boletobiinae, Calpinae, Erebinae, Eulepidotinae, Herminiinae, Hypeninae, Hypenodinae, Hypocalinae, Lymantriinae, Pangraptinae, Rivulinae, Scolecocampinae, Scoliopteryginae, Tinoliinae, Toxocampinae.

Family Arctiinae

Artictiinae are commonly known as tiger moths. All the species are characterised by a pair of dorsal pheromone glands and a peculiar terminal abdominal segment in females. Adults are small to moderately large, from 5 mm to 50 mm (Dowdy and Conner, 2019b). Commonly, they display bright colours with several intricate patterns, often aposematic. There are more than 6,000 species in 750 genera worldwide (Dowdy and Conner, 2019b). The majority of Artictiinae are nocturnal, but some genera can be strictly diurnal, especially in North America (e.g. *Ctenucha* Kirby, 1837). They are particularly numerous in tropical regions. They are polyphagous plant feeders and during immature stages can infest a variety of host plants, including herbaceous or woody plants (Dowdy and Conner, 2019a). Surveys on their distributions revealed that Articiinae are more common in woodland savannah and semideciduous forest than in grassland (Dowdy and Conner, 2019a).

At Takarkori, an astonishing well-preserved Articiinae has been found in the oldest chronological phase, LA1 (Fig. 19).

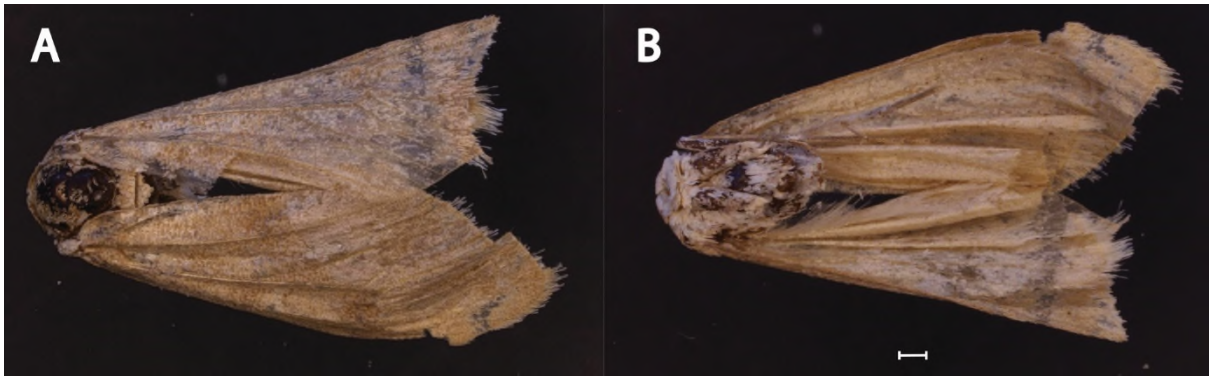

Figure 19: Takarkori Articiinae, A dorsal view, B ventral view, scale bar 500 μm.

Although the preservation status of the sample allowed identification at the family level, the loss of several scales on the wings makes it extremely difficult to have a confident identification at the species level. The taxonomist, Dr Alberto Zilli, suggests that the specimens might be identified as the genus *Teracotona* Butler, 1878.

3.3.3 ISOPTERA

Isoptera, also known as termites, are eusocial insects characterised by two pairs of wings which are very similar in size (wings are present only on reproductive adults). The oldest isopteran fossil has been discovered in a Cretaceous site dating 130 million years ago (Engel and Delclòs, 2010). Currently, there are around 2,600 species worldwide. Africa is the continent with the greatest diversity with over 1,000 species described (Engel *et al*., 2009). They vary from pale white to ivory colour, very rarely black. They undergo incomplete metamorphosis. There are several roles inside the eusocial system of termites, and they all differ morphologically: nymphs, workers, pseudergates, soldiers, and several types of reproductive roles (Snyder, 1949). After the hatching from eggs, nymphs moult at least three times before transforming in adult workers, which are wingless, blind (the only exception are the workers of Hodotermitidae family) and cannot lay eggs (Donovan *et al*., 2000). Pseudergates follow the workers' life cycle, but in case of necessity, they are able to transform into reproductive roles. Workers and pseudergates are the labouring castes and

they are the most abundant termites inside the colony, in charge of foraging and building the nest (Resh and Cardé, 2009). Soldiers oversee the colony protection and for this reason, they are equipped with strong mandibles or other chemical spraying systems. The reproductive roles consist of a royal pair that after the swarming flight to search for a suitable nesting site will lose the wings and will create a new colony (Donovan *et al*., 2000). Most of the species swarm during the rainy season, but dry-wood termites can also swarm during summer evenings. The preferred nesting sites are close to wood (Resh and Cardé, 2009). Isoptera families are divided traditionally in lower or higher families. Lower families are characterised by a symbiotic presence of protozoa and bacteria in the intestinal tract: Mastotermitidae, Kalotermitidae, Termopsidae, Hodotermitidae, Rhinotermitidae, and Serritermitidae (Inward *et al*., 2007). Higher termites have only bacteria inside their intestinal tract: Termitidae. Termites are herbivores, fungivores and humivores (Resh and Cardé, 2009). Their main source of food is the cellulose of plants (alive or dead). Hence, termites are important in recycling systems of wood and plant material (Snyder, 1949).

At Takarkori, several termite fragments have been recovered especially during the early chronological phases (LA1, LA2, LA3) (Fig. 20). An almost sudden disappearance of specimens is visible from the Pastoral phases onwards (refer to Tab. 10).

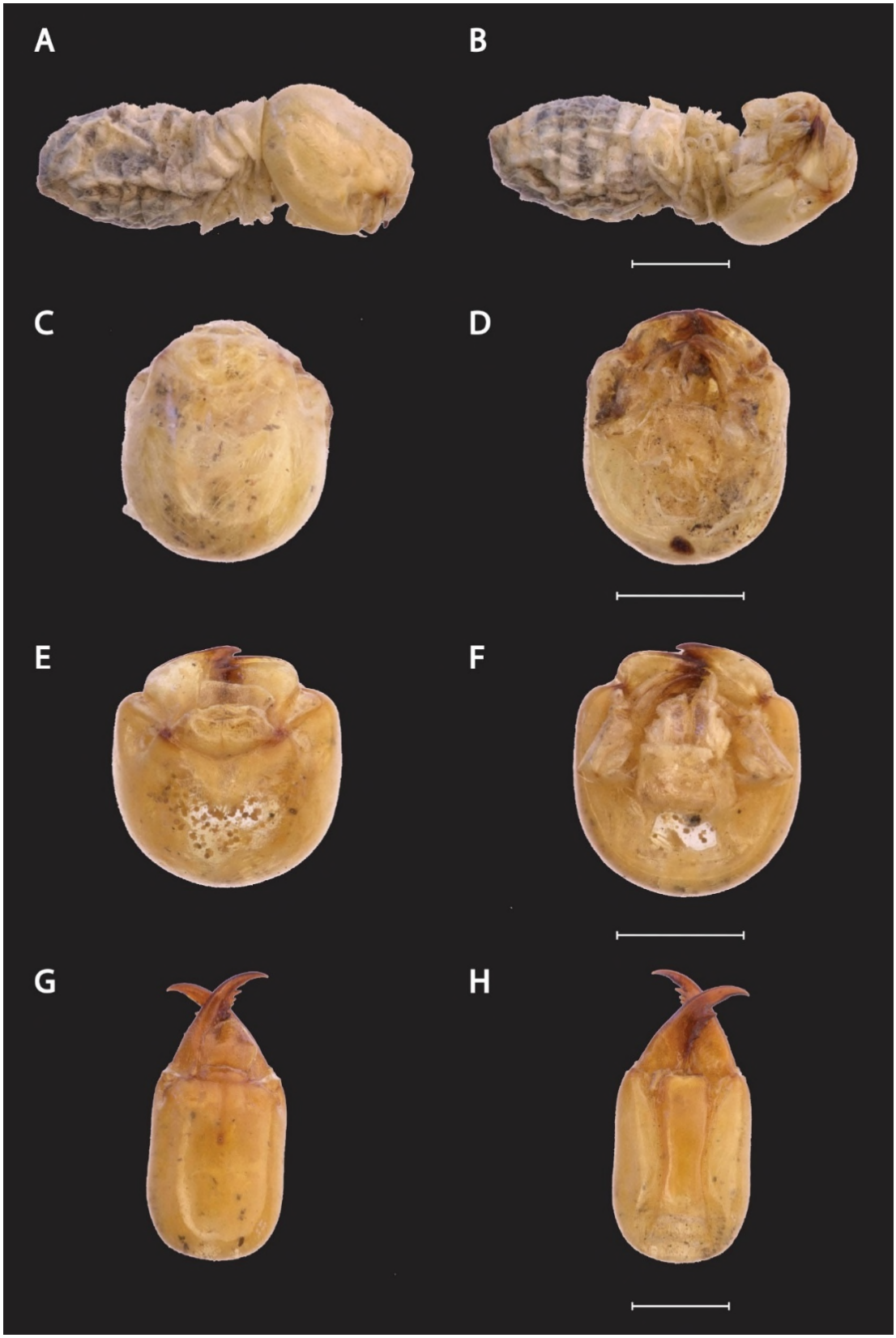

Figure 20: Termite fragments; A latero-dorsal view, B latero-ventral view of a worker; C dorsal view, D ventral view of a termite head; E dorsal view, F ventral view of a different termite head; G dorsal view, H ventral view of a soldier. Scale bars 500 µm.

3.3.4 HYMENOPTERA

Hymenoptera is a megadiverse group of holometabolous insects and encompass sawflies, ants, bees, and wasps. They are cosmopolitan, but they are most diverse in the tropics (Resh and Cardé, 2009). They are characterised by often long antennae multisegmented, thorax composed by three segments or by the incorporation of the first abdominal segment, which creates the common petiolate (tiny waist) look, simple-venation wings with hamules, and fore and hind legs coupled (Richards and Davies, 1977). Their size can range from very small specimens (0.1 mm length) up to large species like the spider-hunting pompilid wasp (12 cm length with wingspan). They have a large variety of eating habits, they include phytophagous, parasitoid, and predator taxa (Richards and Davies, 1977). The group presents solitary, highly social, and eusocial species. Although, historically, Hymenoptera has been divided into two suborders, Symphyta, which do not present the petiolate waist, and Apocrita which have the narrow waist, the terminology is falling in disuse (Doutt, 1959; Malyshev, 1968). Phylogenetic studies modify the complex classification of this group very frequently (Peters *et al*., 2017).

Suborder Apocrita

The Apocrita encompass wasps, bees and ants. The first abdominal segment of Apocrita, also known as propodeum, is fused with the thorax (Resh and Cardé, 2009). The evolution of the narrow waist has a significant impact on oviposition. In fact, the ovipositor can be retracted inside the abdomen or can be extended freely. Some species have also a modified ovipositor developed into a stinger as protection and prey offence (Fitzgerald and Flood, 2006). Apocritan larvae are blind without mobility and they require some level of parental care. Parasitoids often lay eggs inside a host which will become the main food source of larvae. Non-parasitoids species, usually, lay eggs on nest cell and regularly provide larvae with food (Resh and Cardé, 2009). Historically, the suborder has been dived into two groups: Parasitica and Aculeata. Aculeata is a monophyletic group characterised by the modification of ovipositor into a venomous stinger. Ants, bees, and several parasitic and

predatory wasps are among Aculeata. The group also includes all social hymenopterans. Aculeata is divided into several superfamilies and families (Resh and Cardé, 2009).

Family Formicidae

The Formicidae family belongs to the superfamily of Vespoidea and includes 288 genera with about 10,000 ant species described worldwide (Astruc *et al*., 2004). The most ancient fossil of an ant is dated back to the Cretaceous (~120 million years ago), but studies affirm that the radiation has started probably during the Eocene (~45 million years ago) (Resh and Cardé, 2009). Ants are ecologically very successful dominating numerous terrestrial ecosystems (Urbani *et al*., 1992). They are cosmopolitan. The reason for their success lies in their eusociality with the efficient management of their colonies, and on their incredible adaptability to modify the surrounding environment to fit their needs (Wilson, 1980). Ants have an important role in the turnover of soils because they are seed distributors and harvesters. Moreover, they regulate aphid infestation minimizing defoliation (Graham *et al*., 2004). Human interest in ants become apparent when invasive species have been transported through commerce with catastrophic results (Resh and Cardé, 2009). Some species, in fact, are considered pests, like the fire ant *Solenopsis invicta*, Buren, 1972.
At Takarkori three identified genera of ants and one identified specimen at species level have been collected (Fig. 21). Their abundance throughout the chronological sequence is shown in Table 11.

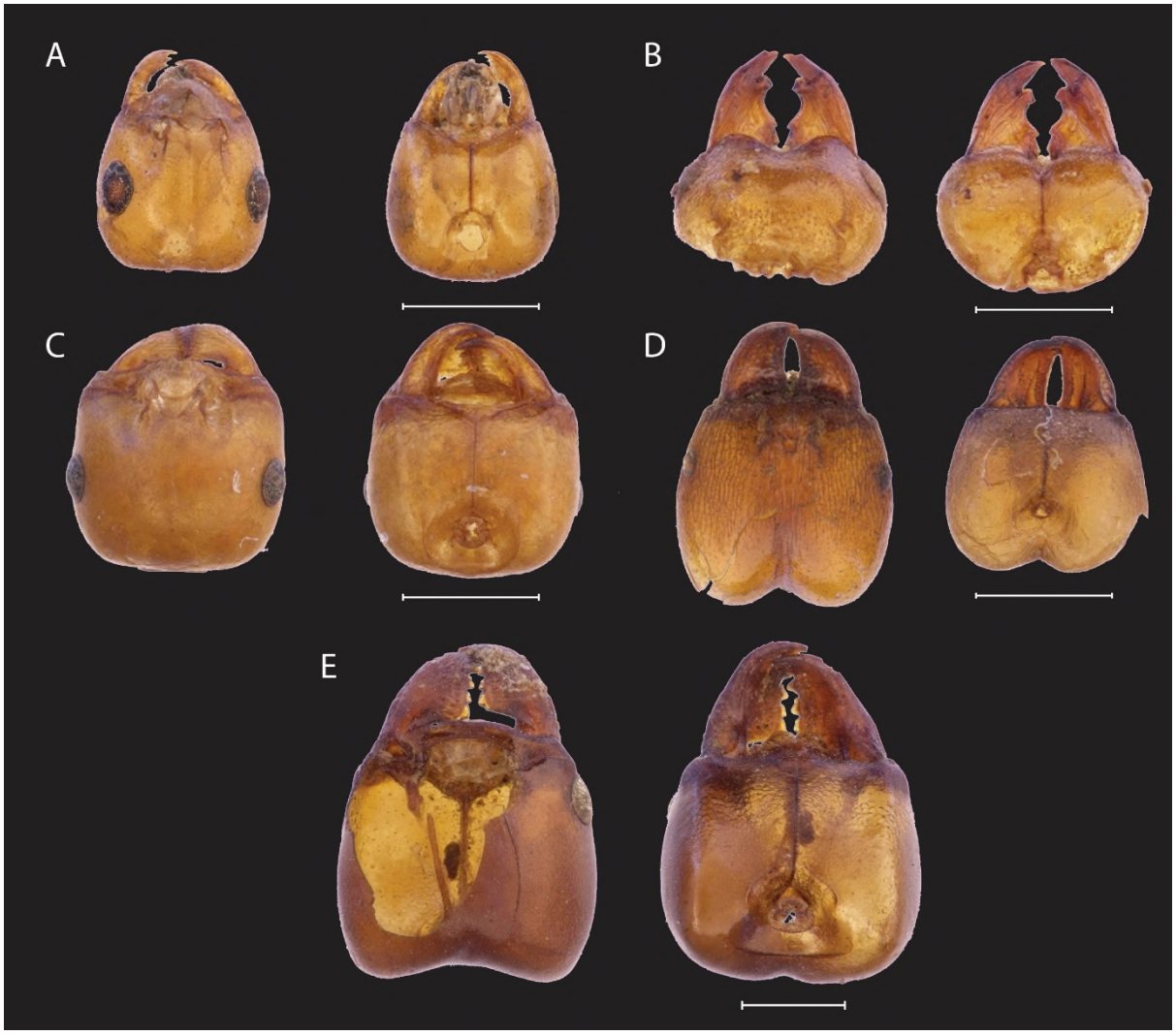

Figure 21: Dorsal and ventral view of ant heads collected at Takarkori. A *Lasius* sp., B Gen. sp., C *Messor* sp., D *Pheidole* sp. major worker, E *Brachyponera sennaarensis*. Scale bars 500 µm.

Table 11: Abundance of each ant in each chronological phase (*= 1, **= 2-10, ***= <100, ****=>100; LA = Late Acacus, EP = Early Pastoral, MP = Middle Pastoral, LP = Late Pastoral).

FORMICIDAE	LA1	LA2	LA3	EP1	EP2	MP1	MP2	LP1
Brachyponera sennaarensis	**	*	\	*	**	\	**	\
Messor sp.	**	**	**	**	**	**	*	**
Pheidole sp.	**	\	\	\	***	*	**	\
Lasius sp.	***	**	**	*	***	**	***	*

Brachyponera sennaarensis

Brachyponera sennaarensis belongs to Ponerinae subfamily and is widely distributed in sub-Saharan regions, where its preferred habitats, savannahs and open forests, are common

(Paknia, 2006). This species is carnivorous, but in a pinch, it is the only species of Ponerinae that can feed on seeds (Levieux and Diomande, 1978; Déjean and Lachaud, 1994). Recent studies affirm that *B. sennaarensis* have shown a significant preference of human-impacted environments, where rubbish dumps and waste ground are available (Lachaud and Déjean, 1994). Its painful sting can cause strong allergic reactions (Collingwood *et al*., 2004). *Brachyponera sennaarensis* is characterised by a head broader than the thorax, first and second gastral tergites divided by a distinct constriction, large size, often bodies of dark colours (from dark brown to black) with antennae, tibiae and tarsi of reddish colours, bodies covered with pubescence (Akbarzadeh *et al*., 2004; Tirgari and Paknia, 2005).

Messor sp.

Messor Forel, 1890 belongs to Myrmicinae subfamily and it includes more than 160 species. It is a cosmopolitan genus, but its highest diversity is in the Palaearctic region (Resh and Cardé, 2009). Predominantly granivorous, it has a fundamental role in seed dispersal. Bolton (1982) affirms they are commonly found in savannahs and grassland, but they have been recovered also from drier environments, such as semi-arid areas and deserts (Bolton, 1982). *Messor* genus is characterised by 2,2 palp formula, strong dimorphic traits, antennae ending in a 3-segmented club, medium size, and strong heavy mandibles (Bolton, 1981).

Lasius sp.

Lasius Fabricius, 1804 is another genus belonging to Myrmicinae subfamily and it is widespread in the Holarctic region with 133 species (Wilson, 1955). This genus has been extensively studied due to its peculiar behaviour: conspicuous nuptial flights, transportation of homopterous insects, and a temporary parasitic phase (Wilson, 1955). It is one of the most abundant genera of the whole Insecta world. Depending on the species, drier heathlands, cornfields, and undisturbed pasture are the most preferred habitats. *Lasius* workers are characterised by equal size (monomorphic appearance, between 2 and 3 mm length), yellow to brown body colours, a notch on dorsal thorax (Wilson, 1955).

Pheidole sp.

Pheidole Westwood, 1839 also belongs to Myrmicinae subfamily and it is a large cosmopolitan genus with more than 1,000 species, their highest diversity is in the Neotropics (Wilson, 2003). Most of *Pheidole* species are dimorphic with a significant difference between minor and major workers (Sarnat *et al*., 2015). The head of major workers is considerably larger, and they can be frequently infested by a parasitoid phorid flies (Diptera). The dipterous immature stages can only survive in the big cephalic capsule of major workers as the head of minor workers do not offer enough food (Resh and Cardé, 2009). The role of major workers is to carry and dissect large food items for minor workers to store them. Most of the species of *Pheidole* are generalist foragers that can feed on seeds, dead or leaving arthropods, and human by-products (Holway *et al*., 2002). The success of the genus is linked to the polygynous behaviour of colonies. The species in this genus are capable to create super-colonies with multiple queens. The genus preferred habitats are low elevation dry grounds (Sarnat *et al*., 2015).

Family Bradynobaenidae

Another family belonging to the superfamily of Vespoidea is Bradynobaenidae, which include wasps that resemble velvet ants (Mutillidae). Like the latter, females of this family are wingless. Bradynobaenidae are characterised by pronounced suture between pronotum and mesonotum and they are typical of arid environments (Pagliano and Romano, 2012, 2018). The family is rare as there are only 155 species (including Cyphotidae which recently have been considered as a separate family) described worldwide. Little is known about their behaviour and habitat preferences, but all of them are parasitoid of other arthropods in semi-arid environments (Soliman *et al*., 2018).

A male specimen of Bradynobaenidae, belonging to subfamily Apterogyninae, has been collected in the oldest chronological phase (LA1) of Takarkori (Fig. 22). The genus of the specimen can be either *Macroocula* or *Apterogyna*. Unfortunately, the head is missing and the diagnostic features to distinguish between the two genera are lost.

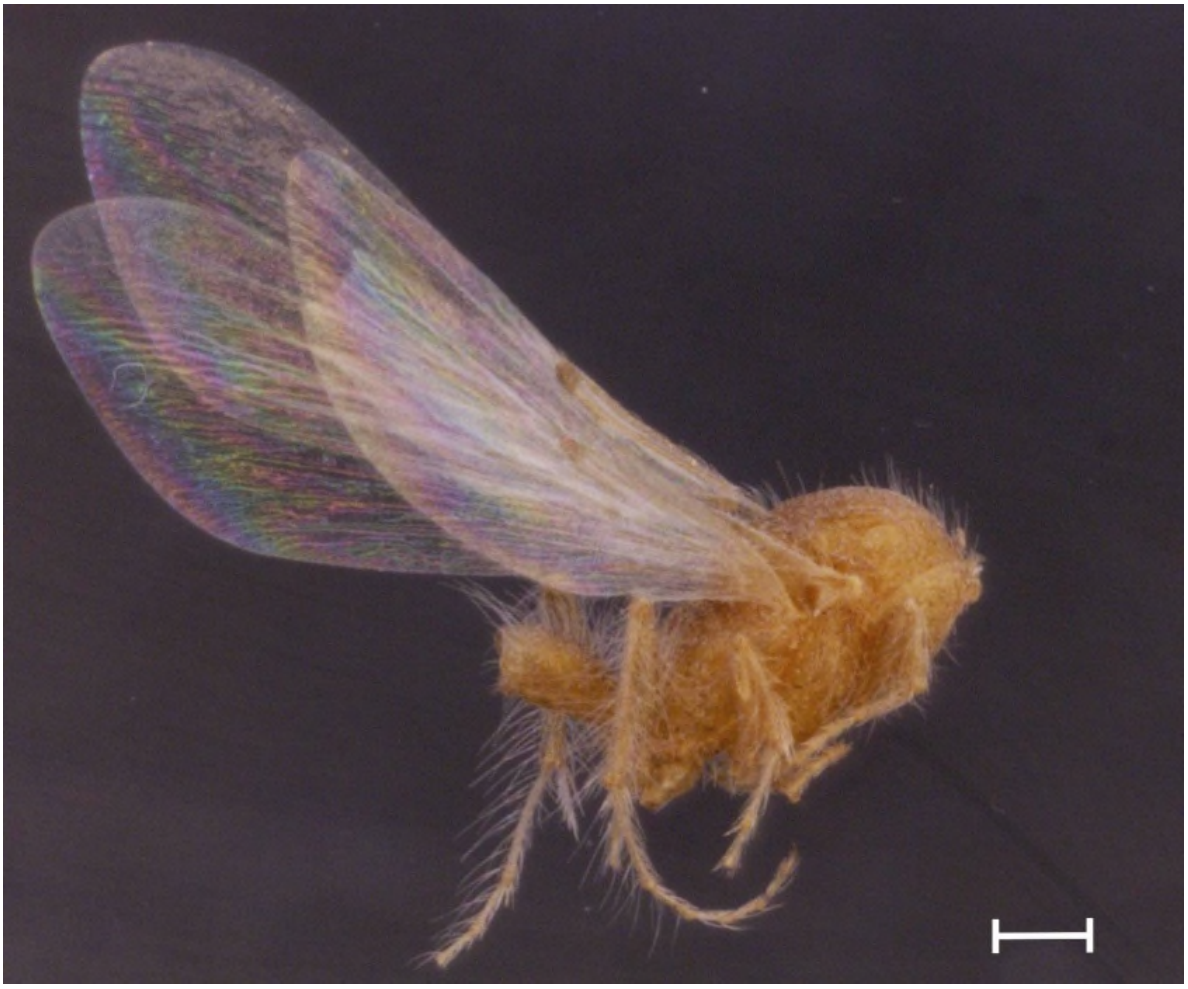

Figure 22: Male specimen of Bradynobaenidae (Apterogyninae) collected at Takarkori. Scale bar 500 μm.

