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NOVEL ICHNOLOGY AND THE TAPHONOMIC SIGNIFICANCE OF INSECT RELATED BIOEROSION IN BONE

By

Alexander Haig Parkinson

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DECLARATION

I declare that this thesis is my own unaided work. It is being submitted for the Degree of Doctor of Philosophy at the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at any other university.

On this the 06th Day of April 2022 in Johannesburg

ABSTRACT

Insects have been known to negatively impact the preservation of skeletal remains for over a century. In archaeological literature a large percentage of manuscripts merely note damage by insects without providing any substantive description of the observed modifications. Whilst, in palaeontology the descriptions are more comprehensive, but formal ichnotaxonomic diagnosis has been limited.

Research in this thesis is presented in the form of six peer-reviewed papers. Papers one to three utilise the ichnotaxonomic method to establish four new ichno-species, across three major continents (Africa, Asia, and South America). The species include *Cubiculum inornatus*, *Osteocallis infestans*, *Cubiculum copperi* and Munitusichnus *pascens*. Additionally, this research represents the first comprehensive descriptions of bone surface modifications by insects from the Middle Triassic of South America. In doing so pushing back the origins of unique behaviour (osteophagia) amongst insects by some 90 million years. Descriptions of bone surface modifications presented herein also represent the earliest evidence of this behaviour from the African and Asian continents, during the Early Jurassic and Middle Jurassic retrospectively. Papers three and four present the first comprehensive descriptions of Plio-Pleistocene aged bone surface modifications by insects, as well as the first reported case of insect damage on hominin skeletal remains, from The Cradle of Humankind, South Africa.

Paper five highlights the application of micro-computed tomographic imaging in differentiating between taphonomic post-mortem bone damage, and peri- or anti- mortem pathologies. The initial hypothesis proposed that bone modifications were related to post-mortem insect damage. However, an in-depth MicroCT analysis in conjunction with a differential diagnosis methodology eliminated insects as the potential causative agent, and identified the second reported instance of Osteomyelitis occurring in a Sauropodomorph dinosaur from the Lower Jurassic deposits of China. The last paper presented in this thesis provides a system of grading bone surface modifications in respect of their usefulness in the identification and differentiation of the associated causal agents. The UID Index could potentially have a broad application across several taphonomic subdisciplines.

Bone surface modifications thus represent a significant body of evidence which can contribute to a better understanding of peri-, ante- and post-mortem taphonimic processes, providing ethnological insights into the origins and dispersal patterns of ancient behaviours, identify potential instances of behavioural convergence, and have indirectly identified an elusive role player in the carrion feeding niche, during the Mesozoic.

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GLOSSARY OF TERMS & ABBREVIATIONS

Abiotic – physical rather than biological i.e. not derived from living organisms.

BSM – Bone surface modifications, term often used interchangeable with trace, trace morphology, or taphonomic signature.

Bioerosion – Bioerosion can be defined as every form of biologic penetration into hard substrates (Pirrone et al., 2014a).

Biotic – relating to or resulting from living organisms.

Causal Agent – A biotic (e.g. insects, carnivores) or abiotic (e.g. fluvial transportation, sedimentary abrasion) process responsible for a taphonomic signature.

***Cubiculum* –** The first ichnogenus diagnosed in bone (Roberts et al., 2007) and interpreted as a representation of insect pupation chambers.

- ***C. ornatus* –** First *Cubiculum* ichnospecies (Roberts et al., 2007).
- ***C. inornatus* –** Ichnotaxonomically diagnosed in Chapter 2 (Xing et al., 2015).
- ***C. levis* –** Second *Cubiculum* ichnospecies (Pirrone et al., 2014b).
- ***C. copperi* -** Ichnotaxonomically diagnosed in Chapter 4 (Parkinson, 2016).

Ethology – the science of organism behaviour.

Ichnotaxonomy – International Commission for Zoological Nomenclature in 1999 incorporated trace fossils into its formal taxonomic naming system. Ichnotaxonomy formally associates both ichnospecies and ichnogenera to unique trace fossils.

***Munitusichnus pascens* –** ichnotaxonomically diagnosed in Chapter 4.

Neotaphonomic ichnology – A term coined in this research which refers to a multidisciplinary method which harnesses the accumulated knowledge and experience from three distinctive but complimentary bodies of knowledge; namely taphonomy, ichnology and neo-taphonomic experimentation.