3.3.5 TAKARKORI ENVIRONMENTAL RECONSTRUCTION

The Central Sahara, during the majority of the Pleistocene, was uninhabited due to severe climatic conditions. Since the Late Pleistocene (~ 14,500 years ago) the onset of a new climatic change gradually modified the region's conditions leading to more humid environments (Nicholson and Flohn, 1980; Street and Gasse, 1981; Cremaschi *et al*., 2010). The re-colonisation of the area started at the beginning of the Holocene due to the amelioration of the climate (Cremaschi, 1998; di Lernia, 1999a; Marshall and Hildebrand, 2002). This period in the study region is also known as "Acacus" phase. Africa underwent a high rainfall period called the African Humid Period (AHP) between 11,500 and 6,000 cal yr BP driven by earth orbital changes (Nicholson and Flohn, 1980; Gasse and Van Campo, 1994; Gasse, 2000; Gasse and Roberts, 2004; Hoelzmann *et al*., 2004; Mayewski *et*

al., 2004; Garcin *et al*., 2007; Demenocal and Tierney, 2012). During this phase of the "Green Sahara", water reservoirs such as Mega-Chad and Mega Fezzan lakes were recharged (Schuster *et al*., 2005; Armitage *et al*., 2007). This theory has been supported by palaeohydrological, geological, botanical and archaeological records of the area (Claussen and Gayler, 1997; Jolly *et al*., 1998; Gasse, 2000; Cremaschi *et al*., 2014). Savannahs and woodlands were the main vegetation coverage of the region sustaining a high diversity fauna (Mercuri, 2008; Cremaschi and Zerboni, 2009; Drake *et al*., 2011; Van Neer *et al*., 2020). This kind of environment might have facilitated the exploitation of new resources by hunter-gatherers (di Lernia, 1996). Caves and rock-shelters typical of the region allowed occupation leading to the introduction of pastoralism as the main economic system (Barich, 1987). However, the favourable climatic conditions were not stable, and they were interspersed with temporary short drier periods. For example, around 8,200 years ago a sudden climatic change caused a drought event (Alley *et al*., 1997; Mayewski *et al*., 2004; Alley and Ágústsdóttir, 2005; Wiersma *et al*., 2006), but wetter conditions were re-established soon after. The AHP came to an end abruptly around 5,500 cal years ago, rapidly spreading a hyper-arid condition which has led to the present world's largest hot desert, the Sahara, which is still expanding its boundaries (Guo, 2000; Kröpelin *et al*., 2008; Demenocal and Tierney, 2012). The cause of this extreme climatic change lies on a shift of planet orbital parameters (Claussen *et al*., 1999). A progressive loss of savannah and woodland vegetation led to a large-scale dust mobilisation resulting in speeding up the desertification process, which has been completed 4,000 years ago (Mercuri, 2008). The onset of a new extreme climate forced the arising populations to adapt to drier environments in order to survive (Cremaschi and di Lernia, 1995; Demenocal and Bloemendal, 1995; Cremaschi, 1998; Richerson *et al*., 2001; Demenocal, 2004; Petraglia *et al*., 2010; Mercuri *et al*., 2011; Prendergast *et al*., 2016). The Late Pastoral phases ended about 3,000 years ago with the setting up of irrigated agriculture, which bypassed the problem of the desertification with the introduction of oasis farming (Cremaschi, 1996).

3.4 CONCLUSION: THE ENTOMOLOGICAL EVIDENCE

The abundance of *Locusta migratoria* during the Late Acacus (especially LA1, LA2) confirms the theory of a green Sahara during the Early Holocene. In fact, the survival of this specific species is strictly linked to moist soils, as eggs cannot survive in dry environments. In addition, the oligophagous diet of *L. migratoria* (Blaney and Winstanley, 1982), enhances the botanical records and reconstruction of the past landscape. As macro-botanical and pollen evidence suggested, woodland and grassland savannahs dominated the region allowing the proliferation of the migratory locust. A sudden drop in abundance is detectable around the first Holocene crisis (soon after LA3, 8,3- 8,0 cal Ka) and the reason of the decrease can be attributed either to the first arid climate shift which happened around that time or to the anthropic activity. The presence of a specific anatomical district, legs, as the only fragments preserved after millennia may indicate a human disturbance. In fact, almost all leg fragments present a breaking pattern similar to the one that modern locusts show after being eaten by humans. Their abundance in early Late Acacus phases may have been overestimated due to their targeted collection for feeding purposes. Further research needs to be conducted in order to clarify this point. Another strong indication of the green landscapes during the early phases of the Holocene is the presence of the moth belonging to Erebidae (Arctiinae) family. The latter is a polyphagous plant feeder, immature stages specifically are phytophagous (Teston and Do Correa, 2015), making it a good bio-indicator of vegetation quality. It is commonly found in semideciduous forests or woodland savannahs and its recovery in LA1 suggests a gradual shift from woodland savannah to grassland savannah during the subsequent phases of LA2 and LA3. Termites are also numerous throughout all ancient phases, emphasizing again the most likely presence of wood in the area. In contrast, ants (Formicidae) have been recovered throughout the entire sequence at Takarkori, from Late Acacus to Late Pastoral. Although some of their preferred habitats are savannahs, open forests, and semi-arid environments, due to their ability to adapt very quickly to climate changes they are not informative enough in the Takarkori case. Lack of information about the behaviour and preferences of

Bradynobaenidae wasp make the specimen not suitable to draw conclusion about environmental reconstructions. A hypothetical chronological sequence of events is represented in Fig. 23.

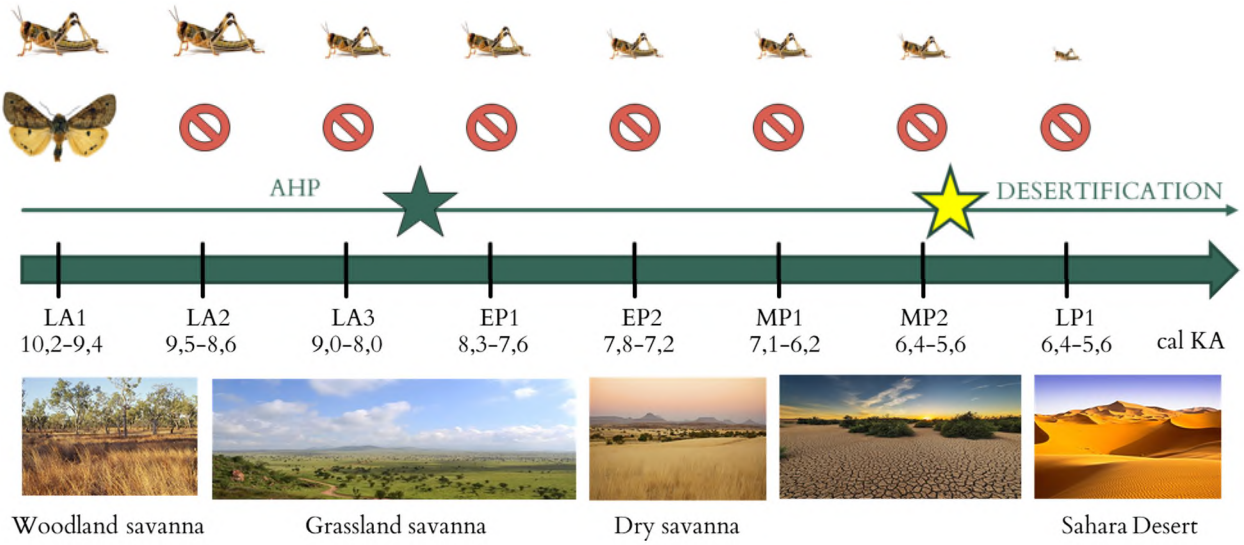

Figure 23: Visual representation of the locust disappearance during the chronological sequence at the Takarkori site caused by environmental changes. Green star marks the 8.2K event, yellow start marks the desertification process (LA = Late Acacus, EP = Early Pastoral, MP = Middle Pastoral, LP = Late Pastoral).

4 SYNANTHROPIC, HUMAN AND ANIMAL PARASITES, FOOD PEST: EVIDENCE OF HUMAN PRESENCE AND ACTIVITIES

4.1 SYNANTHROPIC INSECTS

Over the past sixty years, archaeoentomology has proven to be a powerful tool, not only to reconstruct environments but also to investigate socio-natural interactions. Synanthropic insects can be used to understand the human past (Kenward and Large, 1998; Ponel *et al*., 2000; Huchet and Greenberg, 2010; King, 2014). A synanthrope is a wild insect that interacts with humans and benefits from the association with them. This definition cannot be attributed to domesticated species, such as honeybees or silkworms (Lecocq, 2018). The synanthropic behaviour originated from the exploitation of similar microhabitats. Species did not relocate from natural to artificial habitats but rather took advantage of similarities between the two. The majority of synanthropic species are able to survive in nature (Lecocq, 2018). An exception to this is the highly specialised small group of insects commonly known as obligate synanthropes, like the human louse or certain grain beetles. Obligate synanthropic insects are very rare or sometimes even absent in natural environments due to their incapability to sustain a breeding population without the presence of humans (Plarre, 2013). Throughout the millennia they followed human diasporas around the world becoming fairly cosmopolitan (Bain and King, 2011). From an anthropocentric point of view, most of them are considered pests (Plarre, 2013). In archaeoentomology, insects belonging to this group, such as ectoparasites and stored product pests, are commonly recovered and can help depict a specific past settlement.

The archaeoentomological analysis performed on Takarkori revealed the presence of several synanthropic insects at the site (see Tab. 10 Chapter 3, Paragraph 3.2). In particular, the following species show a various degree of synanthropy or relationship with human and animal life: *Musca domestica*, Linnaeus, 1758, *Pediculus humanus*, Linnaeus, 1758, *Oestrus ovis*, (Linnaeus 1758), and *Sitophilus granarius*, (Linnaeus, 1758). In this chapter, description and typical habits of these insects are presented, discussed, and interpreted to fulfil the thesis's aims.

4.2 DIPTERA

Diptera encompass mosquitos, midges, fruit flies, house flies and many more two-winged insects. Extremely familiar to humans, it is one of the most diverse groups with more than 150,000 described species (Resh and Cardé, 2009). Dipterans are ubiquitous thanks to their outstanding ability to colonise every ecological niche. The tremendous structural variety, the large number of ecological habitats exploited, different diets throughout their entire life cycles, adaptability to extreme conditions, and superb flight-skill contributed to the success of Diptera on our planet (Mcalpine *et al*., 1989). Due to all these characteristics, Diptera have an economic impact on human lives. Several species are considered pests in many significant fields, such as agriculture, veterinary medicine, forestry, and even in medicine (Skevington and Dang, 2002). Although the common negative perception of flies as a nuisance, it is important to underline how many other species inside the group are beneficial to humans. Their fundamental role as scavengers, pollinators, and bioindicators cannot be denied. Besides, some species have been used as animal models in the scientific research field allowing exceptional discoveries (e.g. the midge *Chironomus* spp. and the fruit fly *Drosophila melanogaster* Meigen, 1830) (Resh and Cardé, 2009). True flies are holometabolous undergoing a complete metamorphosis. A typical dipterous life cycle has been briefly summarised in Figure 10 (Chapter 2, Paragraph 2.6.1) and consists of an egg stage (there are a few exceptions inside the order, some species are larviparous), some larval instars (the number of instars can vary according to the families), a pupal stage (during which the metamorphosis happens), and then the adult (Dethier *et al*., 1962). Each

stage usually has a separate ecological role, for example, immature stages are adapted to feeding and adults to reproduction and dispersal. This system reflects their capability to survive in many different habitats (Courtney and Merritt, 2009). Adults are terrestrial, but larvae can also colonise aquatic environments. Historically, dipterans have been divided into two suborders: the lower Diptera (Nematocera) and the higher Diptera (Brachycera). Nematocera are characterised by long segmented antennae, slender bodies, long-legged, and larvae with sclerotised head capsules (Feener Jr and Brown, 1997). Brachycera are characterised by three-segmented antennae, sturdier bodies, and larvae usually hemicephalic or acephalic (Marshall, 2012).

4.2.1 Infamous dipterous families

Several species of true flies are categorised as economically important and have a major impact on everyday human life. Specialised mouthparts for "biting" are common in the group allowing some flies to feed on blood; families like Simuliidae, Ceratopogonidae, Culicidae, Tabanidae, Psychodidae, and Muscidae can cause severe allergic reactions (Resh and Cardé, 2009). However, the "bite" of a fly, despite its direct consequences, can hide a more troublesome unexpected result. Diseases such as onchocerciasis, leishmaniasis, malaria, yellow fever, dengue, sleeping sickness of humans, or trypanosomiases, protozoan and virus infections of both domestic and wild animals, are all transmitted by true flies (Marshall, 2012). Diptera include the highest number of vectors of pathogens to human and animals among the Arthropoda phylum. Immature stages of Calliphoridae and Sarcophagidae families are the cause of myiasis, infestations of tissues on living animals (Zumpt, 1965). Severe discomfort and potentially fatal secondary infections may occur when infested areas are not treated (Hall and Smith, 1993; Hall and Wall, 1995). Despite that, in recent years, induced myiasis have been used to treat wounds to remove necrotic tissues and to promote healing: maggot therapy (Gupta, 2008). The method has shown particularly positive results on burns. Furthermore, since several decades, calliphorids and sarcophagids have been used in forensic entomology to establish the time since death

(Smith, 1986; Tuccia *et al*., 2018). Economic losses of livestock such as reduction of milk production, weight loss, muscle damages, and even death of a great number of cattle, sheep, horses are linked to bot flies (Oestridae) (Colwell *et al*., 2006). Agriculture is also deeply affected by dipterans: for instance, Cecidomyiidae, commonly known as gall gnats, live and feed inside plants. Another example is some species in the family Tephritidae which can infest multiple substrates such as leaves, fruits, stems affecting the entire system of a plant. Similar behaviours are found also in the Anthomyiidae family, which attack roots, and Agromyzidae family, specialised on leaves promoting decay (Resh and Cardé, 2009).

Six families of true flies have been collected from Takarkori samples and all of them have species that can fall in the synanthropic group or are related with human and animal bodies and excrements: Phiophilidae, Sphaeroceridae, Calliphoridae, Sarcophagidae, Muscidae, and Oestridae. Puparia fragments are the only traces of true flies at the site. Identification at species level was possible only for Muscidae and Oestridae families.

4.2.2 PIOPHILIDAE

Piophilidae family encompasses around 80 described species. They are globally distributed, and they are known as consumers of high-protein dead food. Dung, drying tissues and carcasses in advanced decay are the preferred food source (López-García *et al*., 2020). They are commonly named skipper flies because of the peculiar escaping strategy of leaping typical of their larvae. Larval leaping is used to move from the feeding to the pupariation site. A famous example of this behaviour is observed in stored-product pest *Piophila casei*, (Linnaeus, 1758), the cheese-skipper fly. The larva of this species infests a wide range of substrata, from cheese, cured ham to rotten fish. The leaping is obtained by forming a loop with the mouth grabbing the tail and suddenly realising it propelling the entire body away (Marshall, 2012). Cheese skipper flies can cause enteric myiasis, surviving the digestive system and potentially damaging internal organs (Marshall, 2012). Some species of piophilids are parasites of bird nests. Most of Piophilidae occur in the Holarctic region.

Very few fragments of Piophilidae puparia have been collected from LA1, LA3 and EP1 layers at Takarkori. Two different species have been recognised (Fig. 24).

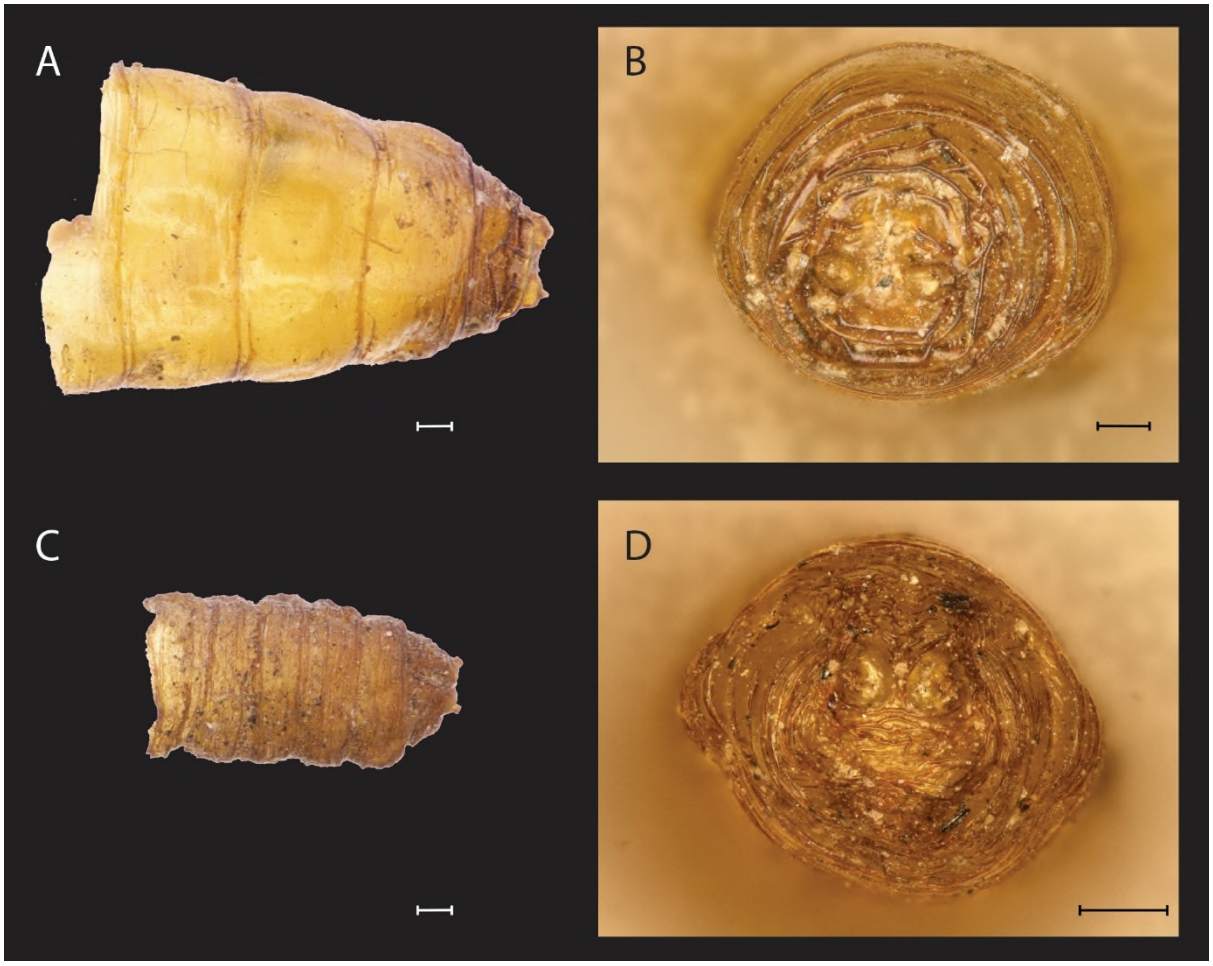

Figure 24: Piophilidae specimens collected at Takarkori. A-B ventral view and close up of posterior spiracles of species 1; C-D ventral view and close up of posterior spiracles of species 2. Scale bar 500 μm.

Their occurrence can be explained by the feasible constant presence of dung at the site. Another explanation could be the presence of dry meat, pelts or decomposing bones.

4.2.3 SPHAEROCERIDAE

Sphaeroceridae family encompasses about 1,600 described species and they are ubiquitous. They are commonly named small dung flies and they are characterised by shortened first tarsomeres in the hind legs, dorsal stiff bristles, and black to grey colour (Marshall, 2012). Most of their larvae are polysaprophagous, feeding on dung, carrion, sewage, decomposing plants and fungi. Some others are highly specialised and develop in association with other

arthropods. Sphaerocerids are very common in humid environments (Resh and Cardé, 2009; Buckland *et al*., 2018).

Fragments of puparia belonging to the family have been collected almost throughout the entire chronological sequence of Takarkori (Fig. 25), indicating a constant presence of dung and decomposing matter at the site.

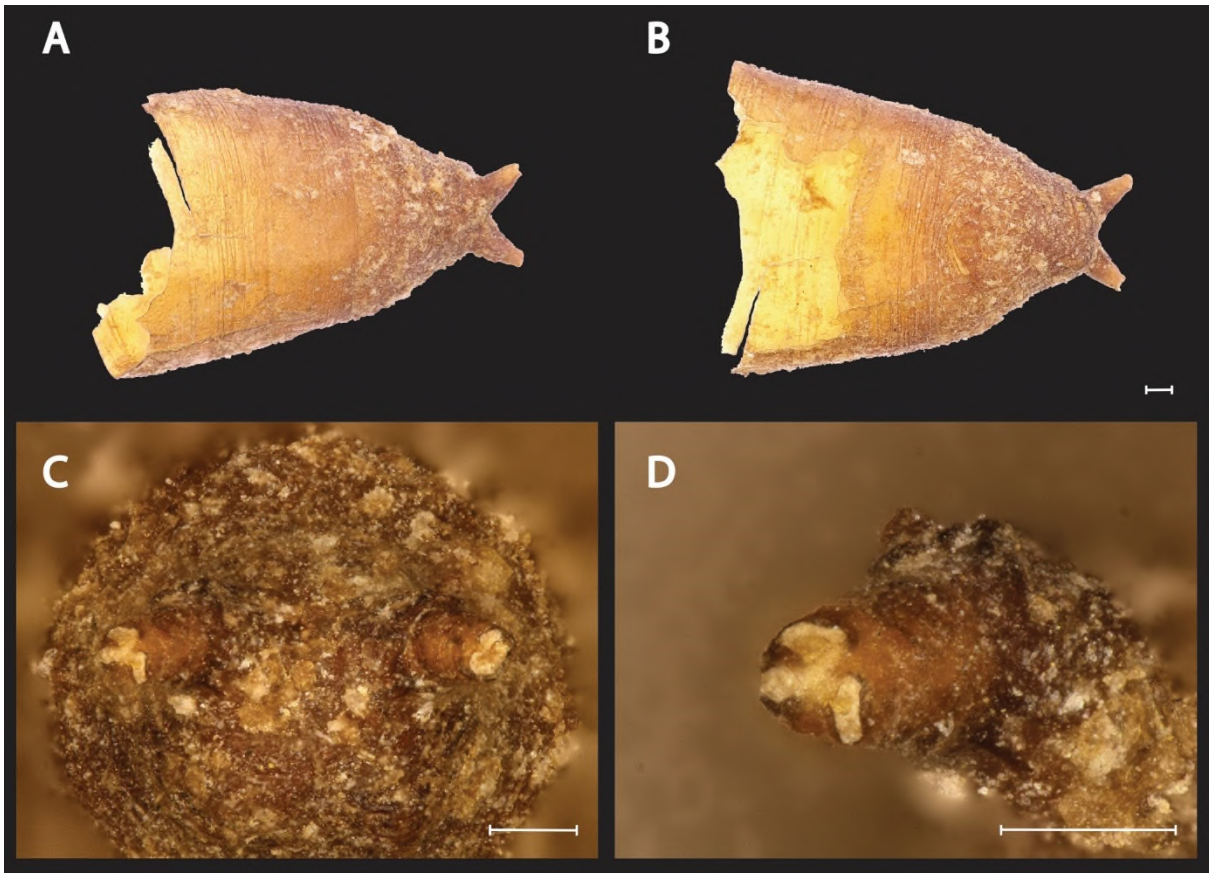

Figure 25: Sphaeroceridae specimen collected at Takarkori site; A-B dorsal and ventral view of the posterior region, C close up of posterior spiracles, D close up of one posterior spiracle. Scale bar 100 μm.

4.2.4 CALLIPHORIDAE

Calliphoridae family belong to the superfamily Oestroidea, which include most of the calyptrate flies, such as Sarcophagidae and Oestridae. Calliphorids are commonly named blowflies and there are about 1,600 described species all around the world. The group includes a wide range of parasitic, predaceous and sarcosaprophagous species and it is well-known to have a strong synanthropic behaviour (Resh and Cardé, 2009). They play an

important ecological role in accelerating the bodies/carcasses decomposition process. Many species are larviparous, but the most common carrion-breeding species can lay several batches of hundreds of eggs. When the eggs hatch, large clusters of larvae (also called maggots) start scraping the food surfaces with their sturdy mouth-hooks, realising proteolytic enzymes produced in their salivary glands (Marshall, 2012). Development is temperature driven and large assemblages of larvae can increase the temperature of the carcass by several degrees (Amendt *et al*., 2004). Pupariation starts after post-feeding third larvae move away from the food source in search of a sheltered site. The life cycle can be completed in little time when temperatures are high and multiple generations can be found at the same time on the same food source (Smith, 1986). Due to their feeding habits and their ability to detect decomposing odours, calliphorids are a useful tool in forensic entomology as they are usually the first coloniser of dead bodies (Amendt *et al*., 2004; Lister, 2009; Vanin *et al*., 2009; Giordani *et al*., 2018).

A single well-preserved fragment of calliphorid puparium has been collected in LA2 layer at Takarkori (Fig. 26). The recovery might indicate the presence of decaying animal matter, most likely to be ascribed to food waste rather than a cadaver/carcass.

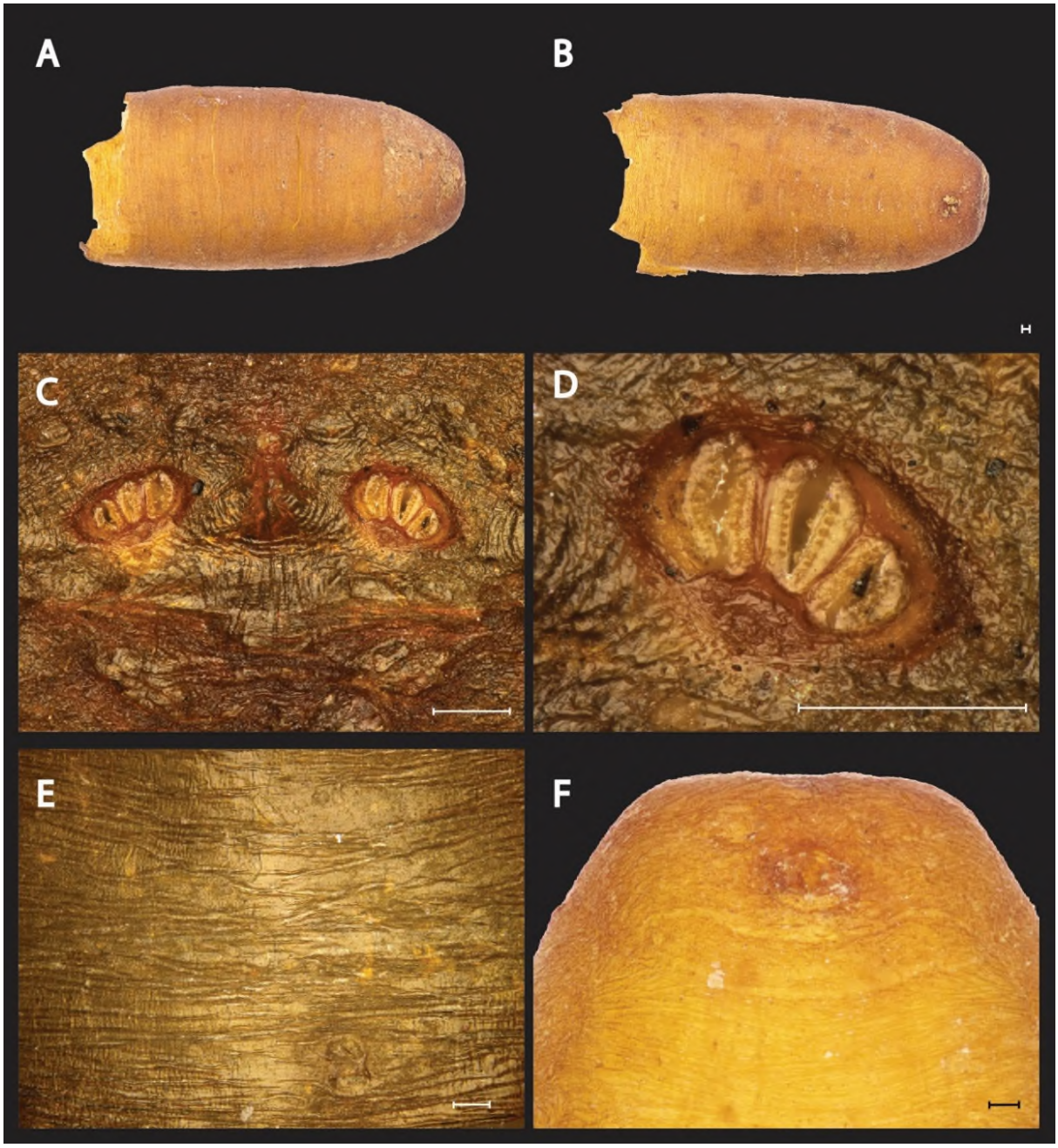

Figure 26: Fragment of Calliphoridae puparium. A-B dorsal and ventral view; C-D close up of posterior spiracles; E close up on speculation; F close up on anal plate. Scale bar 100 µm.

4.2.5 SARCOPHAGIDAE

Sarcophagidae family includes more than 3,000 cosmopolitan species. Sarcophagids are characterised by longitudinal three black stripes on the thorax and tessellated pattern on the abdomen (Resh and Cardé, 2009). They are commonly named flesh flies, which indicates the habits of some species to feed on decomposing meat. Typically, these sturdy

flies have been recorded in urban areas where waste is present in a large amount. The group encompasses also unusual specialists such as parasites of other invertebrates, bat dung scavengers, and pests of wasp nests. Most of sarcophagids are ovoviviparous, meaning that eggs hatch as soon as they are expelled. This adaptation evolved probably due to their ephemeral food choice or evasive hosts (Panagiotakopulu, 2004a; Huchet and Greenberg, 2010; Marshall, 2012).

A few fragments of Sarcophagidae puparia have been collected in the most ancient layer (LA1) (Fig. 27), indicating a probable co-existence between humans and animals at the Takarkori site.

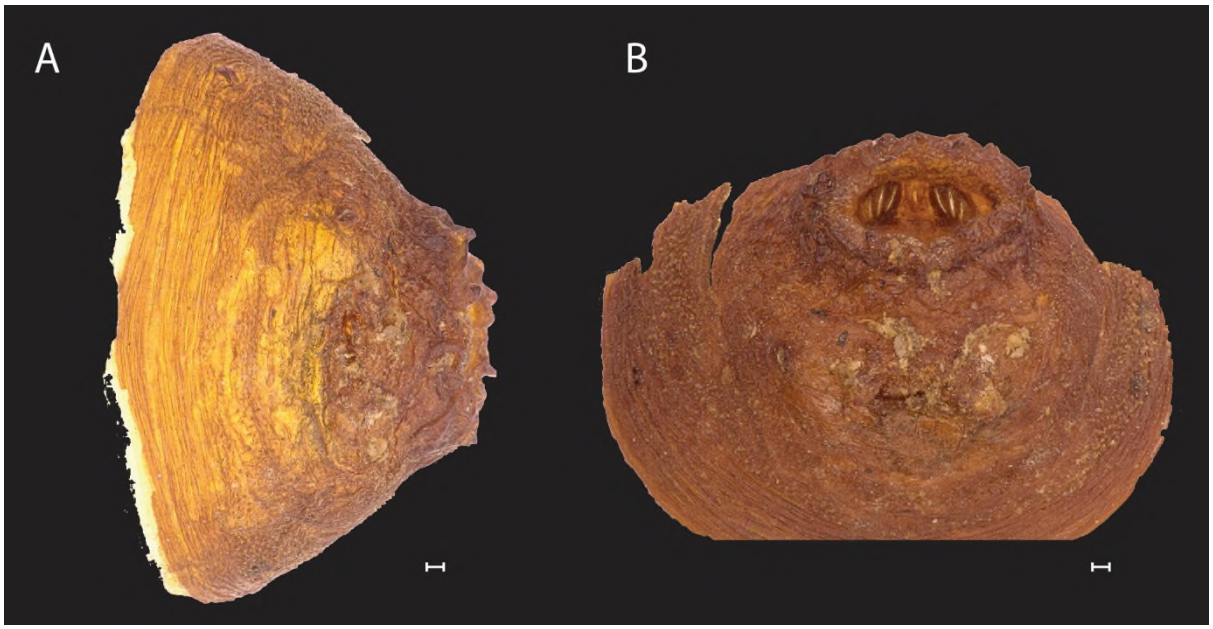

Figure 27: Fragment of Sarcophagidae puparium. A posterior region, ventral plate; B posterior spiracles. Scale bar 500 μm.

4.2.6 MUSCIDAE

Muscidae family belongs to the superfamily Muscoidea, which differs from Oestroidea superfamily by the absence of stout bristles on the meron. Muscids are commonly named house flies due to the ever-present synanthropic genus of *Musca*, Linnaeus, 1758 comprising 60 species one of which *Musca domestica* is the most common and recognised species among the public, but the group includes around 5,200 other described species (Resh and Cardé, 2009). The majority of muscids are saprophagous or predators in several

different habitats. Their biology has been studied and described by Skidmore (1985), who highlighted the common behaviour of larvae to be facultative predators during the late instars. A lot of muscids are significant pests (Marshall, 2012). Many species (e.g. *Musca vetustissima* Walker, 1849, *Musca sorbens* Wiedemann, 1830, *Musca autumnalis* De Geer, 1776, *Synthesiomyia nudiseta* Van Der Wulp, 1883, and *Muscina stabulans* (Fallén, 1817)) feeds on faeces and bacteria-rich waste and this habit can cause the transmission of pathogens to humans causing enteric diseases. Some others, due to the presence of a forward-directed stiffened lower lip (labium), can painfully bite animal skins to feed on blood (e.g. *Stomoxys calcitrans* (Linnaeus, 1758), *Philornis* Meinert genus, *Passeromyia* Rodhain and Villeneuve genus) (Resh and Cardé, 2009).

4.2.6.1 Musca domestica

Musca domestica also known as the house fly, measures around 6 mm long and is characterised by dusty grey colour, thorax with four narrow longitudinal black stripes, grey scutellum, yellow abdomen gradually becoming darker, silky yellow lower face, dark brown antennae, black palpi, proboscis ending in fleshy lobes, black legs, and pale grey with yellow base wings (Schiner, 1864). It is the most widely distributed fly around the planet and one of the species showing a strong synanthropy (Skidmore, 1985; Panagiotakopulu, 2004b). Very rarely found in nature, it has a strict association with humans and domesticated animals. Its distribution ranges from sub-polar regions to the tropics, where large numbers can occur. Local distribution is driven by two main factors, availability of breeding sites and food sources. The species inhabits human settlements being the most abundant insect inside houses (Hewitt, 1914). Usually, it is most common during the warmest months of the year, but micro-climates inside heated buildings are exploited throughout the entire year even during the cold season. It is omnivorous and its indiscriminate feeding habit is one of the reasons for its ecological success. The anatomy of the proboscis and the alimentary tract is adapted to solely sucking function absorbing liquids or liquefied food (Hewitt, 1914). Thus, *M. domestica* is not able to ingest solid food. When dry food such as sugar is available, the fly secretes saliva to moisten and liquefy the

substance. Furthermore, to increase the salivary glands production and to promote better digestion, regurgitation of previously ingested food is frequently observed (Hewitt, 1914). After a meal, the fly usually rests in a quiet place grooming its head and proboscis with its front legs in anthropomorphic rubbing motions. This habit is the main cause of the bacterial contamination on several different surfaces and consequently gives *M. domestica* the title of the deadliest mechanical vector of germ diseases (Hewitt, 1914). Several studies proved the fly can transport the typhoid bacillus, enterobacteria causing summer diarrhoea of infants, and bacteria of several infectious serious diseases such as anthrax, tuberculosis, cholera, ophthalmia, plague, yaws, dysentery and oriental sore (Hewitt, 1914; Greenberg, 1973).

The house fly life cycle starts with the oviposition of approximately 120 eggs per time. Females require high-protein and sugary diets to produce eggs. The preferred oviposition sites are manures, but other decaying substrata can be used when animal faeces are not available. The development is temperature-dependent. The larval and the pupal stages are relatively short during optimal temperatures. Adults have 2 or 3 weeks of life span during summertime (Skidmore, 1985). Dispersal from rearing sites is common even when favourable conditions are present. Its attraction to dark openings also promotes the wide geographical scale dispersal of the species as it is easily transported by humans through the economic routes (Hewitt, 1914).

The earliest record of *M. domestica* in archaeoentomology is from Erkelenz-Kückhoven site in the Rhine Valley, Germany, dating 7,000 years ago (Schmidt, 2012). The recovery of fragments of puparia belonging to it at Takarkori site in ancient layers (LA1, LA2, LA3, EP1, EP2, MP1) becomes the new earliest record of the species in association with human settlements dating between 11,2 and 9,9 cal KA (Fig. 28).

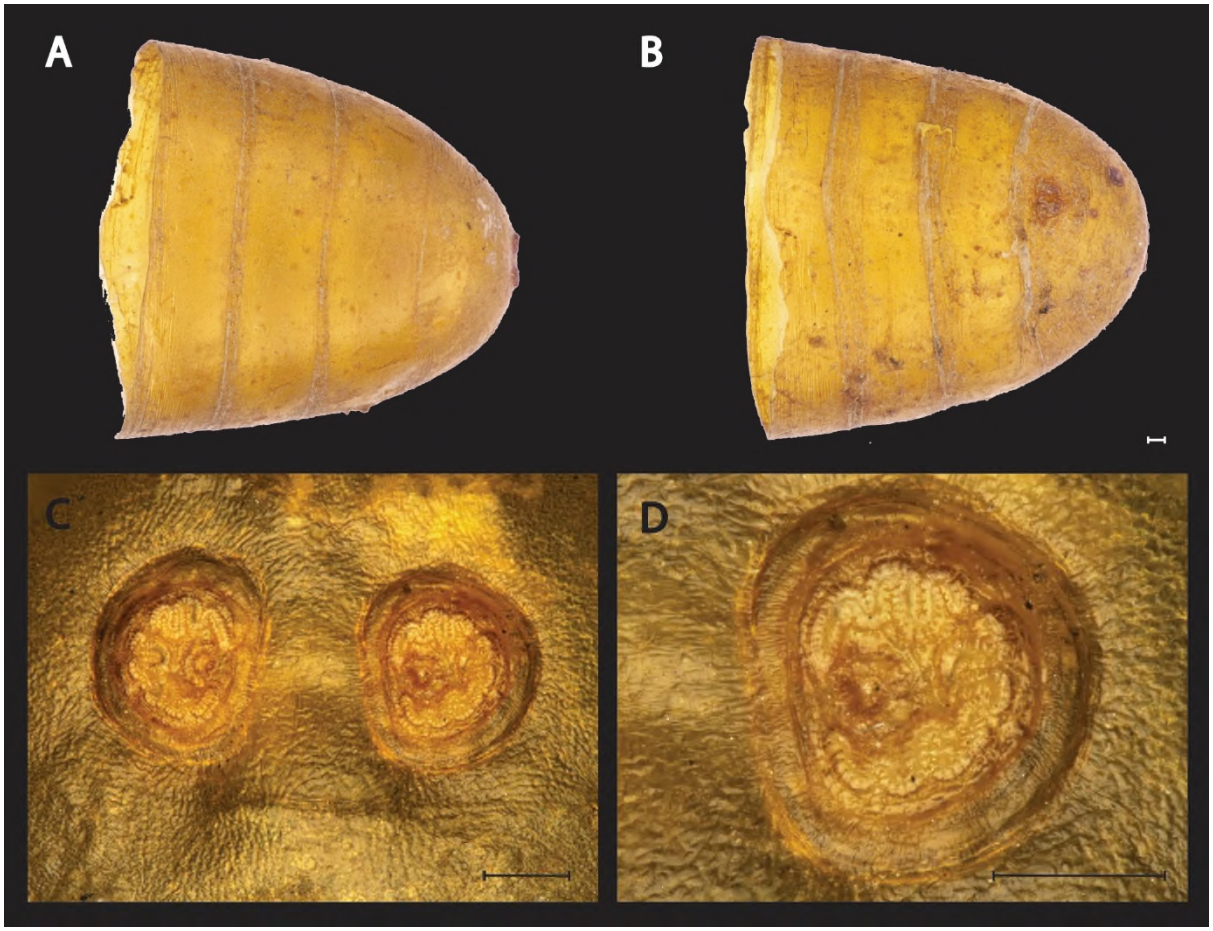

Figure 28: Fragment of *Musca domestica* puparium. A-B dorsal and ventral view; C-D close up to posterior spiracles. Scale bar 100 μm.

The discovery is fundamental to trace back when the synanthropy of the species co-evolved with humans. Moreover, the presence of houseflies at Takarkori can indicate the presence of several food sources of its preference such as, dung, faeces, exposed meat, food waste, and rotting decaying matter, suggesting Takarkori be shared between humans and animals.

4.2.7 OESTRIDAE

Oestridae family is a small group of flies, commonly named bot or warble flies, encompassing around 160 described species (Wood, 1987). All larvae in the family cause myiasis in mammals. Their obligate parasitic behaviour not only impacts the productivity and welfare of domestic animals but also affects many wild endangered large mammals (Colwell *et al*., 2006). The family is divided into four subfamilies categorised according to

the anatomical site they infest (Tab. 12) (James, 1947; Zumpt, 1965; Papavero, 1977): Hypodermatinae, Cuterebrinae, Oestrinae, and Gasterophilinae.

Table 12: Taxonomic classification of oestrid flies and their preferred anatomical site of infestation (modified from Colwell et al., 2006).

Subfamily	Site of infestation	Description of myiasis
Hypodermatinae Cuterebrinae	Dermal/subdermal	Larvae penetrate the host skin and develop in furuncles
Oestrinae	Nasopharyngeal	Larvae are deposited in nostrils or oral cavity developing in sinuses or the pharynx
Gasterophilinae	Intestinal	Eggs or larvae are deposited on the host and ingested during grooming

Mammals evolved between the Late Triassic (237 million years ago) and the Early Jurassic (201 million years ago) (Luo, 2007). Botflies probably co-evolved during the mammal radiation after the Cretaceous-Tertiary boundary around 66 million years ago in the Afrotropical region (Pape, 2001). The little number of species of oestrid flies shows a low evolutionary diversification rate ascribable to restricted niche opportunities caused by their highly selective association (Schaefer, 1979). Zumpt (1965), Papavero (1977) and Schaefer (1979) speculated on the origin of their parasitic behaviour stating they probably derived from less specialised calliphorids with ectoparasitic blood-sucking larvae. They also analysed oestrids relationship with hosts noting that all mammals affected are terrestrial herbivores with gregarious behaviour or with the habit to live in borrows. The only species with the ability to infest carnivores is *Dermatobia hominis* (Linnaeus Jr. in Pallas, 1781), even though it is more often found on herbivores. The reason the latter is preferred by bot flies is that they are more numerous than predators supplying a wider range of habitats (Colwell *et al*., 2006). Herbivores tend to live in herds being easier to be found compared to a single errant animal. Most of the botflies are highly host-specific, not able to fully develop on hosts different from the natural one (Tab. 13) due to the adaptation to each immune system response (Colwell *et al*., 2006).

Table 13: Oestrids and their hosts (modified from Colwell et al., 2006).

Subfamily	Genus	Natural hosts
Hypodermatinae	*Hypoderma*	Bovine
	Oestroderma	Pikas
	Oestromyia	Mice, marmots, pikas
	Pallasiomyia	*Saiga tatarica* (Linnaeus, 1766)
	Pavlovskiata	*Gazella subgutturosa* (Güldenstädt, 1780)
	Portschinskia	Mice, pikas
	Przhevalskiana	Caprine, gazelles
	Strobiloestrus	*Kobus* antelopes
Cuterebrinae	*Cuterebra*	Rodents, lagomorphs, howler monkey
	Dermatobia	Non-specific (large mammals and birds)
	Neocuterebra	*Loxodonta africana* (Blumenbach, 1797) *Loxodonta cyclotis* Matschie, 1900
	Ruttenia	*Loxodonta africana* (Blumenbach, 1797) *Loxodonta cyclotis* Matschie, 1900
Oestrinae	*Cephenemyia*	Cervidae
	Cephalopina	Cameline
	Gedoelstia	Antelopes
	Kirkioestrus	Antelopes
	Oestrus	Ovine, caprine
	Pharyngobolus	Elephants
	Pharyngomyia	Cervidae, zebras, pigs, giraffe, hippopotamus, springbuck, sheep
	Rhinoestrus	Equine
	Tracheomyia	Kangaroos
Gasterophilinae	*Cobboldia*	Elephants
	Gasterophilus	Equine
	Gyrostigma	Rhinoceros

Each oestrid subfamily has a specific life cycle that differs from the others because of the distinctive preferred infestation sites. Oestrinae life cycle is presented below as the species found at Takarkori belongs to this subfamily (Fig. 29).

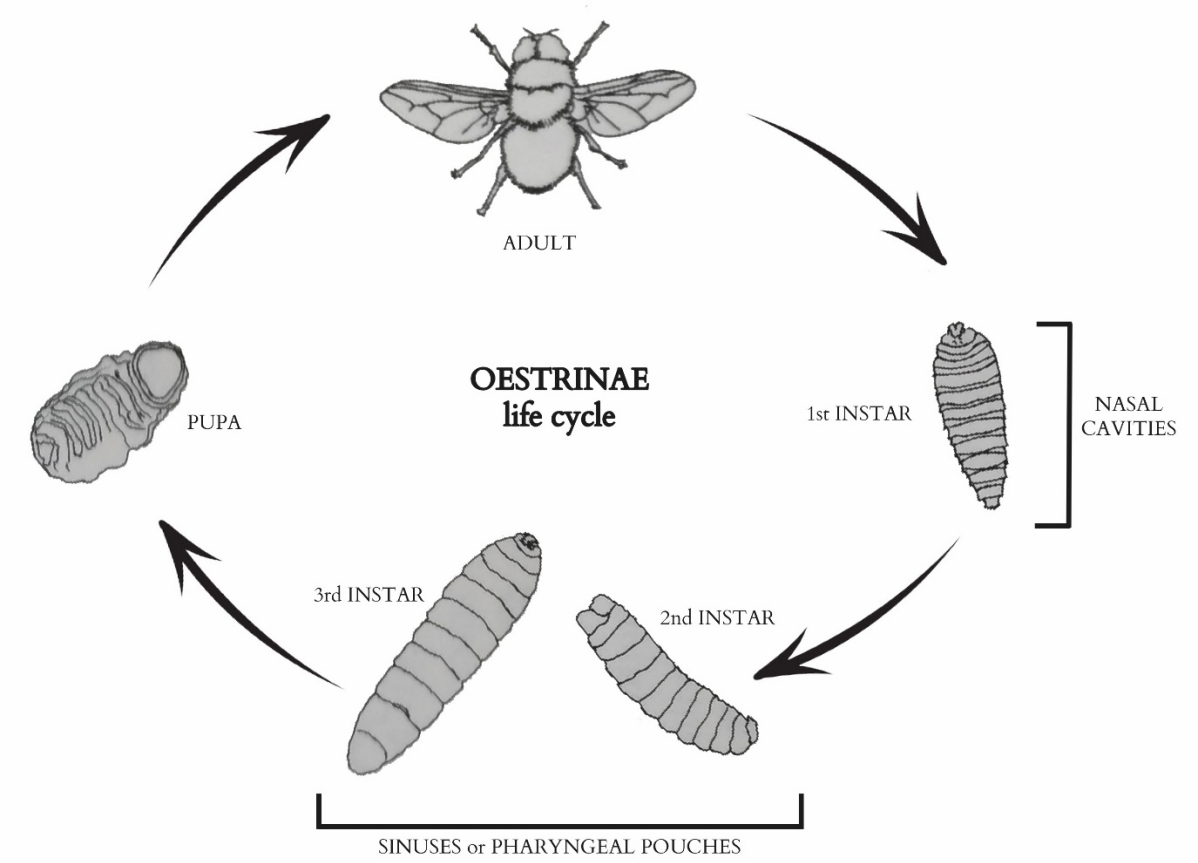

Figure 29: Life cycle of Oestrinae subfamily (modified from Colwell et al., 2006).

Oestrinae females are larviparous and discharge between 2 and 20 first instar larvae around the nasal and oral cavities (some species exploit also the eyes area) of the host (Colwell *et al*., 2006). The larvae are attached to the surface due to a small amount of fluid produced by females. Immediately after the deposition, due to robust mouth hooks, larvae migrate into the outer nasal cavities where they start to develop (Angulo-Valadez *et al*., 2010). First instar larvae, usually, are white dorsoventrally flattened with well-developed spines on ventral and lateral surfaces (Colwell and Scholl, 1995). The spines aid attachment on the host mucosal walls (Guitton *et al*., 1996). A second migration into sinuses or pharyngeal pouches is performed during second and third instar development. Second instar larvae are creamy-white and they are significantly larger compared to the first instar (Colwell and Scholl, 1995). Although they are dorsoventrally flattened like the previous instar, their dorsal surface is rounder giving an ovoid appearance when cross-sectioning them. Spines are reduced in the lateral surfaces of larval bodies (Colwell *et al*., 2006). Third instar larvae are the same creamy colour at the early stages and then they darken during maturation

(Cepeda-Palacios and Scholl, 2000a). The developmental period of larvae varies according to the species and can last from several days to several months (Zumpt, 1965). Post-feeding third larvae, when the development is complete, enter in the so-called "wandering phase" departing by dropping from the host's body) (Cepeda-Palacios and Scholl, 1999, 2000b; Cepeda-Palacios *et al*., 2015). When the ground is reached, the larvae start crawling (even several meters) to find a suitable substratum to hide (Bishopp *et al*., 1926). Some larvae are negatively phototrophic; thus, they search for sheltered sites with shade before starting to dig into the ground (Biggs *et al*., 1998). The burrowing process is completed using their mouth hooks and usually, they do not dig in great depth, stopping between 1 and 10 centimetres (Cepeda-Palacios and Scholl, 2000a). When hidden into the soil with the anterior part facing upwards, larvae retract the cephalopharyngeal apparatus and invaginate the entire head segment into the body. At the same time, posterior spiracles collapse into the anal tubercle and a contraction of longitudinal muscles arch and shrink the entire body, shortening the specimen by 70% of the original length (Cepeda-Palacios and Scholl, 2000b). An interesting fact is the possibility to reverse the process and to resume the wandering phase when larvae experience disturbance during the early phases of the pre-pupariation process (Cepeda-Palacios and Scholl, 2000b). When the latter is completed, the separation of the pupal cuticle from the larval epidermis (apolysis) starts (Catts, 1967). Then, two distinctive phases can be observed: the cryptocephalic and the phanerocephalic pupal stages. The first is characterised by the absence of a visible head, while in the second the head is evaginated and apparent (Baird, 1997). The passage from the first to the second phase is accomplished by an abrupt violent process consisting of the expansion of the thoracic section (Denlinger and Zdárek, 1994). After that, the pharate morphology develops into the final adult structure (Scholl and Weintraub, 1988). Following eclosion, an adult fly with reduced mouthparts emerges (Wood, 1987). Adults do not feed; their role is strictly related to reproduction (Zumpt, 1965). Male reproductive organs are fully developed soon after eclosion allowing insemination shortly after emergence. Females have mature eggs ready before eclosion (Colwell and Milton, 1998).