***Osteocallis* –** An ichnogenus diagnosed in bone (Roberts et al., 2007) and interpreted as a representation of insect gnawing/feeding.

***O. mandibulus* –** First Osteocallis ichnospecies (Roberts et al., 2007).

***O. infestans* –** Second Osteocallis ichnospecies (Neto et al., 2016).

Osteophagia – The behaviour associated to the consumption of bone, its use in the text does not imply eating of bone for nutritional benefits, but more specifically the destruction of bone associated to several different behaviours.

Trace – a shortened version of trace fossil, in the text often used interchangeably with taphonomic signature, or bone surface modification (BSM).

Trace Morphology – specific reference to the shape of a trace e.g. ellipsoidal, circular, oval, etc

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CHAPTER SEVEN: MODERN BONE MODIFICATIONS BY DERMESTES MACULATUS AND CRITERIA FOR THE RECOGNITION OF DERMESTID TRACES IN THE FOSSIL RECORD

INTRODUCTION

This thesis by publication presents six published manuscripts that relate to bone surface modification in the archaeological and palaeontological record, dominantly associated with modifications produced by insects, observed as trace fossils on fossilised bone. The study of such evidence in the fossil record is largely referred to today as ichnology, a sub-field of Taphonomy. Within these papers several substantial contributions to this field are made, and several novel trace fossils were identified and described. Included in this body of work are also novel developments of standards, methods and visualisation that contribute to the greater body of taphonomic studies. This substantial body of work emphasises the importance of a broad based multi-disciplinary understanding and approach to the study of insect damage to bone. All citations are as of date of first submission.

1.1 The General Science of Taphonomy

The term 'Taphonomy' was first coined in 1940 by I.A. Efremov who defined it as "the study of the transition (in all its detail) of animal remains from the biosphere to the lithosphere" but soon after plants were also incorporated into the definition (Lyman, 2010, Efremov, 1940). Taphonomy has its origins in the field of palaeontology in 1940 but its development as a unique branch of science only gained traction during the 1970's, and by the middle of the 1980's taphonomy was broadly recognised as an independently developing branch of scientific investigation (Lyman, 2010, Pickering et al., 2007). During the early developmental stages of taphonomic thinking emphasis was placed on information loss because of taphonomic processes. Nowadays, taphonomy is broadly recognised for its contributions in understanding the diversity of biological causal agents and/or abiotic processes which have impacted on assemblages. Taphonomic methods and knowledge are currently applied across a broad spectrum of subdisciplines within the fields of archaeology, palaeontology and physical anthropology. Its application in physical anthropology has largely focussed on its utilisation within medicolegal investigations (Pokines and Symes, 2013, Haglund and Sorg, 1997, Gifford, 1981, Behrensmeyer et al., 2000, Denys, 2002, Martínez-Delclòs et al., 2004, Fernández-Jalvo et al., 2011, Waller Jr, 2012, Sohn et al., 2015).

The primary aims of taphonomic research, in respect of bone, is the identification of the causal agent or physical process which resulted in the surface modifications. The identification of the associated casual agents is based on inference drawn by analogy which is conceptually

underpinned by uniformitarianism (Andrews, 1995, Behrensmeyer et al., 2000, Brain, 1981, Domínguez-Rodrigo et al., 2011, Fernández-Jalvo et al., 2011, Kusmer, 1990, Pokines and Symes, 2013). The history of uniformitarianism and its conceptual limitation and/or benefits has been debated for several decades (Baker, 1998, Bottjer et al., 1995, Cannon, 1961, Erwin, 2011, Gould, 1965, Krynine, 1956, Montgomery, 1987, Scott, 1963, Shea, 1982). A number of historical reviews relating to taphonomic developments as well as topics of ongoing debate within this field are available (Lyman, 2010, Pickering et al., 2007, Andrews, 1995, Haglund and Sorg, 1997, Behrensmeyer et al., 2000, Denys, 2002, Lyman, 2004, Domínguez-Rodrigo et al., 2011, Fernández-Jalvo et al., 2011, Pokines and Symes, 2013) and this is not discussed in more detail here.