4.2.7.1 Oestrus Linnaeus, 1758

Four species belong to the *Oestrus* genus: *Oestrus ovis*, *Oestrus variolosus* (Loew, 1863), *Oestrus aureoargentatus* Rodhain & Bequaert, 1912, and *Oestrus caucasicus* Grunin, 1948. Adults of all four species are medium-large flies ranging between 10 and 19 centimetres, have mimic black-silver-white pattern, and are larviparous with the ability to deposit larvae without landing on the host (Colwell *et al*., 2006). The larval stages develop in sinuses feeding on mucous and skin cells. The feeding period can last several months allowing the survival even during the coldest months in temperate latitudes (Angulo-Valadez *et al*., 2010). At optimal conditions, larval development is completed in less than 35 days. The third instar larva leaves the feeding site inducing sneezing and being ejected from the host's nostrils (Cepeda-Palacios and Scholl, 2000a). Once burrowed in the ground, pupariation process takes up to 30 days in warmer temperature, longer in cooler seasons (Colwell *et al*., 2006). Two or three generations per year have been recorded. Species-specific hosts and distribution of each species are presented in Table 14.

Table 14: List of natural hosts and distribution of each species inside *Oestrus* genus (modified from Colwell et al., 2006). *Cosmopolitan in domesticated livestock, absent in wild hosts in Sub-Saharan Africa.

Species	**Distribution**	**Natural Hosts**
O. ovis	Cosmopolitan*	*Ovis* Linnaeus, 1758 genus
		Capra Linnaeus, 1758 genus
		Odocoileus Rafinesque, 1832 genus
O. variolosus	Sub-Saharan Africa	*Connoachaetes taurinus* (Burchell, 1823)
		Alcelaphus buselaphus (Pallas, 1776)
		Alcelaphus lichtensteini (Peters, 1849)
		Damaliscus korrigum (Ogilby, 1837)
		Damaliscus dorcas (Pallas, 1767)
		Damaliscus lunatus (Burchell, 1824)
		Hippotragus niger (Harris, 1838)
		Oryx gazelle Linnaeus, 1758
O. aureoargentatus	Sub-Saharan Africa	*Hippotragus niger*
		Hippotragus equinus (É. Geoffroy Saint-Hilaire, 1803)
		Damaliscus korrigum
		Damaliscus lunatus

		Alcelaphus buselaphus
		Alcelaphus lichtensteini
		Connoachaetes taurinus
O. caucasicus	Central Asia	*Capra caucasica* Güldenstädt e Pallas, 1783
		Capra ibex Linnaeus, 1758

Oestrus ovis is the most important species in the genus as it has an economic impact on both domesticated animals and humans (Hall and Wall, 1995). Commonly named sheep nasal bot fly, it is widespread all around the world. There is little information about biology and pathology of the other species inside the genus (Zumpt, 1965). Heavy infestations of *O. ovis* can cause a substantial animal weight loss leading to problems in meat production (as the constant larval deposition decreases animal grazing time). Wool and milk productions are also affected (Ilchmann *et al*., 1986). When the number of larvae per sheep exceeds a certain threshold, overstimulation of lung tissues and an overwhelmed immunological system can result in fatal interstitial pneumonia (Dorchies *et al*., 1998). Nowadays, *O. ovis* infests predominantly domesticated ovicaprids. In the Americas, it has been also recorded on species belonging to *Odocoileus* genus a medium size deer (Capelle, 1966). There is only one report of *O. ovis* infesting *Ammotragus lervia* Pallas, 1777, a wild goat endemic in North Africa, in a recently introduced population in Spain (Barroso *et al*., 2017).

Although humans are not the natural host of *O. ovis*, several cases of infestation from it have been recorded (Pampiglione *et al*., 1997) throughout the last century. In human infestations, flies tend to deposit larvae in the eyes (opthalmomyiasis) causing severe conjunctivitis, lid oedema, and punctate keratopathy (Harvey, 1986).

One single closed puparium of *O. ovis* has been collected in the ancient chronological layer of Takarkori (LA1) (Fig. 30). Observation using synchrotron radiation revealed that the pharate inside the puparium is a male (Fig. 31).

Figure 30: Dorsal, lateral, and ventral view of *Oestrus ovis* specimen. Scale bar 100 µm.

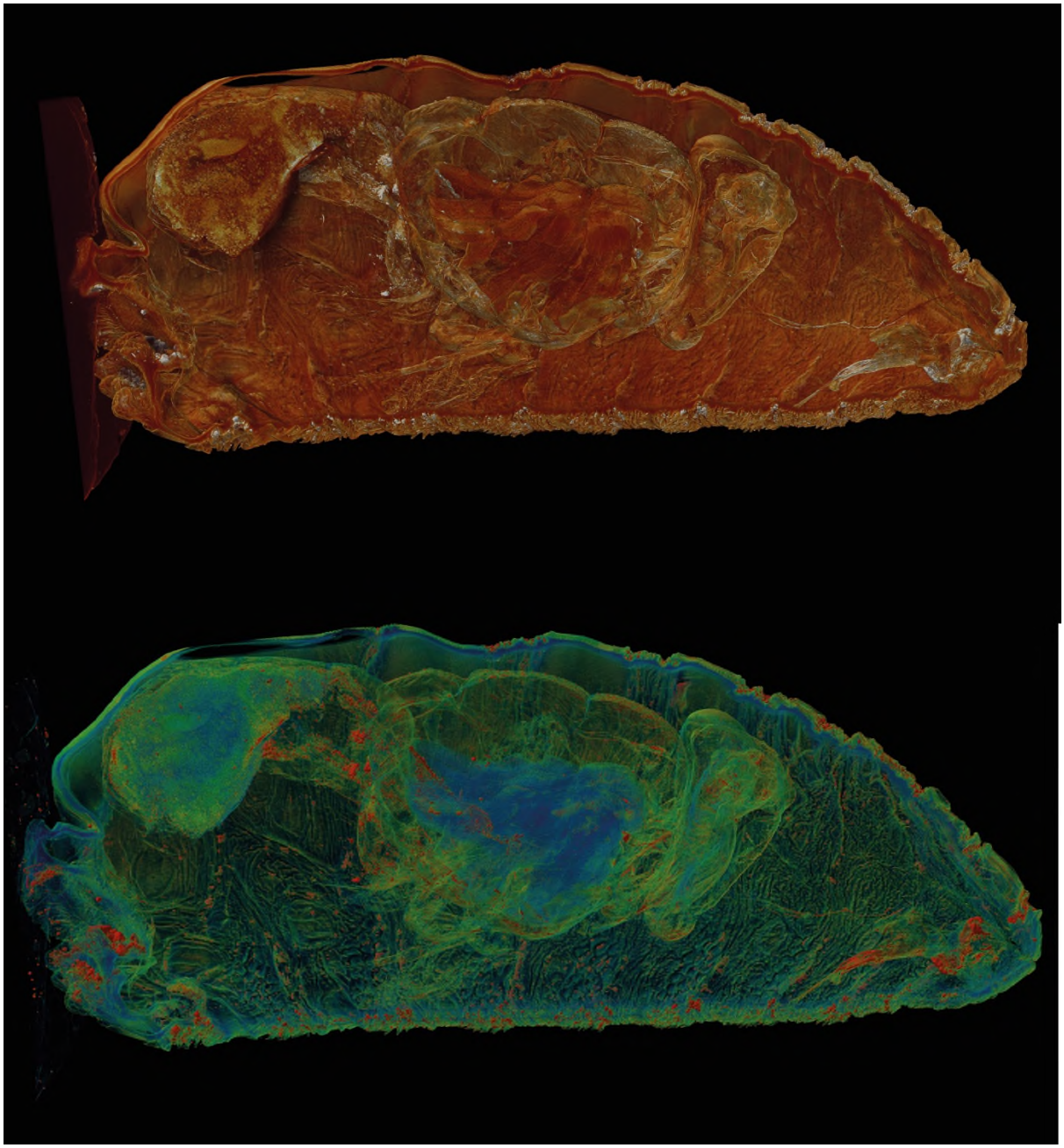

Figure 31: 3D reconstruction of the pharate. Lateral view. Internal organs are highlighted in blue in the bottom image.

The hypothesis that Takarkori was shared between humans and animals is strongly supported not only by archaeozoological and archaeological records (di Lernia and Cremaschi, 1996; di Lernia, 1999b; Dunne *et al*., 2012) but also by *O. ovis*. The oestrid fly is a keystone to understand the evolution of parasitism from wild animals to livestock, and to comprehend how important humans have been in speeding the co-evolution process.

Indeed, the specimen is the first record of a bot fly in an archaeological context and the oldest known *Oestrus ovis* record. The fact that nowadays, the species infest predominantly domesticated animals, with only one report of *O. ovis* infesting *Ammotragus lervia* (Barroso *et al*., 2017), suggests early forms of animal management by hunter and gatherers during the early Holocene phases in North Africa. Several remains of *Ammotragus lervia* have been recovered at Takarkori. The presence of the bot fly in the Late Acacus horizon highlights how less selective it was compared to nowadays. Furthermore, the specimen is a pupa with almost a totally developed pharate inside, which means that the fly was close to complete its life cycle having left the host and burrowed into the ground. This can be explained only if wild goats were gathered at the Takarkori site. *Ammotragus lervia*, in nature, lives in small groups of 3-6 female individuals. Presumably, humans assembled a larger group including males for reproduction, creating a favourable niche for the oestrid parasite. This is also supported by archaeological evidence, with stone fences and fodder accumulation at the site (Rotunno *et al*., 2019).

4.3 PHTHIRAPTERA: THE HUMAN LICE

Phthiraptera order encompasses obligatory ectoparasites of birds and mammals with approximately 5,000 described species. There are four suborders: Amblycera, Ischnocera, Rhynchophthirina, and Anoplura (Resh and Cardé, 2009). The first three are commonly known as chewing lice attacking birds, and the last one is characterised by blood-sucking species typical of mammals. All of them are hemimetabolous (Burgess, 2004). The main features are the absence of wings, single or two-segmented tarsus often with claws, reduced eyes (sometimes totally absent), short antennae, and small dorsoventrally flattened bodies (between 0.3 and 12 millimetres in length) (Maunder, 1977). Chewing lice have mandibles, Anoplura have modified mouthparts into piercing stylets adapted to sucking (Fournier *et al*., 2002). Only a few life cycles of economically important species have been studied in detail. Eggs are called nits and they are typically deposited on hairs or feathers of the host. The entire life cycle is completed on a single host. The egg development period varies from species to species; after the emergence, nymphs usually undergo three moults before

fully developing into adults. They are not able to survive long without the host (Resh and Cardé, 2009). Dispersal occurs through mechanical contact between different host individuals in most of the species. Even though a few species can parasite multiple different hosts, many lice are highly species-specific, specialising to attack the unique natural host (Burgess, 2004). The group has economic importance as lice can cause irritation, inflammation, itching, causing great discomfort to the animal, but more importantly they are vectors of other parasites and threatening diseases (Maunder, 1977). High infestation rates are commonly observed on weakened hosts such as sick birds unable to groom or neglected infants and children in the case of humans. Many species also affect domesticated animals like dogs, cats, rabbits, cattle, etc. Zoos and laboratory colonies might also be an easy target when not controlled properly (Resh and Cardé, 2009). Humans have three species of lice specialised to attack three different body locations: *Pediculus humanus humanus*, known as the body louse (alternatively the clothing louse), *Pediculus humanus capitis*, the head louse, and *Pthirus pubis* (Linnaeus, 1758), the crab or pubic louse (Maunder, 1977). Although their biology is similar, they differ in the ability to remain attached to different types of hairs. Body and pubic hairs are thicker compared to hairs of the head. Soon after the emergence from eggs, nymphs have to feed to prevent starvation and dehydration. The absence of a host is increasing the mortality rate exponentially within a few hours (Fournier *et al*., 2002). All three human louse species belong to the Anoploura suborder being capable to feed by sucking blood (Li *et al*., 2010). During the ingestion of liquid food, the expandable cuticle of the abdomen can increase up to one-third of their regular size. Head and crab lice feed every 4-6 hours; body lice can survive for days without feeding. Females are commonly larger than males and within 2 days after the last moults they start to lay eggs, often in groups of 3-6 per day cementing them with a sort of glue to the hairs. The life cycle is completed in 30-42 days (Resh and Cardé, 2009). Lice have been successfully used to trace and to confirm the human past migrations (Light *et al*., 2008).

Pediculus humanus humanus

The body louse usually does not live directly on the host. Clothing and bedding are the preferred habitats reaching the host just to feed. The nits are commonly deposited on fibres close to clothes seams. Infestations occur when poor hygiene habits are displayed (Raoult and Roux, 1999). Individuals unable to wash themselves living in crowded conditions like in wars, natural disasters, homelessness, or neglection, are most frequently infested. Diseases such as the epidemic typhus, the murine typhus and the relapsing fever are transmitted to humans by contact with contaminated lice faecal pallets (Raoult and Roux, 1999).

Pediculus humanus capitis

On the contrary, the head louse prefers a clean and healthy head to infest. Nowadays, children between 3 and 11 years old are more likely to be the louse targets as during that age head-to-head contacts are more frequent than in adults. The nits are deposited near the scalp where they can exploit the warmth released by the human body (Hunter and Barker, 2003).

Pthirus pubis

The crab louse commonly parasite pubic and perianal areas. However, unconventional sites like beard, moustaches, axillae, eyebrows and eyelashes can be affected. The louse is transmitted by sexual contact. Both head and crab lice can transmit *Streptococcus pyogenes* Rosenbach 1884 and *Staphylococcus aureus* (Burns and Sims, 1988).

The oldest record of a human louse in archaeoentomology is a nit still attached to the hairs of a mummy discovered in Brazil dated 10,000 years ago (Araújo *et al*., 2000); the second oldest record is from Israel dated 9,000 years ago (Mumcuoglu and Zias, 1991). The discovery of a well-preserved specimen of *Pediculus humanus capitis* (Fig. 32) from the ancient horizon dating 10,2-9,4 cal KA at Takarkori makes it one of the earliest evidence in co-evolutionary history between humans and lice. Its presence confirms human settlements and the prolonged residential usage of Takarkori as a shelter during the early

phases of the Holocene. The louse also gives an insight into the hygienic condition of the population. As previously stated, *Pediculus humanus capitis* prefers to infest clean and healthy heads, suggesting good hygiene care within hunters and gatherers of the area.

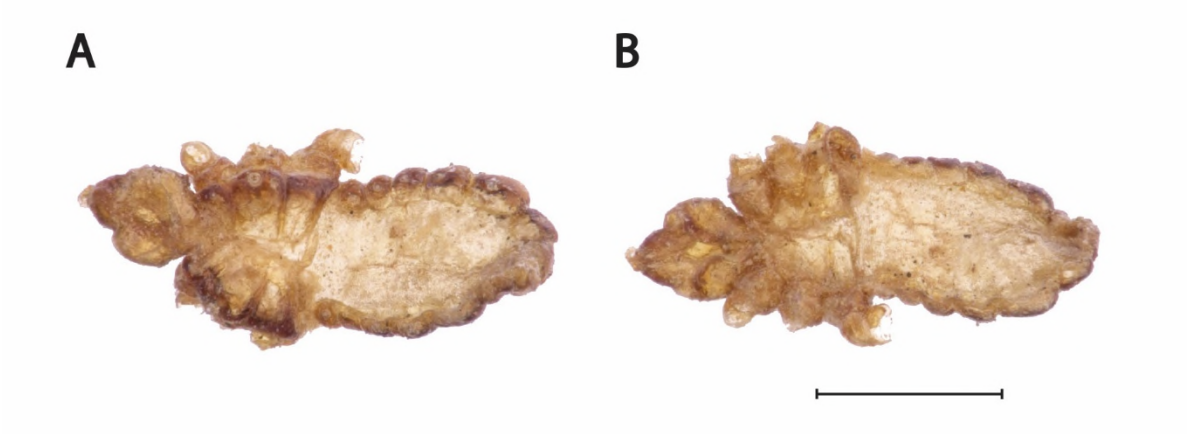

Figure 32: Fragments of head louse collected at Takarkori. A dorsal view, B ventral view. Scale bar 500 μm.

4.4 COLEOPTERA: FOOD PESTS

Coleoptera is the largest order of living animals on Earth encompassing more than 400,000 described species. Thanks to their vast biodiversity, they are represented in all biogeographical regions. A bewildering diversity of morphological features and adaptations is displayed within the group (Marshall, 2018). Commonly known as beetles, adults are characterised by two pair of wings: forewings modified into highly sclerotised elytra and hindwings concealed beneath them. Larval stages are wingless. The other main features are articulated prothorax distinct from the mesothorax, heavily sclerotised abdominal segments, antennae up to 11 segments, and retracted genitalia when not in use (Evans, 2014). All beetles are holometabolous completing a full metamorphosis. Most beetles are herbivores, fungivores or predators. Many species are considered pests of stored products and crops, but many others are beneficial as they can be used as biological control agents. Besides, several species are also good bioindicators used in conservational studies (Hinton and Corbet, 1949). The group is a monophyletic assemblage which has evolved into myriad different life cycles depending on food resources and disparate reproductive systems. Coleoptera is divided into four suborders: Archostemata, Adephaga, Myxophaga,

and Polyphaga (Resh and Cardé, 2009). The reasons for the extraordinary success of the order have been debated since the last century, and many theories tried to explain it. Currently, beetle early origin during the Triassic has been suggested to have had a significant role in their diversification, as several distinct biotas were already colonised before the breakup of the supercontinent Pangea started in the Jurassic era (Resh and Cardé, 2009). A second hypothesis refers to the evolution of the protective elytra as the main factor which has driven speciation (Resh and Cardé, 2009). Another proposed reason indicates the difference in eating habits between immature stages and adults as the cause of the bewildering diversity exploded in the Cretaceous Era when the angiosperm radiation transformed the environment creating separate ecological niches ready to be exploited (Marshall, 2018).

4.4.1 Stored-product insects

Since the dawn of agriculture, stored products of both plant and animal origins have provided insects with shelter, breeding and feeding sites. Their protection has been a difficult task for humans for 10,000 years and different populations from different times have adopted many methods to prevent losses (Stejskal *et al*., 2015). There are hundreds of insects associated with stored products, but only approximately 100 species have a significant economic impact (Hinton and Corbet, 1949). The majority are beetles (Coleoptera), moths (Lepidoptera), and parasitoid wasps (Hymenoptera). According to their food preferences, the group can be split in two: species attacking dry botanicals (commonly grain seeds) and species scavenging animal products (meat, wool, furs, etc.) (Hinton, 1945). In a storage environment all trophic levels are represented, but, because of the instability of this not self-sustainable ecosystem, there is a unidirectional biomass energy flow. Considering the harvested products and processed materials as the producers (1st trophic level), the primary consumers are insects feeding directly on the products being able to penetrate the outer surfaces of seeds to eat or oviposit (2nd trophic level), the secondary consumers feed on the waste products or debris provided by the primary

consumers and they are usually external feeders grazing on already damaged seeds. They are commonly polyphagous. Predators and parasitoids of them become the tertiary consumers (3rd trophic level) (Resh and Cardé, 2009). The consequences of these insect attacks are remarkable; destruction of food and contamination with faeces and exuviae are the main ones. The rate of the infestation is temperature and humidity driven (Stejskal *et al*., 2015). The optimal temperature range for stored product insects to be active is between 8 and 41°C. Diapause and hibernation closer to the extreme limits are the only survival strategies for these insects. Above and below the threshold mortality is close to 100% (Hinton, 1945). The second most important factor to consider is humidity. Although a defined threshold is not present, the majority of stored product insects prefer optimal humidity between 60 and 75%. All stored product insects are food opportunists (Hinton, 1945). Almost every species collected in human-made storages can be found commonly in nature. Natural habitats are usually sheltered seeds collection performed by rodents, birds or other insects (Marshall, 2018). They can survive a long period without feeding and they can have a rapid population growth when conditions are favourable. Dispersal is ensured thanks to their good ability to fly, essential to locate other breeding sites (Evans, 2014). Nowadays, most of them are widely distributed thanks to global trades. However, especially in colder regions, some stored product insects have strictly synanthropic populations, not being able to survive outside heated warehouses or granaries (Resh and Cardé, 2009).

4.4.2 TROGIDAE

Trogidae are also commonly named hide beetles including around 300 described species (Strümpher, 2015). They are cosmopolitan and they are characterised by a robust and dull appearance, from grey to black to red-brown, often encrusted in debris and mites, hypognathous, and visible scutellum (Evans, 2014). Usually, they are associated with dried-out organic material as they almost feed entirely on keratin (Marshall, 2018).

A single complete trogid beetle has been collected in LA1 horizon at Takarkori (Fig. 33).

Figure 33: Trogid beetles. A dorsal view, B ventral view. Scale bar 500 μm.

4.4.3 TENEBRIONIDAE

Tenebrionidae are commonly named darkling beetles and it is a numerous cosmopolitan family encompassing almost 20,000 described species (Resh and Cardé, 2009). They largely vary in size from 1 to 80 millimetres, and they are characterised by 5-5-4 tarsal formula, antennae of 11 segments covered by the canthus, closed procoxal cavities (Marshall, 2018). They have a myriad of habitats and habits allowing their dispersal even in the most extreme environments of the Earth. Many live in dry-environments and are generalist omnivores or predators of other insects' immature stages (Marshall, 2018).

Fragments of darkling beetles have been collected during the LA1 horizon at Takarkori (Fig. 34).

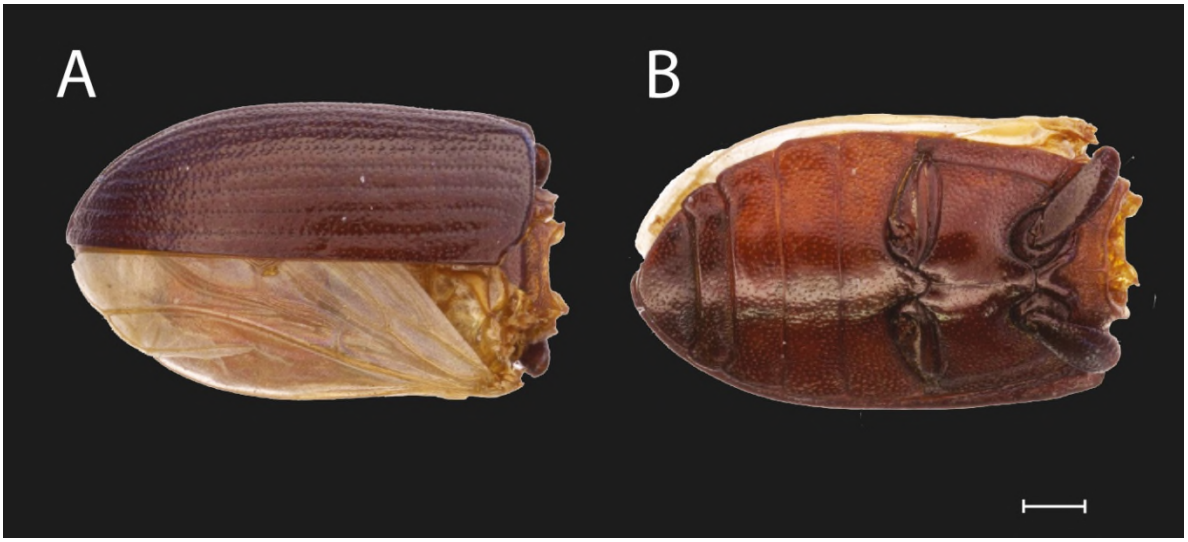

Figure 34: Biggest fragment of Tenebrionidae recovered at Takarkori. A dorsal view, B ventral view. Scale bar 500 µm.

The occurrence of trogid and dermestid beetles, which consume animal products such as leather, fur, hair, suggests employment of Takarkori for a prolonged period and the presence of some sort of human crafted by-products, such as desiccated meat or fur/wool production.

4.4.4 PTINIDAE

Ptinidae are commonly known as spider beetles and death-watch beetles. In recent years, this family underwent a hierarchical re-classification (Marshall, 2018). Now, two previously separated families (Ptinidae and Anobiidae) are considered as a single one. The majority of Ptinidae are woodboring species, a few are detritivores or scavengers, and others are stored-product pests (Hinton, 1945). The family encompasses approximately 2,500 described species and they are characterised by a pointing down prothorax which make the species to look like it is wearing a hood, wider elytra compared to the prothorax, accentuated convex elytra, long legs, long antennae with often lopsided clubs, bodies covered in tiny scales, 5-5-5 tarsal formula, and abdomen divided into five ventrites (Marshall, 2018). Ptinidae includes economical important stored products pests. They inflict damages to tobacco, seeds, cereals, spices, leather, wood, fruits and can attack growing young trees (Hinton and Corbet, 1949).

Mezium sp.

The *Mezium,* Curtis, 1828 genus belongs to the subfamily Ptininae, commonly known as true spider beetles. Its distribution is limited to the Mediterranean area (Iberian Peninsula, Marocco, and Canary Island) and central and southern Africa (Borowski, 2009a). Some species are endemic of small ecological African niches. It is frequent along coasts preferring mild climates. The natural habitat is caves in which it survives feeding on animal faeces. It has often been recorded in barns and poultry farms (Borowski, 2009b).

An enormous amount of *Mezium* fragments have been recovered during the entire chronological sequence at Takarkori (Fig. 35).

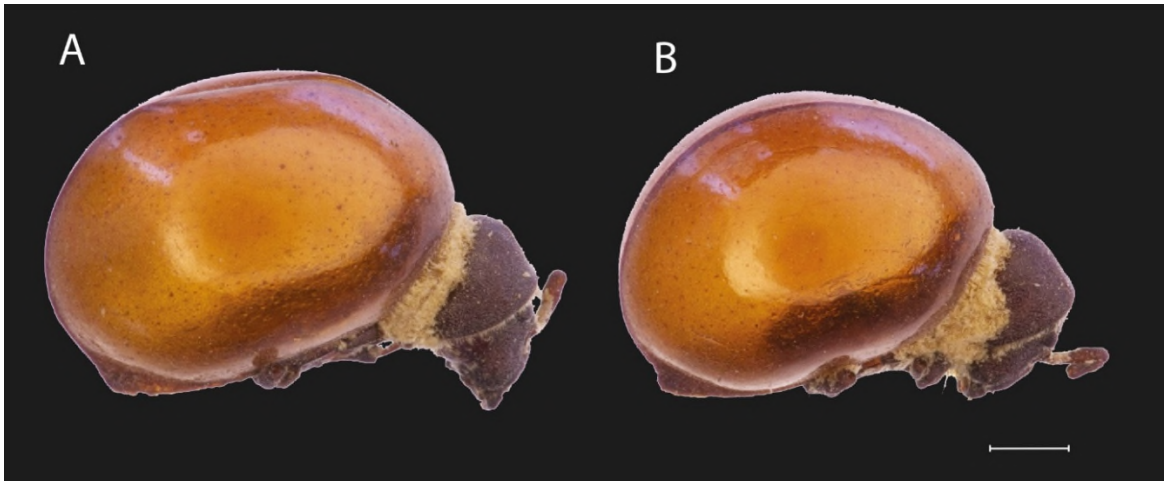

Figure 35: Two specimens of *Mezium* sp. Scale bar 500 μm.

Takarkori is a rock-shelter and it can resemble the ambient of a shallow cave, creating a favourable environment for the ptinids. Furthermore, the presence of dung is confirmed by several records of animal bones in the area (di Lernia, 1999a).

4.4.5 DERMESTIDAE

Dermestidae includes skin or hide beetles, carpet beetles, and larder beetles (Schroeder *et al*., 2002). Adults are quite small, ranging from just 2 mm to 12 mm in length. They are oval and convex or sometimes elongated. Usually, they are covered in hair and they have peculiar bare clubbed antennae (Marshall, 2018). Most of them feed on animal products, including leather, fur, hair, skin, wool, and even dairy products. Due to these eating-habits,

they are often used in taxidermy and by natural history museums to clean animal skeletons (Evans, 2014). However, they can be very dangerous in museums as they can destroy entire insect collections. Dermestidae live in different habitats, where there is a carcass or other source of food available (Peacock, 1993). They typically appear late in the decomposition process, when the corpse begins to dry out (Grassberger and Frank, 2004).

4.4.5.1 *Dermestes maculatus,* De Geer, 1774

Dermestes maculatus is commonly named hide beetle. Adults are dark coloured and hairy; larvae are covered in setae (Richardson and Goff, 2001). Hide beetles feed on advanced decayed cadavers or carcasses and dry animal products. They are cosmopolitan and they are well known to be important pests with a high economic impact (Osuji, 1975). For instance, hide beetles are considered detrimental in the silk industry attacking silkworms causing their death (Shaver and Kaufman, 2009). Another field in which the hide beetle can cause severe damages is poultry production, as they can attack alive turkeys (Samish *et al*., 1992). *Dermestes maculatus* is also commonly used by museums during skeleton preparation as they can remove entirely any residue of flesh from bones (Timm *et al*., 2020). Hide beetles have been recovered in archaeological contexts from all around the world (Giordani *et al*., 2020).

Several adult fragments and many exuviae have been recovered from LA1 and LA2 horizons (Fig. 36), indicating the presence of dry animal products, bones, and advance decaying protein matter at the site.

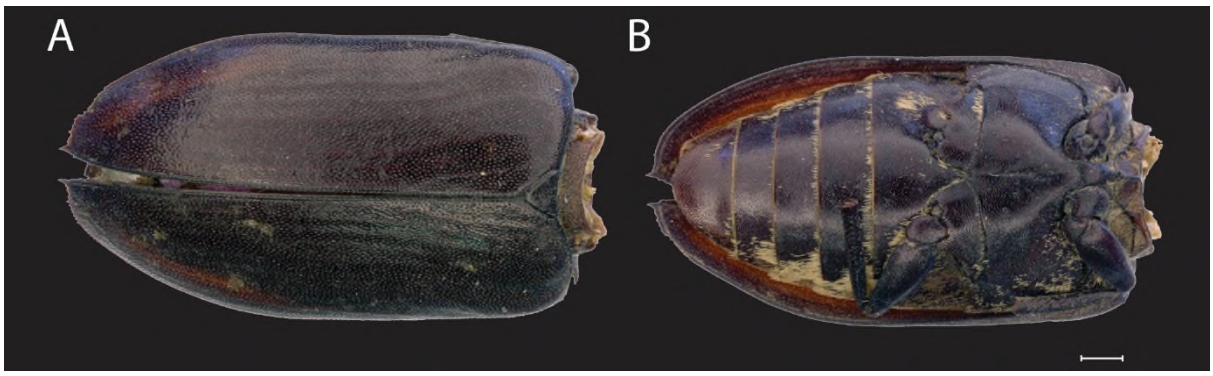

Figure 36: Best preserved dermestid of Takarkori. A dorsal view, B ventral view. Scale bar 500 µm.

4.4.6 CURCULIONIDAE

Curculionidae are commonly known as weevil beetles (Lawrence *et al*., 1982) and it is the biggest family among beetles with 50,000 described species. They have a wide range of size and shapes and are cosmopolitan. They are characterised by distinctive elongated snouts, which are used to penetrate plants to feed or to oviposition (Crowson, 1981). The majority lives inside plant material, but a few weevils are specialised in the consumption of fungi, decaying plant material or other insects (very rare) (Marshall, 2018). They are also known to be serious food and fibres pests.

Sitophilus granarius

The grain weevil is a highly synanthropic species, which, nowadays, cannot be found in natural environments. It is the most common and impactful pest worldwide (Panagiotakopulu, 2000). Adults range between 3 and 5 millimetres in length with the typically elongated snouts of curculionids. They are characterised by reddish-brown colour (Plarre, 2013). They feed on wheat, sorghum, chickpeas, rye, millet, barley, buckwheat, maize, oats, and they have been reported also on chestnuts, cornmeal, and acorns (Hoffmann, 1954). Adults are not able to fly, and females lay between 35 and 254 eggs depositing them one by one inside each grain kernel. The process involves creating a small hole by chewing the kernel surface and then seal it back after deposition. Legless larvae feed and complete the entire life cycle inside their kernel (Woodbury, 2008). After pupariation, the pupa already presents the typical stout with which, after finishing the metamorphosis, it drills a hole to emerge. The life cycle lasts around five weeks in the warmer season and tropical regions and can take up to 20 weeks in the cooler season and colder regions. Adults retain a long life span of approximately eight months (Panagiotakopulu, 2000).

An almost complete specimen of *Sitophilus granarius* has been collected in LA1 horizon at Takarkori (Fig. 37). The specimen is currently the oldest record of *Sitophilus granarius* as

the previous one was recorded at Servia (Macedonia) during the Early Neolithic phase dated 7,000-6,800 cal BP by Hubbard, (Cressida Ridley *et al*., 1973).

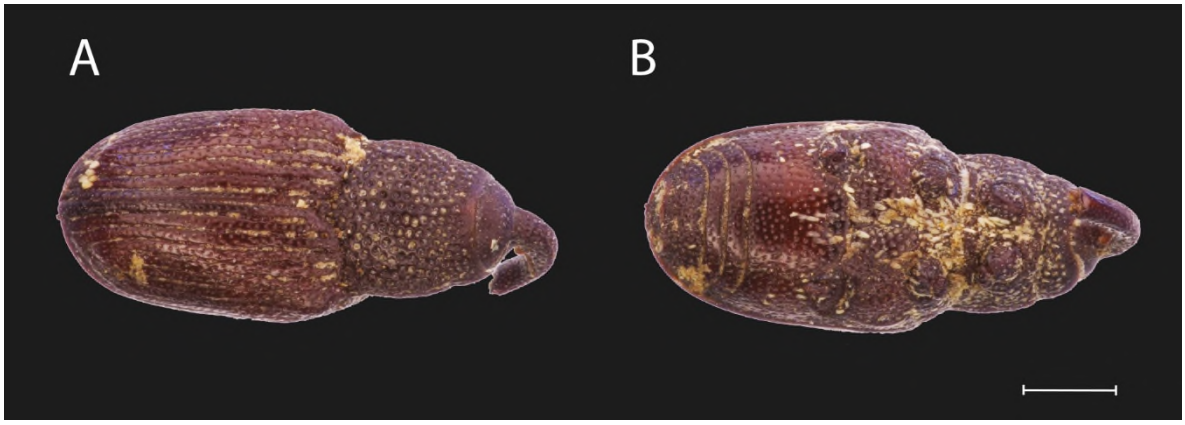

Figure 37: *Sitophilus granarius* specimen recovered from Takarkori. A dorsal view, B ventral view. Scale bar 500 μm.

The use of Takarkori as storage for seeds and cereals has been confirmed by the botanical records (Fornaciari *et al*., 2014; Fornaciari *et al*., 2018; Mercuri *et al*., 2018). The occurrence of the grain weevil during the early phases of the Holocene, when the domestication of plants has yet to be completed, in association with wild cereal gives a new perspective in its co-evolution with humans. The anthropic tendency to collect bigger grains and accumulate them in one single site may have driven the species-specific evolution of *Sitophilus* Schoenherr, 1838 genus (Plarre, 2013). Nowadays, many species belonging to the genus infests only one type of domesticated cereal grain or seed highlighting their impossibility to survive in nature. *Sitophilus granarius* has been recorded on modern sorghum and at Takarkori the analysis of macro-botanical residues confirms the presence of wild sorghum assemblage at the site.

4.5 CONCLUSION

The presence of humans in Takarkori is well documented by the discovery of a few burials belonging to the Pastoral cultural period (di Lernia and Tafuri, 2013). However, insects can reinforce the theory of human residential or semi-residential use of the site before the Pastoral phase. The record of the head louse in the Late Acacus layers supports the exploitation of the rock-shelter since the early phases of the Holocene, suggesting a

preliminary form of a sedentary lifestyle. Also, flies give further information about the human activities performed at the site. Most of synanthropic flies analysed show preferences towards decomposing protein matter, dung and faeces as main the food source, indicating a probable tendency to process meat and/or consume meals without properly disposed food waste. The attraction to dung and faeces of the flies and spider beetles also suggests that Takarkori was shared between humans and animals. This hypothesis is strongly supported by the animal parasite: *Oestrus ovis*. The oestrid fly is a key to understand the evolution of parasitism from wild animals to livestock, and to comprehend how important humans have been in speeding up the co-evolution process. Indeed, the specimen is the first record of a bot fly in an archaeological context. The extraordinary conservation status of the specimen and the use of synchrotron radiation allowed the identification at the species level, becoming the oldest known *Oestrus ovis* record. Currently, the species infest predominantly domesticated animals, preferring the common sheep as its prevalent natural host. There is only one report of *O. ovis* infesting *Ammotragus lervia*, a wild goat endemic in North Africa, in a recently introduced population in Spain (Barroso *et al*., 2017), whereas no record of oestrid myiasis on *Ammotragus* from Africa has ever been published. The transition from hunter and gatherers to pastoralism during the early Holocene phases in North Africa involved the domestication of animals. At the preliminary phases of the process, several remains of *Ammotragus lervia* have been recovered at Takarkori, whereas the genus *Ovis* (domesticated sheep) was not present in the region yet. The presence of the bot fly in the Late Acacus horizon highlights how less selective it was compared to nowadays. Furthermore, the specimen is a pupa with almost a totally developed pharate inside, which means that the fly was closed to complete its life cycle having left the host and burrowed into the ground. This can be explained only if wild goats were gathered at the site. *Ammotragus lervia*, in nature, lives in small groups of 3-6 female individuals. Presumably, humans assembled few animals, probably including males for reproduction, creating a favourable niche for the oestrid parasite. The beetle fauna also helps in the reconstruction of human activities at Takarkori. Most of them like

trogid and dermestid beetles consume animal products, leather, fur, hair, suggesting employment of the rock-shelter for a prolonged period. They might also suggest the presence of some sort of human crafted by-products, such as desiccated meat or fur/wool production. The grain weevil indicates that Takarkori was used as a storage of seeds or cereals. Botanical analyses have confirmed the presence of sorghum deposits in the area (Fornaciari *et al*., 2014; Fornaciari *et al*., 2018).

5 FINAL CONCLUSIONS

This thesis presents the archaeoentomological investigation from the Takarkori archaeological site, South West Libya. The study is the first from the area and it can be used as a reference for future studies in similar sites. Fifty-seven soil samples with a total weight of 24,5 kg of material have been analysed and more than 10,000 insect fragments have been collected. Despite several identification issues encountered, most of them have been identified at the lowest taxonomic level possible. The entire work is commented in relation to the aims given in paragraph 1.5.

i) **Optimisation of cleaning techniques to aid identification.**

Due to the dry nature of the samples, flotation and wet sieving techniques are inappropriate to collect insect fragment from soils. Hand sorting the sample is currently the best collection technique to increase the biodiversity of the recovered assemblage. An extensive overview of every cleaning technique and their optimisation shows that when morphological and molecular analyses are taken into account together, the best methods, with positive results in both analyses, are the warm water/soap, sonication and sodium hydroxide solutions. Hydrochloric acid/sodium bicarbonate solutions, bleach, and glacial acetic acid immersion are, therefore, not recommended to clean entomological samples when the molecular analysis is required.

ii) **Potential evaluation of synchrotron radiation micro-CT scan technique to aid identification of immature stages from archaeological contexts.**

The use of synchrotron radiation to produce advance micro-CT images for insect identification has been a success. The novel technique not only allowed to visualise diagnostic features in a non-destructive way, but also allowed to observe and measure the internal structures to study organisation and shape of internal organs providing the necessary information for the insect development evaluation. The technique also gave the possibility to have a 3D reconstruction, allowing a morphological comparison with modern samples.

iii) **The interpretation of Takarkori past environments and human activities** is summarised in Table 15 below.

Table 15: Summary of the entomological contributions in the understanding of Takarkori settlement.

TOPIC	HYPOTHESIS	BIOLOGICAL AND ARCHAEOLOGICAL EVIDENCE	INSECT SPECIES	ENTOMOLOGICAL INFORMATION	ENTOMOLOGICAL CONTRIBUTION AND NOVELTIES
CLIMATE CHANGE	During the Early phases of Holocene, North Africa underwent the African Humid Period (AHP) between 11,000 and 6,000 cal yr BP. The landscape was dominated by continuous grassland, typical of savannah.	Botanical evidence (Mercuri *et al*., 2011) Hydrological evidence (Gasse, 2000) Zoological evidence (Van Neer *et al*., 2020)	*Locusta migratoria*	The migratory locust survival is strictly linked to moist soils, as its eggs cannot survive in dry environments.	For the first time, paleo-reconstruction of Early Holocene environments have been performed in North Africa to trace the desertification process.
			Lepidoptera (Erebidae moth)	The larvae of Erebidae moth feeds on plants.	
			Isoptera (termites)	Termites prefer environments with decaying woods.	
HUMAN PRESENCE	Due to the amelioration of the climate, re-colonisation of the region started by hunter-gatherers.	Archaeological evidence: -rock arts (di Lernia and Zampetti, 2008; di Lernia and Gallinaro, 2010) -pottery (Biagetti *et al*., 2004; Eramo *et al*., 2020) -tools (di Lernia *et al*., 2016) -human burials (di Lernia and Tafuri, 2013)	*Pediculus humanus capitis*	The head louse infests only humans.	The Takarkori louse is one of the earliest evidence in co-evolutionary history between humans and lice, giving information about the health of early hunter-gather population.
	The site was exploited by human as a residential or semi-residential area at the beginning of Holocene.		Diptera	Flies give information about the human activities performed at the site as most of them show preferences towards decomposing	The presence of *Musca domestica* at Takarkori is the earliest record of the species in association with human settlements.

				protein matter, dung and faeces indicating a probable tendency to process meat and/or consume meals without properly dispose of food waste.	The recovery is very important to trace back when the synanthropy of the species co-evolved with humans.
LIVESTOCK PRESENCE	Takarkori was shared between humans and animals during the early phases of domestication.	Archaeological evidence: -rock arts (di Lernia and Zampetti, 2008; di Lernia and Gallinaro, 2010) -milk/cheese production (Dunne *et al*., 2012; Dunne *et al*., 2013; Dunne *et al*., 2018) Zoological evidence: -bones assemblages (di Lernia *et al*., 2012)	Diptera *Oestrus ovis* Ptinidae *Mezium* sp	The attraction to dung and faeces of the flies, support that animals were present at the site. The presence of *Oestrus ovis*, a parasite which usually infest domesticated animals also strongly support the hypothesis. The natural habitat of spider beetles *Mezium* is caves in which it survives feeding on animal faeces.	The oestrid specimen is the first record of a bot fly in an archaeological context and the oldest known *Oestrus ovis* record. This discovery is important to understand the evolution of parasitism from wild animals to livestock, and to comprehend how important humans have been in the co-evolution process.
FOOD STORAGE	Takarkori was used as a storage of seeds and cereals.	Botanical evidence (Fornaciari *et al*., 2014; Fornaciari *et al*., 2018) Archaeological evidence (cooking soup) (Dunne *et al*., 2016)	*Sitophilus granarius*	It feeds on wheat, sorghum, chickpeas, rye, millet, barley, buckwheat, maize, oats, and they have been reported also on chestnuts, cornmeal,	The sample is the oldest granary weevil ever recovered and it is extremely important to trace back the dispersal of the species and its synanthropy as

				and acorns. It is the most common and impactful pests worldwide.	nowadays *Sitophilus* is not found any longer in nature.
OTHER HUMAN ACTIVITIES	Prolonged use of Takarkori site by humans during Late Acacus phase.	Archaeological evidence (di Lernia *et al*., 2012; Biagetti and di Lernia, 2013; Rotunno *et al*., 2019)	Trogidae *Dermestes maculatus*	Trogid and dermestid beetles consume animal products, leather, fur, hair.	Their eating habits suggest the presence of some sort of human crafted by-products, such as desiccated meat or fur/wool production.

The thesis demonstrates how insects can support other archaeological disciplines to reconstruct environments and to understand human past habits giving original insights on several activities. Furthermore, in this case, insects showed the ability to highlight the health conditions of both humans and livestock at the site. At last, several synanthropic species analysed in the study are the oldest record ever discovered, presenting the remarkable opportunity to investigate the co-evolution between them and humans.

5.1 FURTHER RESEARCH

Due to the current global pandemic (COVID-19) and the resulting closure of laboratories, some analyses have been suspended:

- a selected batch of Ptinidae fragments has been selected to perform an aDNA extraction trial, both using classical techniques and Next-Generation Sequencing (NGS), as they are the most numerous insect fragment recovered from Takarkori.
- an immersion aDNA extraction trial is also planned for the sheep nasal bot fly puparium.
- an entomological collection targeting soil samples from Takarkori areas where houses or barns were located has to be performed to recover more specimens of lice and animal parasites.

Further research on Takarkori entomological material is planned:

- Ideally, it would be useful to organise an expedition to North Africa to collect modern reference insect samples to create a morphological and molecular database to use for future analyses. Despite the current political situation of several Northern African countries, this plan could be developed in southern Tunisia, where the Archaeological Mission in the Sahara operates today.

5.2 SCIENTIFIC OUTPUTS

From this thesis, four papers are in preparation for publication:

- Holocene climate changes and human adaptation at Takarkori rock-shelter (SW Libya): An archaeoentomological perspective.

- From wild to domesticated: A study of the evolution of the parasite-host relationship in livestock.
- The synanthropism of *Musca domestica*, Linnaeus, 1758: The Late Acacus record.
- Hunters and gatherers eating habits: North African entomophagy.

REFERENCES

Abrami, A., Arfelli, F., Barroso, R., Bergamaschi, A., Bille, F., Bregant, P., Brizzi, F., Casarin, K., Castelli, E., and Chenda, V. (2005). Medical applications of synchrotron radiation at the SYRMEP beamline of ELETTRA. *Nuclear Instruments and Methods in Physics Research Section A: Accelerators, Spectrometers, Detectors and Associated Equipment, 548*(1-2), 221-227.

Ahmed, W. B., Henchiri, M., Mastouri, A., and S'himi, N. S. (2018). Calcified aquatic insect larval constructions in the Pleistocene tufa of Jebel El Mida, Gafsa, southern Tunisia: Recognition and paleoenvironmental significance. *Journal of African Earth Sciences, 140*, 256-266.

Akbarzadeh, K., Tirgari, S., and Nateghpur, M. (2004). *First record of presence of stinging ants* Pachycondyla sennaarensis *(Formicidae: Hymenoptera) in Sistan and Baluchestan province.* Paper presented at the Proceedings of 16th Iran Plant Protection Congress, Tabriz.

Alfieri, A. (1956). La véritable identité du scarabée sacré de l'Egypte pharaonique. *Bulletin de la Société entomologique d'Egypte, 40*, 451-452.

Alley, R. B., and Ágústsdóttir, A. M. (2005). The 8k event: Cause and consequences of a major Holocene abrupt climate change. *Quaternary Science Reviews, 24*(10-11), 1123-1149.

Alley, R. B., Mayewski, P. A., Sowers, T., Stuiver, M., Taylor, K. C., and Clark, P. U. (1997). Holocene climatic instability: A prominent, widespread event 8200 yr ago. *Geology, 25*(6), 483-486.

Aly, S. M., and Wen, J. (2013). Applicability of partial characterization of cytochrome oxidase I in identification of forensically important flies (Diptera) from China and Egypt. *Parasitology Research, 112*(7), 2667-2674.

Amendt, J., Goff, M. L., Campobasso, C. P., and Grassberger, M. (2010). *Current concepts in forensic entomology*. Dordrecht: Springer.

Amendt, J., Krettek, R., and Zehner, R. (2004). Forensic entomology. *Naturwissenschaften, 91*(2), 51-65.

Anankware, P., Fening, K. O., Osekre, E., and Obeng-Ofori, D. (2015). Insects as food and feed: A review. *International Journal of Agricultural Research and Review, 3*(1), 143-151.

Andersen, J. (1993). Beetle remains as indicators of the climate in the Quaternary. *Journal of Biogeography*, 557-562.

Angulo-Valadez, C. E., Scholl, P. J., Cepeda-Palacios, R., Jacquiet, P., and Dorchies, P. (2010). Nasal bots… a fascinating world! *Veterinary Parasitology, 174*(1-2), 19-25.

Anketell, J., and Ghellali, S. (1984). Nests of a tube-dwelling bee in Quaternary sediments of the Jeffara plain, SPLAJ. *Libyan Studies, 15*, 137-141.

Araújo, A., Ferreira, L., Guidon, N., da Serra Freire, N. M., Reinhard, K., and Dittmar, K. (2000). Ten thousand years of head lice infection. *Parasitology Today, 16*(7), 269.

Armitage, S., Drake, N., Stokes, S., El-Hawat, A., Salem, M., White, K., Turner, P., and McLaren, S. (2007). Multiple phases of North African humidity recorded in lacustrine sediments from the Fazzan Basin, Libyan Sahara. *Quaternary Geochronology, 2*(1-4), 181-186.

Astruc, C., Julien, J., Errard, C., and Lenoir, A. (2004). Phylogeny of ants (Formicidae) based on morphology and DNA sequence data. *Molecular Phylogenetics and Evolution, 31*(3), 880-893.

Audouin, J. (1835). Observation sur la découverte de *Gibbium scotias* dans un ancien tombeau a Thébes. *Annales de la Société Entomologie de France, 5*, 5-6.

Austin, J. J., Ross, A. J., Smith, A. B., Fortey, R. A., and Thomas, R. H. (1997). Problems of reproducibility–does geologically ancient DNA survive in amber–preserved insects? *Proceedings of the Royal Society of London. Series B: Biological Sciences, 264*(1381), 467-474.

Badek, A., Dabert, M., Mironov, S. V., and Dabert, J. (2008). A new species of the genus *Proctophyllodes* (Analgoidea: Proctophyllodidae) from Cetti's Warbler *Cettia cetti* (Passeriformes: Sylviidae) with DNA Barcode Data. *Annales Zoologici, 58*(2), 397-402, 396.

Bain, A. (1998). A seventeenth-century beetle fauna from colonial Boston. *Historical Archaeology, 32*(3), 38-48.

Bain, A. (1999). *Archaeoentomological and archaeoparasitological reconstructions at Îlot Hunt (CeEt-110): New perspectives in historical archaeology (1850-1900).* PhD Thesis. Laval University, Quebec.

Bain, A. (2001). *Archaeoentomological and Archaeoparasitological Reconstructions at Îlot Hunt (CeEt-110): New Perspectives in Historic Archaeology:(1850-1900)* (Vol. 973). British Archaeological Reports Limited.

Bain, A., and King, G. (2011). Asylum for wayward immigrants: Historic ports and colonial settlements in northeast North America. *Journal of the North Atlantic, 2*(sp1), 109-124.

Bain, A., and Prévost, M.-A. (2010). Environmental archaeology and landscape transformation at the seventeenth-century Ferryland site, Newfoundland. *Historical Archaeology, 44*(3), 21-35.

Baird, C. R. (1997). Bionomics of *Cuterebra austeni* (Diptera: Cuterebridae) and its association with *Neotoma albigula* (Rodentia: Cricetidae) in the southwestern United States. *Journal of Medical Entomology, 34*(6), 690-695.

Barich, B. E. (1987). *Adaptation in archaeology: An example from the Libyan Sahara*. Dallas: Southern Methodist University Press.

Barroso, P., Ruiz-de-Ybáñez, R., Martínez-Carrasco, C., Gens, M. J., Escribano, F., Sánchez, A., and Pérez, J. M. (2017). First report of oestrosis in aoudad from southeastern Spain. *Parasitology Research, 116*(7), 2053-2055.

Benfekih, L., Chara, B., and Doumandji-Mitiche, B. (2002). Influence of anthropogenic impact on the habitats and swarming risks of *Dociostaurus maroccanus* and *Locusta migratoria* (Orthoptera, Acrididae) in the Algerian Sahara and the semiarid zone. *Journal of Orthoptera Research*, 243-250.

Benfekih, L., and Petit, D. (2010). *The annual cycle of Saharan populations of Locusta migratoria cinerascens (Orthoptera: Acrididae: Oedipodinae) in Algeria.* Paper presented at the Annales de la Société entomologique de France.

Benson, D. A., Clark, K., Karsch-Mizrachi, I., Lipman, D. J., Ostell, J., and Sayers, E. W. (2015). GenBank. *Nucleic Acids Research, 43*, D30-D35.

Béthoux, O., and Nel, A. (2001). Venation pattern of Orthoptera. *Journal of Orthoptera Research, 10*(2), 195-198.

Biagetti, S., and di Lernia, S. (2013). Holocene deposits of Saharan rock shelters: The case of Takarkori and other sites from the Tadrart Acacus Mountains (Southwest Libya). *African Archaeological Review, 30*(3), 305-338.

Biagetti, S., Merighi, F., and di Lernia, S. (2004). Decoding an Early Holocene Saharan stratified site. Ceramic dispersion and site formation processes in the Takarkori rock-shelter, Acacus Mountains, Libya. *Journal of African Archaeology, 2*(1), 3-21.

Biggs, H., McClain, E., Muller, G., Anthonissen, M., and Hare, K. (1998). A prediction model for strike in the sheep nasal fly, *Oestrus ovis*, in Namibia. *Preventive Veterinary Medicine, 33*(1-4), 267-282.

Bishopp, F. C., Brundrett, H. M., Laake, E. W., and Wells, R. W. (1926). *The cattle grubs or ox warbles, their biologies and suggestions for control*. Washington: US Department of Agriculture.

Blair, K. (1935). *Some ancient beetles from Egypt and Mesopotamia.* Paper presented at the Proceedings of the Royal Entomological Society of London.

Blaisdell, F. (1927). *A blind beetle excavated from an Egyptian city's ruins dating between 117 and 235 AD.* Paper presented at the Proceedings of the Entomological Society of Washington.

Blaney, W., and Winstanley, C. (1982). *Food selection behaviour in* Locusta migratoria. Paper presented at the Proceedings of the 5th International Symposium on Insect-Plant Relations, Pudoc, Wageningen.

Boheme, P., Amendt, J., and Zehner, R. (2011). The use of COI barcodes for molecular identification of forensically important fly species in Germany. *Parasitology Research, 110*, 2325-2332.

Bolton, B. (1981). A revision of the ant genera *Meranoplus* F. Smith, *Dicroaspis* Emery and *Calyptomyrmex* Emery (Hymenoptera, Formicidae) in the Ethiopian Zoogeographical Region. *Bulletin of the British Museum (Natural History). Entomology, 42*(2), 43-81.

Bolton, B. (1982). Afrotropical species of the myrmicine ant genera *Cardiocondyla*, *Leptothorax*, *Mellisotarsus, Messor* and *Lataulacus* (Formicidae). *Bulletin of the British Museum (Natural History). Entomology, 45*(4), 307-370.

Borowski, J. (2009a). The spider beetles of the continental Africa (Coleoptera: Ptinidae). Part I—Genus *Mezium,* Curtis. *Studies and reports of District Museum Prague-East, Taxonomical Series, 5*, 1-10.

Borowski, J. (2009b). The spider beetles of the continental Africa. Part II—*Dignomorphus danielssoni* n. gen. and n. sp. from South Africa (Coleoptera: Ptinidae). *Genus, 20*(2), 257-261.

Bortolini, S., Giordani, G., Tuccia, F., Maistrello, L., and Vanin, S. (2018). Do longer sequences improve the accuracy of identification of forensically important Calliphoridae species? *PeerJ, 6*, e5962.

Bostock, E., Mancini, L., and Vanin, S. (2014). *Pupal age estimation in* Megaselia scalaris*: From microscopy to synchrotron.* Paper presented at the 11th Meeting of the European Association for Forensic Entomology, Lille, France.

Buckland, P. (1976). The use of insect remains in the interpretation of archaeological environments. *Geoarcheology: Earth science and the past*, 369-396.

Buckland, P., Greig, J., and Kenward, H. (1974). York: An early medieval site. *Antiquity, 48*(189), 25-33.

Buckland, P., and Wagner, P. (2001). Is there an insect signal for the" Little Ice Age"? *Climatic Change, 48*(1), 137-149.

Buckland, P. C., Amorosi, T., Barlow, L. K., Dugmore, A. J., Mayewski, P. A., McGovern, T. H., Ogilvie, A. E., Sadler, J. P., and Skidmore, P. (1995). Bioarchaeological and climatological evidence for the fate of Norse farmers in medieval Greenland. *Antiquity, 70*(267), 88.

Buckland, P. C., Buckland, P. I., and Panagiotakopulu, E. (2018). Caught in a trap: Landscape and climate implications of the insect fauna from a Roman well in Sherwood Forest. *Archaeological and Anthropological Sciences, 10*(1), 125-140.

Buckland, P. C., Edwards, K. J., Panagiotakopulu, E., and Schofield, J. E. (2009). Palaeoecological and historical evidence for manuring and irrigation at Garðar (Igaliku), Norse Eastern Settlement, Greenland. *The Holocene, 19*(1), 105-116.

Burgess, I. F. (2004). Human lice and their control. *Annual Reviews in Entomology, 49*(1), 457-481.

Burleigh, R., and Southgate, B. (1975). Insect infestation of stored Egyptian lentils in antiquity. *Journal of Archaeological Science, 2*(4), 391-392.

Burns, D., and Sims, T. (1988). A closer look at *Pthirus pubis*. *British Journal of Dermatology, 118*(4), 497-503.

Cano, R. J. (1996). Analysing ancient DNA. *Endeavour, 20*(4), 162-167.

Cano, R. J., Poinar, H., and Poinar, G. (1992). Isolation and partial characterisation of DNA from the bee *Proplebeia dominicana* (Apidae: Hymenoptera) in 25-40 million year old amber. *Medical Science Research, 20*(7), 249-251.

Cano, R. J., Poinar, H. N., Pieniazek, N. J., Acra, A., and Poinar, G. O. (1993). Amplification and sequencing of DNA from a 120–135-million-year-old weevil. *Nature, 363*(6429), 536-538.

Capelle, K. J. (1966). The occurrence of *Oestrus ovis* L.(Diptera: Oestridae) in the bighorn sheep from Wyoming and Montana. *The Journal of Parasitology*, 618-621.

Carrott, J., and Kenward, H. (2001). Species associations among insect remains from urban archaeological deposits and their significance in reconstructing the past human environment. *Journal of Archaeological Science, 28*(8), 887-905.

Castalanelli, M. A., Severtson, D. L., Brumley, C. J., Szito, A., Foottit, R. G., Grimm, M., Munyard, K., and Groth, D. M. (2010). A rapid non-destructive DNA extraction method for insects and other arthropods. *Journal of Asia-Pacific Entomology, 13*(3), 243-248.

Catts, E. (1967). Biology of a California rodent bot fly *Cuterebra latifrons* Coquillett (Diptera: Cuterebridae). *Journal of Medical Entomology, 4*(2), 87-101.

Cepeda-Palacios, R., Ruiz-Ochoa, C., Ramirez-Orduna, R., Angulo-Valadez, C., and Dorchies, P. (2015). The puparium structure of the sheep nasal botfly (*Oestrus ovis* L.). *Revista de la Facultad de Agronomía y Veterinaria, La Plata, 166*, 215-220.

Cepeda-Palacios, R., and Scholl, P. (1999). Gonotrophic development in *Oestrus ovis* (Diptera: Oestridae). *Journal of Medical Entomology, 36*(4), 435-440.

Cepeda-Palacios, R., and Scholl, P. (2000a). Factors affecting the larvipositional activity of *Oestrus ovis* gravid females (Diptera: Oestridae). *Veterinary Parasitology, 91*(1-2), 93-105.

Cepeda-Palacios, R., and Scholl, P. (2000b). Intra-puparial development in *Oestrus ovis* (Diptera: Oestridae). *Journal of Medical Entomology, 37*(2), 239-245.

Chaddick, P., and Filce Leek, F. (1972). Further specimens of stoned products insects found in ancient Egyptian tombs. *Journal of stored products research, 8*(1), 83-86.

Chapco, W., and Litzenberger, G. (2004). A DNA investigation into the mysterious disappearance of the Rocky Mountain grasshopper, mega-pest of the 1800s. *Molecular Phylogenetics and Evolution, 30*(3), 810-814.

Chapuis, M. P. (2006). *Génétique des populations d'un insecte pullulant, le criquet migrateur,* Locusta migratoria. PhD Thesis. University of Montpellier, France.

Claussen, M., and Gayler, V. (1997). The greening of the Sahara during the mid-Holocene: Results of an interactive atmosphere-biome model. *Global Ecology and Biogeography Letters*, 369-377.

Claussen, M., Kubatzki, C., Brovkin, V., Ganopolski, A., Hoelzmann, P., and Pachur, H. J. (1999). Simulation of an abrupt change in Saharan vegetation in the mid-Holocene. *Geophysical research letters, 26*(14), 2037-2040.

Collingwood, C. A., Pohl, H., Gusten, R., Wranik, W., and van Harten, A. (2004). The ants (Insecta: Hymenoptera: Formicidae) of the Socotra Archipelago. *Fauna of Arabia, 20*, 473-496.

Colwell, D. D., Hall, M. J., and Scholl, P. J. (2006). *The oestrid flies: Biology, host-parasite relationships, impact and management*. Wallingford, UK: CABI.

Colwell, D. D., and Milton, K. (1998). Development of *Alouattamyia baeri* (Diptera: Oestridae) from howler monkeys (Primates: Cebidae) on Barro Colorado Island, Panama. *Journal of Medical Entomology, 35*(5), 674-680.

Colwell, D. D., and Scholl, P. J. (1995). Cuticular sensilla on newly hatched larvae of *Gasterophilus intestinalis* and *Oestrus ovis*. *Medical and Veterinary Entomology, 9*(1), 85-93.

Coope, G. (1970). Interpretations of Quaternary insect fossils. *Annual review of entomology, 15*(1), 97-121.

Coope, G., and Lemdahl, G. (1996). Validations for the use of beetle remains as reliable indicators of Quaternary climates: A reply to the criticisms by Johan Andersen. *Journal of Biogeography, 23*(1), 115-120.

Coope, G. R., and Brophy, J. A. (1972). Late Glacial environmental changes indicated by a coleopteran succession from North Wales. *Boreas, 1*(2), 97-142.

Coope, G. R., and Osborne, P. J. (1967). *Report on the coleopterous fauna of the Roman well at Barnsley Park, Gloucestershire*. Bristol: Bristol and Gloucestershire Archaeological Society.

Corwin, D., Clifford, C. M., and Keirans, J. E. (1979). An improved method for cleaning and preparing ticks for examination with the scanning electron microscope. *Journal of Medical Entomology, 16*(4), 352-353.

Cosmi, F., and Biasi, G. (2011). A preliminary study on sclerofoam morphology. Étude préliminaire de la morphologie des mousses sclérosantes. *Phlébologie, 64*, 3.

Courtney, G., and Merritt, R. (2009). Diptera (non-biting flies). In G. Linkens (Ed.), *Encyclopedia of Inland Waters* (Vol. 2). Oxford: Elsevier Limited.

Cremaschi, M. (1996). The rock varnish in the Messak Settafet (Fezzan, Libyan Sahara), age, archaeological context, and paleo-environmental implication. *Geoarchaeology, 11*(5), 393-421.

Cremaschi, M. (1998). Late Quaternary geological evidence for environmental changes in South Western Fezzan (Libyan Sahara). In M. Cremaschi and S. di Lernia (Eds.), *Wadi teshuinat palaeoenvironment and prehistory in South-Western Fezzan (Libyan Sahara): Survey and excavations in the Tadrart Acacus, Erg Uan Kasa, Messak Settafet and Edeyen of Murzuq, 1990-1995*. Milano: CNR.

Cremaschi, M., and di Lernia, S. (1995). The transition between Late Pleistocene and Early Holocene in the Uan Afuda cave (Tadrart Acacus, Libyan Sahara). Environmental changes and human occupation. *Quaternaire, 6*(3), 173-189.

Cremaschi, M., and di Lernia, S. (1998). *Wadi Teshuinat*. Milano: CNR.

Cremaschi, M., and Zerboni, A. (2009). Early to Middle Holocene landscape exploitation in a drying environment: Two case studies compared from the central Sahara (SW Fezzan, Libya). *Comptes Rendus Geoscience, 341*(8-9), 689-702.

Cremaschi, M., Zerboni, A., Mercuri, A. M., Olmi, L., Biagetti, S., and di Lernia, S. (2014). Takarkori rock shelter (SW Libya): An archive of Holocene climate and environmental changes in the central Sahara. *Quaternary Science Reviews, 101*, 36-60.

Cremaschi, M., Zerboni, A., Spötl, C., and Felletti, F. (2010). The calcareous tufa in the Tadrart Acacus Mt.(SW Fezzan, Libya): An early Holocene palaeoclimate archive in the central Sahara. *Palaeogeography, Palaeoclimatology, Palaeoecology, 287*(1-4), 81-94.

Cressida Ridley, M., Wardle, K., Hubbard, R., Watson, J., and Jones, R. (1973). Rescue excavations at Servia 1971-1973: A preliminary report. *The Annual of the British School at Athens, 74*, 185-230.

Crowson, R. (1981). *The Biology of the Coleoptera.* London, UK: Academic Press.

Defaut, B. (2005). Sur la répartition de *Locusta migratoria migratoria* Linné en France (Caelifera, Acrididae, Oedipodinae). *Matériaux orthoptériques et entomocénotiques, 10*, 87-92.

Defaut, B. (2013). Biométrie des types des Caelifères de France (Orthoptera). 3. Mensurations chez les Acrididae Melanoplinae et Locustinae. *Matériaux orthoptériques et entomocénotiques, 18*, 5-44.

Defaut, B., Morichon, D., Morin, D., and Puissant, S. (2012). *Locusta cinerascens* (Fabricius), espèce distincte de *Locusta migratoria* L. (Caelifera, Acrididae, Locustinae). *Matériaux orthoptériques et entomocénotiques, 17*, 5-7.

Déjean, A., and Lachaud, J.-P. (1994). Ecology and behavior of the seed-eating ponerine ant *Brachyponera senaarensis* (Mayr). *Insectes Sociaux, 41*(2), 191-210.

deMenocal, P. B. (2004). African climate change and faunal evolution during the Pliocene–Pleistocene. *Earth and Planetary Science Letters, 220*(1-2), 3-24.

deMenocal, P. B., and Bloemendal, J. (1995). Plio-Pleistocene climatic variability in subtropical Africa and the paleoenvironment of hominid evolution: A combined data-model approach. *Paleoclimate and evolution, with emphasis on human origins*, 262-288.

deMenocal, P. B., and Tierney, J. E. (2012). Green Sahara: African humid periods paced by Earth's orbital changes. *Nature Education Knowledge, 3*(10), 12.

Denlinger, D. L., and Zdárek, J. (1994). Metamorphosis behavior of flies. *Annual review of entomology, 39*(1), 243-266.

DeSalle, R., Gatesy, J., Wheeler, W., and Grimaldi, D. (1992). DNA sequences from a fossil termite in Oligo-Miocene amber and their phylogenetic implications. *Science, 257*(5078), 1933-1936.

Dethier, V., Larsen, J., and Adams, J. (1962). *The fine structure of the olfactory receptors of the blowfly.* Paper presented at the Olfaction and Taste: Proceedings of the 1st International Symposium, Stockholm.

di Lernia, S. (1996). Changing adaptative strategies: A Long-Term process in the Central Saharan Massifs from Late Pleistocene to Early Holocene. The Tadrart Acacus perspective (Libyan Sahara). In G. Aumassip, J. D. Clark, and F. Mori (Eds.), *The Prehistory of Africa, XIII Congress UISPP, Colloquium XXX* (Vol. 15). Forli: ABACO.

di Lernia, S. (1999a). Discussing pastoralism. The case of the Acacus and surroundings (Libyan Sahara). *Sahara* (11), 7-20.

di Lernia, S. (1999b). *The Uan Afuda Cave. Hunter-gatherer societies of Central Sahara* (Vol. 1). Firenze: All'Insegna del Giglio.

di Lernia, S., Bruni, S., Cislaghi, I., Cremaschi, M., Gallinaro, M., Gugliemi, V., Mercuri, A. M., Poggi, G., and Zerboni, A. (2016). Colour in context. Pigments and other coloured residues from the Early-Middle Holocene site of Takarkori (SW Libya). *Archaeological and Anthropological Sciences, 8*(2), 381-402.

di Lernia, S., and Cremaschi, M. (1996). Taming barbary sheep: Wild animal management by Early Holocene hunter-gatherers at Uan Afuda (Libyan Sahara). *Nyame akuma*(46), 43-54.

di Lernia, S., and Gallinaro, M. (2010). The date and context of Neolithic rock art in the Sahara: Engravings and ceremonial monuments from Messak Settafet (South-West Libya). *Antiquity, 84*(326), 954-975.

di Lernia, S., N'siala, I. M., and Mercuri, A. M. (2012). Saharan prehistoric basketry. Archaeological and archaeobotanical analysis of the early-middle Holocene assemblage from Takarkori (Acacus Mts., SW Libya). *Journal of Archaeological Science, 39*(6), 1837-1853.

di Lernia, S., and Tafuri, M. A. (2013). Persistent deathplaces and mobile landmarks: The Holocene mortuary and isotopic record from Wadi Takarkori (SW Libya). *Journal of Anthropological Archaeology, 32*(1), 1-15.

di Lernia, S., and Zampetti, D. (2008). *La memoria dell'arte*. Firenze: All'Insegna del Giglio.

Donovan, S., Jones, D., Sands, W., and Eggleton, P. (2000). Morphological phylogenetics of termites (Isoptera). *Biological Journal of the Linnean Society, 70*(3), 467-513.

Dorchies, P., Duranton, C., and Jacquiet, P. (1998). Pathophysiology of *Oestrus ovis* infection in sheep and goats: A review. *Veterinary Record, 142*(18), 487-489.

Doutt, R. L. (1959). The biology of parasitic Hymenoptera. *Annual review of entomology, 4*(1), 161-182.

Dowdy, N., and Conner, W. (2019a). Nonchalant flight in tiger moths (Erebidae: Arctiinae) is correlated with unpalatability. *Frontiers in Ecology and Evolution,* 7, 480.

Dowdy, N. J., and Conner, W. E. (2019b). Characteristics of tiger moth (Erebidae: Arctiinae) anti-bat sounds can be predicted from tymbal morphology. *Frontiers in zoology, 16*(1), 45.

Downe, A. E. (1961). Serological studies of insect proteins. *Canadian Journal of Zoology 40*(6), 957-967.

Drake, N. A., Blench, R. M., Armitage, S. J., Bristow, C. S., and White, K. H. (2011). Ancient watercourses and biogeography of the Sahara explain the peopling of the desert. *Proceedings of the National Academy of Sciences, 108*(2), 458-462.

Dunne, J., di Lernia, S., Chłodnicki, M., Kherbouche, F., and Evershed, R. Á. (2018). Timing and pace of dairying inception and animal husbandry practices across Holocene North Africa. *Quaternary International, 471*, 147-159.

Dunne, J., Evershed, R. P., Cramp, L., Bruni, S., Biagetti, S., and di Lernia, S. (2013). The beginnings of dairying as practised by pastoralists in 'green' Saharan Africa in the 5th millennium BC. *Documenta Praehistorica, 40: 118-30*.

Dunne, J., Evershed, R. P., Salque, M., Cramp, L., Bruni, S., Ryan, K., Biagetti, S., and di Lernia, S. (2012). First dairying in green Saharan Africa in the fifth millennium BC. *Nature, 486*(7403), 390-394.

Dunne, J., Mercuri, A. M., Evershed, R. P., Bruni, S., and di Lernia, S. (2016). Earliest direct evidence of plant processing in prehistoric Saharan pottery. *Nature Plants, 3*(1), 1-6.

Dussault, F., Bain, A., and LeMoine, G. (2014). Early Thule winter houses: An archaeoentomological analysis. *Arctic Anthropology, 51*(1), 101-117.

Eggermont, H., Verschuren, D., Fagot, M., Rumes, B., Van Bocxlaer, B., and Kröpelin, S. (2008). Aquatic community response in a groundwater-fed desert lake to Holocene desiccation of the Sahara. *Quaternary Science Reviews, 27*(25-26), 2411-2425.

El-ghali, M. A. K. (2005). Depositional environments and sequence stratigraphy of paralic glacial, paraglacial and postglacial Upper Ordovician siliciclastic deposits in the Murzuq Basin, SW Libya. *Sedimentary Geology, 177*(3-4), 145-173.

Elias, S. A. (1985). Paleoenvironmental interpretations of Holocene insect fossil assemblages from four high-altitude sites in the Front Range, Colorado, USA. *Arctic and Alpine Research, 17*(1), 31-48.

Elias, S. A. (1994). *Quaternary insects and their environments*. Washington: Smithsonian Institution Press.

Elias, S. A. (2009). *Advances in Quaternary Entomology*. Amsterdam: Elsevier.

Engel, M. S., and Delclòs, X. (2010). Primitive termites in Cretaceous amber from Spain and Canada (Isoptera). *Journal of the Kansas Entomological Society, 83*(2), 111-128.

Engel, M. S., Grimaldi, D. A., and Krishna, K. (2009). Termites (Isoptera): Their phylogeny, classification, and rise to ecological dominance. *American Museum Novitates, 2009*(3650), 1-27.

Eramo, G., Muntoni, I. M., Aprile, A., Pallara, M., Rotunno, R., Zerboni, A., and di Lernia, S. (2020). Networking through pottery characterisation at Takarkori rock shelter (Libyan Sahara, 10,200–4650 cal BP). *Archaeological and Anthropological Sciences, 12*(9), 1-33.

Erickson, J. M. (1988). Fossil oribatid mites as tools for Quaternary paleoecologists: Preservation quality, quantities, and taphonomy. *Bulletin of the Buffalo Society of Natural Sciences, 33*, 207-226.

Evans, A. V. (2014). *Beetles of Eastern North America*. Princeton: Princeton University Press.

Farrow, R., and Colless, D. (1980). Analysis of the interrelationships of geographical races of *Locusta migratoria* (Linnaeus) (Orthoptera: Acrididae), by numerical taxonomy, with special reference to sub-speciation, in the tropics and affinities of the Australian race. *Acrida, 9*(2), 77-99.

Favret, C. (2005). *New non-destructive DNA extraction and specimen clearing technique for aphids (Hemiptera).* Paper presented at the Proceedings of the Entomological Society of Washington.

Feener Jr, D. H., and Brown, B. V. (1997). Diptera as parasitoids. *Annual review of entomology, 42*(1), 73-97.

Fenwick, C. (2012). North Africa (History of Archaeology). In N. A. Silberman (Ed.) *The Oxford Companion to Archaeology* (2nd edition). Oxford: Oxford University Press.

Fitzgerald, K. T., and Flood, A. A. (2006). Hymenoptera stings. *Clinical techniques in small animal practice, 21*(4), 194-204.

Folmer, O., Hoeh, W., Black, M., and Vrijenhoek, R. (1994). Conserved primers for PCR amplification of mitochondrial DNA from different invertebrate phyla. *Molecular Marine Biology and Biotechnology, 3*(5), 294-299.

Fornaciari, R., Fornaciari, S., Francia, E., Mercuri, A. M., and Arru, L. (2018). *Panicum* spikelets from the Early Holocene Takarkori rockshelter (SW Libya): Archaeo-molecular and-botanical investigations. *Plant Biosystems, 152*(1), 1-13.

Fornaciari, R., Olmi, L., Arru, L., Mercuri, A. M., and Lernia, S. d. (2014). *Wild cereals from Holocene central Sahara archaeological sites: aDNA and archaeobotany from the Takarkori rockshelter.* Paper presented at the 9th European Palaeobotany and Palynology Conference, Padova.

Foubert Jr, E. L., and Stier, R. (1958). Antigenic relationships between honeybees, wasps, yellow hornets, black hornets, and yellow jackets. *Journal of Allergy, 29*(1), 13-23.

Fournier, P.-E., Ndihokubwayo, J.-B., Guidran, J., Kelly, P. J., and Raoult, D. (2002). Human pathogens in body and head lice. *Emerging Infectious Diseases, 8*(12), 1515.

Fulton, T. L., and Stiller, M. (2012). PCR amplification, cloning, and sequencing of ancient DNA. In B. Shapiro and M. Hofreiter (Eds.) *Ancient DNA: Methods and Protocols, Methods in Molecular Biology* (Vol. 840). Dordrecht: Springer.

Garcin, Y., Vincens, A., Williamson, D., Buchet, G., and Guiot, J. (2007). Abrupt resumption of the African Monsoon at the Younger Dryas—Holocene climatic transition. *Quaternary Science Reviews, 26*(5-6), 690-704.

Gasse, F. (2000). Hydrological changes in the African tropics since the Last Glacial Maximum. *Quaternary Science Reviews, 19*(1-5), 189-211.

Gasse, F., and Roberts, C. N. (2004). Late Quaternary hydrologic changes in the arid and semiarid belt of northern Africa. In H. F. Diaz and R. S. Bradley (Eds.) *The Hadley circulation: Present, past and future*. Dordrecht: Springer.

Gasse, F., and Van Campo, E. (1994). Abrupt post-glacial climate events in West Asia and North Africa monsoon domains. *Earth and Planetary Science Letters, 126*(4), 435-456.

Gibson, J. F., Kelso, S., Jackson, M. D., Kits, J. H., Miranda, G. F. G., and Skevington, J. H. (2011). Dipteraspecific polymerase chain reaction amplification primers of use in molecular phylogenetic research. *Annals of the Entomological Society of America, 104*, 976–997.

Gifawesen, C., Funke, B., and Proshold, F. (1975). Control of antifungal-resistant strains of *Aspergillus niger* mold contaminants in insect rearing media. *Journal of Economic Entomology, 68*(4), 441-444.

GilArriortua, M., Salona-Bordas, M. I., Caine, L. M., Pinheiro, F., and de Pancorbo, M. M. (2015). Mitochondrial and nuclear DNA approaches for reliable identification of *Lucilia* (Diptera, Calliphoridae) species of forensic interest from Southern Europe. *Forensic Science International, 257*, 393-397.

GilArriortua, M., Salona Bordas, M. I., Caine, L. M., Pinheiro, F., and de Pancorbo, M. M. (2013). Cytochrome b as a useful tool for the identification of blowflies of forensic interest (Diptera, Calliphoridae). *Forensic Science International, 228*(1-3), 132-136.

GilArriortua, M., Salona Bordas, M. I., Kohnemann, S., Pfeiffer, H., and de Pancorbo, M. M. (2014). Molecular differentiation of central European blowfly species (Diptera, Calliphoridae) using mitochondrial and nuclear genetic markers. *Forensic Science International, 242*, 274-282.

Gilbert, M. T. P., Binladen, J., Miller, W., Wiuf, C., Willerslev, E., Poinar, H., Carlson, J. E., Leebens-Mack, J. H., and Schuster, S. C. (2007). Recharacterization of ancient DNA miscoding lesions: Insights in the era of sequencing-by-synthesis. *Nucleic acids research, 35*(1), 1-10.

Giordani, G. (2019). *Non-invasive approaches to morphological and molecular identification of insects from museum, archaeo-funerary and forensic contexts.* PhD Thesis. University of Huddersfield.

Giordani, G., Erauw, C., Eeckhout, P. A., Owens, L. S., and Vanin, S. (2020). Patterns of camelid sacrifice at the site of Pachacamac, Peruvian Central Coast, during the Late Intermediate Period (AD1000–1470): Perspectives from funerary archaeoentomology. *Journal of Archaeological Science, 114*, 105065.

Giordani, G., Grzywacz, A., and Vanin, S. (2019). Characterization and identification of puparia of *Hydrotaea* Robineau-Desvoidy, 1830 (Diptera: Muscidae) from forensic and archaeological contexts. *Journal of Medical Entomology, 56*(1), 45-54.

Giordani, G., Tuccia, F., Floris, I., and Vanin, S. (2018). First record of *Phormia regina* (Meigen, 1826) (Diptera: Calliphoridae) from mummies at the Sant'Antonio Abate Cathedral of Castelsardo, Sardinia, Italy. *PeerJ, 6*, e4176.

Giraldo, H. P. A., Uribe, S. S. I., and Lopez, R. A. (2011). Analysis of mitochondrial DNA sequences (Cytb and ND1) in *Lucilia eximia* (Diptera: Calliphoridae). *Revista Colombiana de Entomologia, 37*, 273–278.

Glocke, I., and Meyer, M. (2017). Extending the spectrum of DNA sequences retrieved from ancient bones and teeth. *Genome research, 27*(7), 1230-1237.

Graham, J. H., Hughie, H. H., Jones, S., Wrinn, K., Krzysik, A. J., Duda, J. J., Freeman, D. C., Emlen, J. M., Zak, J. C., and Kovacic, D. A. (2004). Habitat disturbance and the diversity and abundance of ants (Formicidae) in the Southeastern Fall-Line Sandhills. *Journal of Insect Science, 4*(1), 30.

Grassberger, M., and Frank, C. (2004). Initial study of arthropod succession on pig carrion in a central European urban habitat. *Journal of Medical Entomology, 41*(3), 511-523.

Greenberg, B. (1973). *Flics and disease. Vol. II. II. Biology and disease transmission*. Princeton: Princeton University Press.

Grimaldi, D., and Engel, M. S. (2005). *Evolution of the Insects*. Cambridge: Cambridge University Press.

Guitton, C., Dorchies, M., and Morand, S. (1996). Scanning electron microscopy of larval instars and imago of *Rhinoestrus usbekistanicus* Gan, 1947 (Oestridae). *Parasite, 3*(2), 155-159.

Gullan, P. J., and Cranston, P. S. (2014). *The insects: An outline of entomology*. London, UK: John Wiley & Sons.

Guo, Q. (2000). Climate change and biodiversity conservation in Great Plains agroecosystems. *Global Environmental Change, 10*(4), 289-298.

Guo, Y., Zha, L., Yan, W., Li, P., Cai, J., and Wu, L. (2014). Identification of forensically important sarcophagid flies (Diptera: Sarcophagidae) in China based on COI and Period gene. *International Journal of Legal Medicine, 128*(1), 221-228.

Gupta, M. (2008). Efficacy and economics of biorationals and their admixtures against incidence of major insect pests of soybean. *Annals of Plant Protection Sciences, 16*(2), 282-288.

Gurney, A. B., Kramer, J. P., and Steyskal, G. C. (1964). Some techniques for the preparation, study, and storage in microvials of insect genitalia. *Annals of the Entomological Society of America, 57*(2), 240-242.

Hajibabaei, M., Janzen, D. H., Burns, J. M., Hallwachs, W., and Hebert, P. D. (2006). DNA barcodes distinguish species of tropical Lepidoptera. *Proceedings of the National Academy of Sciences, 103*(4), 968-971.

Hajibabaei, M., Singer, G. A., Hebert, P. D., and Hickey, D. A. (2007). DNA barcoding: How it complements taxonomy, molecular phylogenetics and population genetics. *TRENDS in Genetics, 23*(4), 167-172.

Hall, M., and Wall, R. (1995). Myiasis of humans and domestic animals. In J.R. Baker, R. Muller, D. Rollinson (Eds.) *Advances in Parasitology* (Vol. 35). Amsterdam: Elsevier.

Hall, M. J., and Martín-Vega, D. (2019). Visualization of insect metamorphosis. *Philosophical Transactions of the Royal Society 374*(1783), 0071.

Hall, M. J., and Smith, K. G. (1993). Diptera causing myiasis in man. In R. Lane and R. Crosskey (Eds.), *Medical insects and arachnids*. Amsterdam: Springer Netherlands.

Handt, O., Höss, M., Krings, M., and Pääbo, S. (1994). Ancient DNA: Methodological challenges. *Experientia, 50*(6), 524-529.

Harrison, J. d. G. (2012). Cleaning and preparing adult beetles (Coleoptera) for light and scanning electron microscopy. *African Entomology, 20*(2), 395-401.

Harvey, J. (1986). Sheep botfly: Ophthalmomyiasis externa. *Canadian Journal of Ophthalmology, 21*(3), 92-95.

Hebert, P. D., Ratnasingham, S., and De Waard, J. R. (2003). Barcoding animal life: Cytochrome c oxidase subunit 1 divergences among closely related species. *Proceedings of the Royal Society of London. Series B: Biological Sciences, 270*(1), S96-S99.

Heintzman, P. D. (2013). Patterns in palaeontology: An introduction to ancient DNA. *Palaeontology Online*, 01.

Heller, K., Korsunovskaya, O., Ragge, D., Vedenina, V., Willemse, F., Zhantiev, R., and Frantsevich, L. (1998). Check-list of European Orthoptera. *Articulata,* 7, 1-61.

Hellqvist, M., and Lemdahl, G. (1996). Insect assemblages and local environment in the mediaeval town of Uppsala, Sweden. *Journal of Archaeological Science, 23*(6), 873-881.

Henríquez-Valido, P., Morales, J., Vidal-Matutano, P., Moreno-Benítez, M., Marchante-Ortega, Á., Rodríguez-Rodríguez, A., and Huchet, J.-B. (2020). Archaeoentomological indicators of long-term food plant storage at the Prehispanic granary of La Fortaleza (Gran Canaria, Spain). *Journal of Archaeological Science, 120*, 105179.

Hewitt, C. G. (1914). *The house-fly, Musca domestica Linn*. Cambridge University Press.

Heyn, P., Stenzel, U., Briggs, A. W., Kircher, M., Hofreiter, M., and Meyer, M. (2010). Road blocks on paleogenomes—polymerase extension profiling reveals the frequency of blocking lesions in ancient DNA. *Nucleic acids research, 38*(16), e161-e161.

Hinton, H. E. (1945). *A Monograph of the Beetles associated with stored Products. Volume I*. London: British Museum.

Hinton, H. E., and Corbet, A. S. (1949). *Common insect pests of stored food products. A guide to their identification* (Vol. 15). London: British museum.

Hoelzmann, P., Gasse, F., Dupont, L. M., Salzmann, U., Staubwasser, M., Leuschner, D. C., and Sirocko, F. (2004). Palaeoenvironmental changes in the arid and sub arid belt (Sahara-Sahel-Arabian Peninsula) from 150 kyr to present. In R. W. Battarbee, F. Gasse, and C. E. Stickley (Eds.) *Past climate variability through Europe and Africa* (pp. 219-256). Amsterdam: Springer Netherlands.

Hoffmann, A. (1954). Coléoptères Curculionides, deuxième partie. *Faune de France, 59*.

Hofreiter, M., Jaenicke, V., Serre, D., Haeseler, A. v., and Pääbo, S. (2001). DNA sequences from multiple amplifications reveal artifacts induced by cytosine deamination in ancient DNA. *Nucleic acids research, 29*(23), 4793-4799.

Holway, D. A., Lach, L., Suarez, A. V., Tsutsui, N. D., and Case, T. J. (2002). The causes and consequences of ant invasions. *Annual review of ecology and systematics, 33*(1), 181-233.

Hope, F. (1842). Observations on the Coleoptera of Port Essington. *Annals and Magazine of Natural History 1*, 423-430.

Huchet, J.-B. (2010). Within the mummy Of Namenkhet Amun (San Lazzaro Armenian Monastery, Venice/Italy). *Advances in Egyptology, 1*, 59-80.

Huchet, J.-B., and Greenberg, B. (2010). Flies, Mochicas and burial practices: A case study from Huaca de la Luna, Peru. *Journal of Archaeological Science, 37*(11), 2846-2856.

Hunt, C., Gilbertson, D., Jenkinson, R., Van der Veen, M., Yates, G., and Buckland, P. (1987). ULVS XVII: Palaeoecology and agriculture of an abandonment phase at gasr Mm 10, Wadi Mimoun, Tripolitania. *Libyan Studies, 18*, 1-13.

Hunter, J., and Barker, S. (2003). Susceptibility of head lice (*Pediculus humanus capitis*) to pediculicides in Australia. *Parasitology Research, 90*(6), 476-478.

Hunter, S. J., Goodall, T. I., Walsh, K. A., Owen, R., and Day, J. C. (2008). Nondestructive DNA extraction from blackflies (Diptera: Simuliidae): Retaining voucher specimens for DNA barcoding projects. *Molecular Ecology Resources, 8*(1), 56-61.

Ilchmann, G., Betke, P., Grafe, D., and Gossing, S. (1986). Studies into estrosis and its control in the mongolian peoples-republic. *Monatshefte fur Veterinarmedizin, 41*(4), 128-132.

Inward, D. J., Vogler, A. P., and Eggleton, P. (2007). A comprehensive phylogenetic analysis of termites (Isoptera) illuminates key aspects of their evolutionary biology. *Molecular Phylogenetics and Evolution, 44*(3), 953-967.

James, M. T. (1947). *The flies that cause myiasis in man*. Washington: US Department of Agriculture.

Jenkins, J. (1991). A simple device to clean insect specimens for museums and scanning electron microscopy. *Proceedings of the Entomological Society of Washington, 93*(1), 204-205.

Jinbo, U., Kato, T., and Ito, M. (2011). Current progress in DNA barcoding and future implications for entomology. *Entomological Science, 14*(2), 107-124.

Jolly, D., Harrison, S., Damnati, B., and Bonnefille, R. (1998). Simulated climate and biomes of Africa during the Late Quaternary: Comparison with pollen and lake status data. *Quaternary Science Reviews, 17*(6-7), 629-657.

Jordaens, K., Sonet, G., Richet, R., Dupont, E., Braet, Y., and Desmyter, S. (2013). Identification of forensically important *Sarcophaga* species (Diptera: Sarcophagidae) using the mitochondrial COI gene. *International Journal of Legal Medicine, 127*(2), 491-504.

Keirans, J., Clifford, C., and Corwin, D. (1976). *Ixodes sigelos*, n. sp.(Acarina: Ixodidae), a parasite of rodents in Chile, with a method for preparing ticks for examination by scanning electron microscopy. *Acarologia, 18*(2), 217-225.

Kenward, H. (1974). Methods for palaeo-entomology on site and in the laboratory. *Science and Archaeology, 13*(1).

Kenward, H., and Large, F. (1998). Recording the preservational condition of archaeological insect fossils. *Environmental Archaeology, 2*(1), 49-60.

Kenward, H. K. (1976). Reconstructing ancient ecological conditions from insect remains; some problems and an experimental approach. *Ecological Entomology, 1*(1), 7-17.

Kenward, H. K., Hall, A. R., and Jones, A. K. (1980). A tested set of techniques for the extraction of plant and animal macrofossils from water-logged archaeological deposits. *Science and Archaeology, 22*, 3-15.

Khajehzadeh, Y. (2002). Investigation on taxonomy of *Locusta migratoria* L. and its phase status in sugercane and rice fields in Khuzestan province. *The Scientific Journal of Agriculture 24*(2), 51-73.

Kim, K. J. (1989). *Characteristics of synchrotron radiation.* Paper presented at the AIP conference proceedings.

King, C. E., Debruyne, R., Kuch, M., Schwarz, C., and Poinar, H. N. (2009). A quantitative approach to detect and overcome PCR inhibition in ancient DNA extracts. *BioTechniques, 47*(5), 941-949.

King, G. A. (2014). Exaptation and synanthropic insects: A diachronic interplay between biology and culture. *Environmental Archaeology, 19*(1), 12-22.

Kislev, M., Simchoni, O., Fersṭer, G., and Netser, E. (2007). Hygiene and insect damage of crops and food at Masada. In J. Aviram, G. Foerster, E. Netzer, and G. Stiebel (Eds.), *Masada VIII*. Jerusalem: Israel Exploration Siciety and the Hebrew University Jerusalem.

Kröpelin, S., Verschuren, D., Lézine, A.-M., Eggermont, H., Cocquyt, C., Francus, P., Cazet, J.-P., Fagot, M., Rumes, B., and Russell, J. (2008). Climate-driven ecosystem succession in the Sahara: The past 6000 years. *Science, 320*(5877), 765-768.

Kunz, C. (1974). *Synchrotron radiation*. Paper presented at the Meeting on Technology arising from High-energy Physics, Geneva, Switzerland.

Lachaud, J. P., and Déjean, A. (1994). Predatory behavior of a seed-eating ant: *Brachyponera senaarensis*. *Entomologia Experimentalis et Applicata, 72*(2), 145-155.

Lam, N. L., Smith, K. R., Gauthier, A., and Bates, M. N. (2012). Kerosene: A review of household uses and their hazards in low-and middle-income countries. *Journal of Toxicology and Environmental Health, Part B, 15*(6), 396-432.

Latchininsky, A. (2011). *Locusts and us: The 21st century science vs. the Biblical enemy.* Paper presented at the Fundamental problems of Entomology in XXI century, Saint-Petersburg.

Latchininsky, A. (2013). Locusts and remote sensing: A review. *Journal of Applied Remote Sensing,* 7, 5099.

Lavoie, C., Elias, S., and Payette, S. (1997). Holocene fossil beetles from a treeline peatland in subarctic Québec. *Canadian Journal of Zoology,* 75(2), 227-236.

Lawrence, John F, Newton Jr, and F, A. (1982). Evolution and classification of beetles. *Annual review of ecology and systematics, 13*(1), 261-290.

Lecocq, T. (2018). *Insects:The disregarded domestication histories*. In press: IntechOpen.

Levieux, J., and Diomande, T. (1978). La nutrition des fourmis granivores II. Cycle d'activité et régime alimentaire de *Brachyponera senaarensis* (Mayr) (Hymenoptera: Formicidae). *Insectes Sociaux, 25*(3), 187-196.

Li, W., Ortiz, G., Fournier, P. E., Gimenez, G., Reed, D. L., Pittendrigh, B., and Raoult, D. (2010). Genotyping of human lice suggests multiple emergencies of body lice from local head louse populations. *PLOS Neglected Tropical Diseases, 4*(3), e641.

Light, J. E., Allen, J. M., Long, L. M., Carter, T. E., Barrow, L., Suren, G., Raoult, D., and Reed, D. L. (2008). Geographic distributions and origins of human head lice (*Pediculus humanus capitis*) based on mitochondrial data. *Journal of Parasitology, 94*(6), 1275-1281.

Lister, A. M. (2009). Late-glacial mammoth skeletons (*Mammuthus primigenius*) from Condover (Shropshire, UK): Anatomy, pathology, taphonomy and chronological significance. *Geological Journal, 44*(4), 447-479.

Liverani, M. (2000). The Garamantes: A fresh approach. *Libyan Studies, 31*, 17-28.

Liverani, M. (2004). Rediscovering the Garamantes: Archaeology and history. *Libyan Studies, 35*, 191-200.

Liverani, M., Cremaschi, M., and di Lernia, S. (2000). The «Archaeological Park» of the Tadrart Acacus and Messak Settafet (south-western Fezzan, Libya). *Sahara, 12*(2001), 121-140.

Longo, R., Arfelli, F., Bellazzini, R., Bottigli, U., Brez, A., Brun, F., Brunetti, A., Delogu, P., Di Lillo, F., and Dreossi, D. (2016). Towards breast tomography with synchrotron radiation at Elettra: First images. *Physics in Medicine and Biology, 61*(4), 1634.

López-García, J., Angell, C., and Martín-Vega, D. (2020). Wing morphometrics for the identification of Nearctic and Palaearctic Piophilidae (Diptera) of forensic relevance. *Forensic Science International, 309*, 110192.

Lorcin, P. M. (2007). *Curing the colonizers: Hydrotherapy, climatology, and French colonial spas*. North Carolina: Duke University Press.

Luo, Z.-X. (2007). Transformation and diversification in early mammal evolution. *Nature, 450*(7172), 1011-1019.

Malyshev, S. I. (1968). *Genesis of the Hymenoptera and the phases of their evolution*. Dordrecht: Springer.

Marchetti, D., Arena, E., Boschi, I., and Vanin, S. (2013). Human DNA extraction from empty puparia. *Forensic Science International, 229*(1-3), e26-e29.

Marshall, F., and Hildebrand, E. (2002). Cattle before crops: The beginnings of food production in Africa. *Journal of World prehistory, 16*(2), 99-143.

Marshall, S. (2012). *Flies*. Canada: Firefly Books (US) Incorporated.

Marshall, S. A. (2018). *Beetles: the natural history and diversity of Coleoptera*. Canada: Firefly Books (US) Incorporated.

Martín-Vega, D., Hall, M. J., and Simonsen, T. J. (2016). Resolving confusion in the use of concepts and terminology in intrapuparial development studies of cyclorrhaphous Diptera. *Journal of Medical Entomology, 53*(6), 1249-1251.

Matthews, J. V. (1979). Tertiary and Quaternary environments: Historical background for an analysis of the Canadian insect fauna. *The Memoirs of the Entomological Society of Canada, 111*(108), 31-86.

Mattingly, D. (2011). *Imperialism, Power and Identity. Experiencing the Roman Empire*. Princeton: Princeton University Press.

Mattingly, D., and Wilson, A. (2003). Farming the Sahara: the Garamantian contribution in southern Libya. *Arid lands in Roman times*, 37-50.

Maunder, J. (1977). Human lice—biology and control. *Royal Society of Health Journal, 97*(1), 29-32.

Mayewski, P. A., Rohling, E. E., Stager, J. C., Karlén, W., Maasch, K. A., Meeker, L. D., Meyerson, E. A., Gasse, F., van Kreveld, S., and Holmgren, K. (2004). Holocene climate variability. *Quaternary Research, 62*(3), 243-255.

McAlpine, J. F., Peterson, B. V., Shewell, G., Teskey, H., Vockeroth, J., and Wood, D. (1989). *Manual of nearctic Diptera* (Vol. 3). Canada: Agriculture Canada.

McDonagh, L. M., and Stevens, J. R. (2011). The molecular systematics of blowflies and screwworm flies (Diptera: Calliphoridae) using 28S rRNA, COX1 and EF-1α: Insights into the evolution of dipteran parasitism. *Parasitology, 138*, 1760–1777.

Meier, R. (2008). DNA sequences in taxonomy, opportunities and challenges. *The new taxonomy*, 65-127.

Meiklejohn, K. A., Wallman, J. F., and Dowton, M. (2013). DNA barcoding identifies all immature life stages of forensically important flesh fly (Diptera: Sarcophagidae). *Journal of Forensic Sciences, 58*(1), 184-187.

Mercuri, A. M. (2008). Human influence, plant landscape evolution and climate inferences from the archaeobotanical records of the Wadi Teshuinat area (Libyan Sahara). *Journal of Arid Environments, 72*(10), 1950-1967.

Mercuri, A. M., Fornaciari, R., Gallinaro, M., Vanin, S., and di Lernia, S. (2018). Plant behaviour from human imprints and the cultivation of wild cereals in Holocene Sahara. *Nature Plants, 4*(2), 71-81.

Mercuri, A. M., Sadori, L., and Uzquiano Ollero, P. (2011). Mediterranean and north-African cultural adaptations to mid-Holocene environmental and climatic changes. *The Holocene, 21*(1), 189-206.

Meyer, C. P., and Paulay, G. (2005). DNA barcoding: Error rates based on comprehensive sampling. *PLoS Biology, 3*(12), e422.

Mmari, M. W., Kinyuru, J. N., Laswai, H. S., and Okoth, J. K. (2017). Traditions, beliefs and indigenous technologies in connection with the edible longhorn grasshopper *Ruspolia differens* (Serville 1838) in Tanzania. *Journal of ethnobiology and ethnomedicine, 13*(1), 60.

Morgan, A. V., and Morgan, A. (1980). Beetle bits—the science of paleoentomology. *Geoscience Canada*.

Morgan, A. V., Ruddiman, W., and Wright, H. (1987). Late Wisconsin and early Holocene paleoenvironments of east-central North America based on assemblages of fossil Coleoptera. *The Geology of North America, 3*, 353-370.

Mori, L. (2013). *Life and death of a rural village in Garamantian times. Archaeological investigations in the oasis of Fewet (Libyan Sahara)* (Vol. 6). Firenze: All'Insegna del Giglio.

Moritz, C., and Cicero, C. (2004). DNA barcoding: Promise and pitfalls. *PLoS Biology, 2*(10), e354.

Mumcuoglu, K., and Zias, J. (1991). Pre-pottery neolithic B head lice found in Nahal Hemar Cave and dated 6,900-6,300 BCE (uncalibrated). *Atikot, 20*, 167-168.

Munzi, M. (2004). *La decolonizzazione del passato: archeologia e politica in Libia dall'amministrazione alleata al regno di Idris* (Vol. 22). Rome: L'Erma di Bretschneider.

Naegele, A. (1960). Curieuses coques minéralisées au Sahara. *Nature, 3304*, 327.

Nelson, H. G. (1949). A method of cleaning insects for study. *The Coleopterists' Bulletin*, 89-92.

Nicholson, S. E., and Flohn, H. (1980). African environmental and climatic changes and the general atmospheric circulation in late Pleistocene and Holocene. *Climatic Change, 2*(4), 313-348.

Nielsen, B. O., Mahler, V., and Rasmussen, P. (2000). An arthropod assemblage and the ecological conditions in a byre at the Neolithic settlement of Weier, Switzerland. *Journal of Archaeological Science, 27*(3), 209-218.

Osborne, P. (1988). A Late Bronze Age insect fauna from the River Avon, Warwickshire, England: Its implications for the terrestrial and fluvial environment and for climate. *Journal of Archaeological Science, 15*(6), 715-727.

Osuji, F. N. (1975). Some aspects of the biology of *Dermestes maculatus* DeGeer (Coleoptera, Dermestidae) in dried fish. *Journal of stored products research, 11*(1), 25-31.

Oulebsir, N. (2004). *Les usages du patrimoine: Monuments, musées et politique coloniale en Algérie, 1830-1930*. Paris: Les Editions de la MSH.

Pääbo, S., Higuchi, R. G., and Wilson, A. C. (1989). Ancient DNA and the polymerase chain reaction: The emerging field of molecular archaeology (Minireview). *The Journal of biological chemistry, 264*(17), 9709-9712.

Pagliano, G., and Romano, M. (2012). Three new species of African Apterogyninae from Kenya, Namibia and Senegal (Insecta, Hymenoptera, Bradynobaenidae). *Il Naturalista Valtellinese, 23*, 15-41.

Pagliano, G., and Romano, M. (2018). World list of all known species of Bradynobaenidae (Hymenoptera). *Il Naturalista Valtellinese, 28*, 15-46.

Paknia, O. (2006). Distribution of the introduced ponerine ant *Pachycondyla sennaarensis* (Hymenoptera: Formicidae) in Iran. *Myrmecologische Nachrichten, 8*, 235-238.

Pampiglione, S., Giannetto, S., and Virga, A. (1997). Persistence of human myiasis by *Oestrus ovis* L. (Diptera: Oestridae) among shepherds of the Etnean area (Sicily) for over 150 years. *Parassitologia, 39*(4), 415-418.

Panagiotakopulu, E. (1999). An examination of biological materials from coprolites from XVIII Dynasty Amarna, Egypt. *Journal of Archaeological Science, 26*(5), 547-551.

Panagiotakopulu, E. (2000). *Archaeology and Entomology in the Eastern Mediterranean: Research into the history of insect synanthropy in Greece and Egypt*. Oxford: BAR publishing.

Panagiotakopulu, E. (2001). New records for ancient pests: archaeoentomology in Egypt. *Journal of Archaeological Science, 28*(11), 1235-1246.

Panagiotakopulu, E. (2004a). Dipterous remains and archaeological interpretation. *Journal of Archaeological Science, 31*(12), 1675-1684.

Panagiotakopulu, E. (2004b). Pharaonic Egypt and the origins of plague. *Journal of Biogeography, 31*(2), 269-275.

Panagiotakopulu, E., and Buckland, P. (2009). Environment, insects and the archaeology of Egypt. *Beyond the horizon: Studies in Egyptian Art, 2*, 347-361.

Panagiotakopulu, E., Buckland, P. C., and Kemp, B. J. (2010). Underneath Ranefer's floors–urban environments on the desert edge. *Journal of Archaeological Science, 37*(3), 474-481.

Panagiotakopulu, E., Skidmore, P., and Buckland, P. (2007). Fossil insect evidence for the end of the Western Settlement in Norse Greenland. *Naturwissenschaften, 94*(4), 300-306.

Papavero, N. (1977). *The World Oestridae (Diptera), mammals and continental drift*. Amsterdam: Springer Netherlands.

Pape, T. (2001). Phylogeny of Oestridae (Insecta: Diptera). *Systematic Entomology, 26*(2), 133-171.

Pasqualini, D., Bianchi, C. C., Paolino, D. S., Mancini, L., Cemenasco, A., Cantatore, G., Castellucci, A., and Berutti, E. (2012). Computed micro-tomographic evaluation of glide path with nickel-titanium rotary PathFile in maxillary first molars curved canals. *Journal of endodontics, 38*(3), 389-393.

Peacock, E. R. (1993). *Adults and larvae of hide, larder and carpet beetles and their relatives (Coleoptera: Dermestidae) and of derodontid beetles (Coleoptera: Derodontidae)* (Vol. 5). London: Natural History Museum.

Pedersen, M. W., Overballe-Petersen, S., Ermini, L., Sarkissian, C. D., Haile, J., Hellstrom, M., Spens, J., Thomsen, P. F., Bohmann, K., and Cappellini, E. (2015). Ancient and modern environmental DNA. *Philosophical Transactions of the Royal Society B: Biological Sciences, 370*(1660), 20130383.

Peters, R. S., Krogmann, L., Mayer, C., Donath, A., Gunkel, S., Meusemann, K., Kozlov, A., Podsiadlowski, L., Petersen, M., and Lanfear, R. (2017). Evolutionary history of the Hymenoptera. *Current Biology, 27*(7), 1013-1018.

Petraglia, M. D., Haslam, M., Fuller, D. Q., Boivin, N., and Clarkson, C. (2010). Out of Africa: New hypotheses and evidence for the dispersal of *Homo sapiens* along the Indian Ocean rim. *Annals of human biology, 37*(3), 288-311.

Pinheiro, L., and Gaal-Haszler, S. (2015). Illustrated catalogue of Neotropical *Ctenuchina*, *Euchromiina* and *Pericopina* types (Lepidoptera, Erebidae, Arctiinae, Arctiini) described by Hans Zerny, with discussion on their taxonomic status. *Zootaxa, 3925*(4), 505-535.

Plarre, R. (2013). An attempt to reconstruct the natural and cultural history of the granary weevil, *Sitophilus granarius* (Coleoptera: Curculionidae). *European Journal of Entomology, 107*(1), 1-11.

Poinar, G. (1994). The range of life in amber: significance and implications in DNA studies. *Experientia, 50*(6), 536-542.

Ponel, P., Matterne, V., Coulthard, N., and Yvinec, J.-H. (2000). La Tène and Gallo-Roman natural environments and human impact at the Touffréville rural settlement, reconstructed from Coleoptera and plant macroremains (Calvados, France). *Journal of Archaeological Science, 27*(11), 1055-1072.

Pons, J., Barraclough, T. G., Gomez-Zurita, J., Cardoso, A., Duran, D. P., Hazell, S., Kamoun, S., Sumlin, W. D., and Vogler, A. P. (2006). Sequence-based species delimitation for the DNA taxonomy of undescribed insects. *Systematic Biology, 55*(4), 595-609.

Pradelli, J., Rossetti, C., Tuccia, F., Giordani, G., Licata, M., Birkhoff, J., Verzeletti, A., and Vanin, S. (2019). Environmental necrophagous fauna selection in a funerary hypogeal context: The putridarium of the Franciscan monastery of Azzio (northern Italy). *Journal of Archaeological Science: Reports, 24*, 683-692.

Prendergast, A., Stevens, R., O'Connell, T., Hill, E., Hunt, C., and Barker, G. (2016). A late Pleistocene refugium in Mediterranean North Africa? Palaeoenvironmental reconstruction from stable isotope analyses of land snail shells (Haua Fteah, Libya). *Quaternary Science Reviews, 139*, 94-109.

Ramdani, M., Flower, R. J., Elkhiati, N., Kraïem, M. M., and Fathi, A. A. (2001). Zooplankton (Cladocera, Ostracoda), Chironomidae and other benthic faunal remains in sediment cores from nine North African wetland lakes: the CASSARINA Project. *Aquatic Ecology, 35*(3-4), 389-403.

Raoult, D., and Roux, V. (1999). The body louse as a vector of reemerging human diseases. *Clinical Infectious Diseases, 29*(4), 888-911.

Rasnitsyn, A. P., and Quicke, D. L. (2007). *History of insects*. Berlin: Springer Science & Business Media.

Ratnasingham, S., and Hebert, P. D. (2007). BOLD: The Barcode of Life Data System (http://www.barcodinglife.org). *Molecular ecology notes,* 7(3), 355-364.

Reiss, R. A. (2006). Ancient DNA from ice age insects: Proceed with caution. *Quaternary Science Reviews, 25*(15-16), 1877-1893.

Renwick, J., and Chew, F. (1994). Oviposition behavior in Lepidoptera. *Annual review of entomology, 39*(1), 377-400.

Resh, V. H., and Cardé, R. T. (2009). *Encyclopedia of insects*. Cambridge: Academic press.

Richards, O. W., and Davies, R. (1977). Hymenoptera. In A. D. Imms, O. W. Richards and R. G. Davies (Eds.) *Imms' General Textbook of Entomology*. Dordrecht: Springer.

Richardson, M. S., and Goff, M. L. (2001). Effects of temperature and intraspecific interaction on the development of *Dermestes maculatus* (Coleoptera: Dermestidae). *Journal of Medical Entomology, 38*(3), 347-351.

Richerson, P. J., Boyd, R., and Bettinger, R. L. (2001). Was agriculture impossible during the Pleistocene but mandatory during the Holocene? A climate change hypothesis. *American Antiquity, 66*(3), 387-411.

Ronderos, M. M., Spinelli, G. R., and Sarmiento, P. (2000). Preparation and mounting of biting midges of the genus *Culicoides* Latreille (Diptera: Ceratopogonidae) to be observed with a scanning electron microscope. *Transactions of the American Entomological Society*, 125-132.

Rotunno, R., Mercuri, A. M., Florenzano, A., Zerboni, A., and di Lernia, S. (2019). Coprolites from rock shelters: Hunter-gatherers "herding" Barbary sheep in the Early Holocene Sahara. *Journal of African Archaeology, 17*(1), 76-94.

Rousseau, M. (2011). Paraffin flotation for archaeoentomological research: Is it really efficient? *Environmental Archaeology, 16*(1), 58-64.

Rowley, D. L., Coddington, J. A., Gates, M. W., Norrbom, A. L., Ochoa, R. A., Vandenberg, N. J., and Greenstone, M. H. (2007). Vouchering DNA-barcoded specimens: Test of a nondestructive extraction protocol for terrestrial arthropods. *Molecular ecology notes,* 7(6), 915-924.

Saltini, A. (1984). *Storia delle scienze agrarie* (Vol. 2). Bologna: Edagricole.

Samish, M., Argaman, Q., and Perelman, D. (1992). Research Note: The hide beetle, *Dermestes maculatus DeGeer* (Dermestidae), feeds on live turkeys. *Poultry science, 71*(2), 388-390.

Sarnat, E. M., Fischer, G., Guénard, B., and Economo, E. P. (2015). Introduced *Pheidole* of the world: Taxonomy, biology and distribution. *ZooKeys*(543), 1.

Sarpong, D., Oduro-Kwarteng, S., Gyasi, S., Buamah, R., Donkor, E., Awuah, E., and Baah, M. (2019). Biodegradation by composting of municipal organic solid waste into organic fertilizer using the black soldier fly (*Hermetia illucens*)(Diptera: Stratiomyidae) larvae. *International Journal of Recycling of Organic Waste in Agriculture, 8*(1), 45-54.

Schaefer, C. (1979). Feeding habits and hosts of Calyptrate flies (Diptera: Brachycera: Cyclorrhapha). *Entomologia Generalis, 5*(3), 193-200.

Schelvis, J. (1997). *Remains of mites (Acari) as palaeo-ecological indicators: dwelling mounds and medieval reclamations in The Netherlands.* Paper presented at the Environment and subsistence in Medieval Europe, Brugge.

Schindel, D. E., and Miller, S. E. (2005). DNA barcoding a useful tool for taxonomists. *Nature, 435*(7038), 17-18.

Schiner, I. R. (1864). *Fauna austriaca: Die Fliegen (Diptera)* (Vol. 2). Wien: C. Gerold's Sohn.

Schmidt, E. (2012). *Vorratsschädlinge im Mitteleuropa des 5. Jahrtausends.* Paper presented at the Mitteleuropa im 5. Jahrtausend vor Christus: Beiträge zur internationalen Konferenz, Münster.

Schneeberg, K., Bauernfeind, R., and Pohl, H. (2017). Comparison of cleaning methods for delicate insect specimens for scanning electron microscopy. *Microscopy research and technique, 80*(11), 1199-1204.

Scholl, P., and Weintraub, J. (1988). Gonotrophic development in *Hypoderma lineatum* and *H. bovis* (Diptera: Oestridae), with notes on reproductive capacity. *Annals of the Entomological Society of America, 81*(2), 318-324.

Schroeder, H., Klotzbach, H., Oesterhelweg, L., and Püschel, K. (2002). Larder beetles (Coleoptera, Dermestidae) as an accelerating factor for decomposition of a human corpse. *Forensic Science International, 127*(3), 231-236.

Schuster, M., Roquin, C., Duringer, P., Brunet, M., Caugy, M., Fontugne, M., Mackaye, H. T., Vignaud, P., and Ghienne, J.-F. (2005). Holocene lake Mega-Chad palaeoshorelines from space. *Quaternary Science Reviews, 24*(16-17), 1821-1827.

Schwert, D. P. (1996). Effect of Euro–American settlement on an insect fauna: Paleontological analysis of the recent chitin record of beetles (Coleoptera) from Northeastern Iowa. *Annals of the Entomological Society of America, 89*(1), 53-63.

Schwert, D. P., Anderson, T. W., Morgan, A., Morgan, A. V., and Karrow, P. F. (1985). Changes in late Quaternary vegetation and insect communities in southwestern Ontario. *Quaternary Research, 23*(2), 205-226.

Schwert, D. P., and Ashworth, A. C. (1988). Late Quaternary history of the northern beetle fauna of North America: A synthesis of fossil and distributional evidence. *The Memoirs of the Entomological Society of Canada, 120*(144), 93-107.

Scoble, M. J. (1992). *The Lepidoptera. Form, function and diversity*. Oxford: Oxford University Press.

Setti, L., Francia, E., Pulvirenti, A., Gigliano, S., Zaccardelli, M., Pane, C., Caradonia, F., Bortolini, S., Maistrello, L., and Ronga, D. (2019). Use of black soldier fly (*Hermetia illucens* (L.), Diptera: Stratiomyidae) larvae processing residue in peat-based growing media. *Waste Management, 95*, 278-288.

Shaver, B., and Kaufman, P. (2009). Hide beetle *Dermestes maculatus* DeGeer. *Gainesville, FL: University of Florida/IFAS Extension*.

Simpson, S. J., Sword, G. A., Lorch, P. D., and Couzin, I. D. (2006). Cannibal crickets on a forced march for protein and salt. *Proceedings of the National Academy of Sciences, 103*(11), 4152-4156.

Skevington, J. H., and Dang, P. (2002). Exploring the diversity of flies (Diptera). *Biodiversity, 3*(4), 3-27.

Skidmore, P. (1985). *The biology of the Muscidae of the World* (Vol. 29). Berlin: Springer Science & Business Media.

Smith, C. I., Chamberlain, A. T., Riley, M. S., Stringer, C., and Collins, M. J. (2003). The thermal history of human fossils and the likelihood of successful DNA amplification. *Journal of human evolution, 45*(3), 203-217.

Smith, K. G. (1986). *A manual of forensic entomology*. New York: Comstok Publishing Associates.

Snyder, T. E. (1949). Catalog of the termites (Isoptera) of the World. *Smithsonian Miscellaneous Collections, 112*(1), 1-490.

Soliman, A. M., Gadallah, N. S., and Al Dhafer, H. M. (2018). An illustrated key to the Saudi Arabian species of the genus *Macroocula* Panfilov, 1954, with the description of a new species and the previously unknown female of *M. andreai* Pagliano (Hymenoptera, Bradynobaenidae, Apterogyninae). *ZooKeys*(742), 35.

Song, H. (2011). Density-dependent phase polyphenism in nonmodel locusts: A minireview. *Psyche, 2011*.

Sperling, F. A., Anderson, G. S., and Hickey, D. A. (1994). A DNA-based approach to the identification of insect species used for postmortem interval estimation. *Journal of Forensic Sciences, 39*(2), 418-427.

Stejskal, V., Hubert, J., Aulicky, R., and Kucerova, Z. (2015). Overview of present and past and pest-associated risks in stored food and feed products: European perspective. *Journal of stored products research, 64*, 122-132.

Stevens, J., and Wall, R. (2001). Genetic relationships between blowflies (Calliphoridae) of forensic importance. *Forensic Science International, 120*(1-2), 116-123.

Street, A., and Gasse, F. (1981). Recent developments in research into the Quaternary climatic history of the Sahara. In J. Allan (Ed.) *The Sahara: Ecological change and early economic history*. Boulder: Westview Press.

Strümpher, W. P. (2015). Phylogenetics of the keratin-feeding beetle family Trogidae (Coleoptera: Scarabaeoidea). *Systematic Entomology, 11*(3), 355-363.

Stueben, M., and Linsenmair, K. E. (2008). Advances in insect preparation: Bleaching, clearing and relaxing ants (Hymenoptera: Formicidae). *Myrmecological news, 12*, 15-21.

Sukontason, K. L., Ngern-Klun, R., Sripakdee, D., and Sukontason, K. (2007). Identifying fly puparia by clearing technique: Application to forensic entomology. *Parasitology Research, 101*(5), 1407-1416.

Teston, J. A., and do Correa, D. C. (2015). The Arctiini (Lepidoptera, Erebidae, Arctiinae) fauna of the Serra do Pardo National Park, Pará, Brazil. *Check List, 11*(2), 1613.

Thomsen, P. F., Elias, S., Gilbert, M. T. P., Haile, J., Munch, K., Kuzmina, S., Froese, D. G., Sher, A., Holdaway, R. N., and Willerslev, E. (2009). Non-destructive sampling of ancient insect DNA. *PloS one, 4*(4), e5048.

Timm, R. M., McLaren, S. B., and Genoways, H. H. (2020). Innovations that changed Mammalogy: dermestid beetles—the better way to clean skulls. *Journal of Mammalogy, 101*(4), 923-925.

Tirgari, S., and Paknia, O. (2005). First record of the ponerine ant *Pachycondyla sennaarensis* (Hymenoptera: Formicidae) in Iran, with notes on its ecology. *Zoology in the Middle East, 34*(1), 67-70.

Trevisan Grandi, G., Mercuri, A. M., and Cremaschi, M. (1993). Palynology and stratigraphy of deposits from the Wadi Aramas Cave (Messak Sattafet-Libyan Sahara). *Giornale Botanico Italiano, 127*(3), 696.

Tromba, G., Longo, R., Abrami, A., Arfelli, F., Astolfo, A., Bregant, P., Brun, F., Casarin, K., Chenda, V., and Dreossi, D. (2010). *The SYRMEP Beamline of Elettra: Clinical Mammography and Bio-medical Applications.* Paper presented at the AIP Conference Proceedings, College Park, Maryland.

Tuccia, F., Giordani, G., and Vanin, S. (2018). Forensic entomology: An overview. *Crime, Security and Society, 1*, 1-19.

Tuniz, C., Bernardini, F., Cicuttin, A., Crespo, M.-L., Dreossi, D., Gianoncelli, A., Mancini, L., Cuevas, A. M., Sodini, N., and Tromba, G. (2013). The ICTP-Elettra X-ray laboratory for cultural heritage and archaeology. *Nuclear Instruments and Methods in Physics Research Section A: Accelerators, Spectrometers, Detectors and Associated Equipment, 711*, 106-110.

Urbani, C. B., Bolton, B., and Ward, P. S. (1992). The internal phylogeny of ants (Hymenoptera: Formicidae). *Systematic Entomology, 17*(4), 301-329.

Uvarov, B. P. (1944). The locust plague. *Journal of Economic Entomology, 37*(1), 93-99.

Van Neer, W., Alhaique, F., Wouters, W., Dierickx, K., Gala, M., Goffette, Q., Mariani, G. S., Zerboni, A., and di Lernia, S. (2020). Aquatic fauna from the Takarkori rock shelter reveals the Holocene central Saharan climate and palaeohydrography. *PloS one, 15*(2), e0228588.

Vanin, S., and Huchet, J. (2017). Forensic Entomology and Funerary Archaeoentomology. In E. Schotsmans, N. Marquez-Grant, and S. Forbes (Eds.), *Taphonomy of Human remains: Analysis of the death and the depositional environments* (pp. 176-186). Oxford: John Wiley & Sons Ltd.

Vanin, S., Turchetto, M., Galassi, A., and Cattaneo, C. (2009). Forensic entomology and the archaeology of war. *Journal of Conflict Archaeology, 5*(1), 127-139.

Vickers, K., Bending, J., Buckland, P. C., Edwards, K. J., Hansen, S. S., and Cook, G. (2005). Toftanes: The paleoecology of a Faroese Landnám farm. *Human Ecology, 33*(5), 685.

Wiedemann, H. (2003) *Particle Accelerator Physics*. Dordrecht: Springer.

Wiemers, M., and Fiedler, K. (2007). Does the DNA barcoding gap exist? A case study in blue butterflies (Lepidoptera: Lycaenidae). *Frontiers in Zoology, 4*(1), 8.

Wiersma, A., Renssen, H., Goosse, H., and Fichefet, T. (2006). Evaluation of different freshwater forcing scenarios for the 8.2 ka BP event in a coupled climate model. *Climate Dynamics, 27*(7-8), 831-849.

Wilson, E. O. (1955). A monographic revision of the ant genus *Lasius*. *Bulletin of the museum of Comparative Zoology, Harvard, 113*, 1-201.

Wilson, E. O. (1980). Caste and division of labor in leaf-cutter ants (Hymenoptera: Formicidae: *Atta*). *Behavioral ecology and sociobiology,* 7(2), 157-165.

Wilson, E. O. (2003). Pheidole *in the New World: A dominant, hyperdiverse ant genus* (Vol. 1). Harvard: Harvard University Press.

Wilson, I. G. (1997). Inhibition and facilitation of nucleic acid amplification. *Applied and environmental microbiology, 63*(10), 3741.

Wood, D. (1987). Oestridae. *Manual of Nearctic Diptera, 2*, 1147-1158.

Woodbury, N. (2008). Infanticide avoidance by the granary weevil, *Sitophilus granarius* (L.) (Coleoptera: Curculionidae): The role of harbourage markers, oviposition markers, and egg-plugs. *Journal of Insect Behavior, 21*(2), 55-62.

Yang, D. Y., and Watt, K. (2005). Contamination controls when preparing archaeological remains for ancient DNA analysis. *Journal of Archaeological Science, 32*(3), 331-336.

Yusseff-Vanegas, S. Z., and Agnarsson, I. (2017). DNA-barcoding of forensically important blow flies (Diptera: Calliphoridae) in the Caribbean Region. *PeerJ, 5*, e3516.

Zaidi, F. H., and Chen, X. x. (2011). A preliminary survey of carrion breeding insects associated with the Eid ul Azha festival in remote Pakistan. *Forensic Science International, 209*(1-3), 186-194.

Zangheri, P. (1981). *Il naturalista esploratore, raccoglitore, preparatore, imbalsamatore*. Milan: Hoepli Editore.

Zehner, R., Amendt, J., Schütt, S., Sauer, J., Krettek, R., and Povolný, D. (2004). Genetic identification of forensically important flesh flies (Diptera: Sarcophagidae). *International Journal of Legal Medicine, 118*(4), 245-247.

Zumpt, F. (1965). *Myiasis in man and animals in the Old World. A textbook for physicians, veterinarians and zoologists*. London: Butterworths.