Taphonomic research has often focussed on identifying the causal agents or physical processes which have resulted in bone surface modifications (BSM). Often direct evidence of the causal agents or abiotic processes to which skeletal remains have been exposed are not physically preserved in the archaeological or palaeontological records (Fernandez-Lopez, 1991). Therefore, taphonomic studies contribute additional data to research efforts despite the actual taphonomic processes themselves being inherently destructive. A thorough understanding of BSM enables the reconstruction of a comprehensive sequence of peri- and/or postmortem processes. The history of individual bones / skeletons ultimately culminates in a better understanding of site formation processes as well as the total taphonomic history of the deposit / assemblage under investigation (Fernandez-Jalvo and Andrews, 2016). The taphonomic procedure begins with the identification of the BSM on individual bones / skeletons. Modification types are established and described. A taphonomic history is then compiled based on available data. The individual histories of the bones / skeletons are then interpreted in respect of the entire assemblage, producing a more rigorous and comprehensive reconstruction of site formation processes (Fernandez-Jalvo and Andrews, 2016).

Bone surface modifications can either be a result of a living-organism, or else can be a result of a physical non-organic abiotic process. The best way to explain the complexity of interaction between biotic and abiotic taphonomic processes is by providing a detailed sequence of events to which a hypothetical corpse is exposed post-mortem. The following scenario is posited;

1. A large herbivore dies of natural causes at the side of a river.
2. Flies and other insect soon arrive and begin to feed on the soft tissue.

3. Over a few days, vultures, a jackal, and a lion feed on the corpse.
4. A flash flood sweeps the remains into the river, and it is transported some 100 km downstream.
5. After redeposition at the side of the riverbank, a leopard transports a portion of the remains inside of a dolomitic cave to feed.
6. Eventually the remains completely skeletonise and are buried.
7. The bones become fossilised within a dolomitic cave.
8. Two million years later a palaeontologist finds the deposits.
9. The bones are removed from encasing breccia using a mechanical preparation technique.
10. Finally, a taphonomic analysis begins.

The above scenario presents a sequence of events during which a myriad of biotic and abiotic taphonomic processes have affected the skeletal remains post-mortem. Many of the processes posited above could result in BSM, whilst other taphonomic processes may result in the destruction thereof. The above scenario merely seeks to highlight the potential biological agents and abiotic processes to which skeletal remains may be exposed. A very brief overview of potential taphonomic processes is presented below but is by no means comprehensive. Rather it seeks to highlight the taphonomic complexity to which a single carcass can be exposed postmortem. Insects arrives within minutes after death, but it is unlikely that insects will leave traces on the bones during the early stages of decomposition. It is more widely accepted that the insects modify bone during the later stages of decomposition. The subsequent flurry of carnivore activity may result in BSM, disarticulation and dispersal. The signatures of the BSM between the various carnivores may be difficult to differentiate. The transportation of skeletal remains in the river presents the first major array of abiotic processes. Fluvial transportation may result in a wide range of BSM, dispersal and/or disarticulation, as well as fracturing of the bones due to impact damage. Fluvial transportation could also result in the removal of BSM and/or other taphonomic signatures pre-existing on the bones prior to transportation. Post-mortem the skeletal remains would have been exposed to variable climatic conditions and weathering of the bones may have taken place during various stages of exposure. Final deposition within a cave and subsequent burial of the remains could result in sediment loading prompting deformation or fracturing, BSM due to microbial activity, bone fracturing because of cave collapse, and BSM due to exposure to subterranean insect activity. Finally damage to the bones during mechanical preparation may results in erroneous identification of other processes. (McGee, 2010, Müller et al., 2011, Turner-Walker, 2008, Brain, 1981, Hill, 1976, Hill, 1980, Wylie et al., 1987, Byrd and Castner, 2009,

Haglund and Sorg, 1997, Haglund and Sorg, 2001, Kulshrestha and Satpathy, 2001, Pokines and Symes, 2013, Blumenschine et al., 2007, Egeland, 2008, Faith and Behrensmeyer, 2006, Pante and Blumenschine, 2010, Pante et al., 2012, Richardson, 1980, Fernandez-Jalvo and Andrews, 2016, Voorhies, 1969, Myers et al., 1980, Hill and Behrensmeyer, 1984, Fernández-Jalvo and Andrews, 2003, Fernández-Jalvo and Monfort, 2008, Wiest et al., 2018, Behrensmeyer, 1978, Lyman and Fox, 1989, Clarke, 2019).

1.2 The Importance of Insects

Terrestrial insects are a highly successful group of organisms. Insects can be found in almost every corner of the terrestrial realm from subpolar to extreme deserts conditions. Insects occupy many hyper specialised niches in our ecosystems for example; bees are prolific pollinators, termites and ants are recognised as soil producing agents, while flies and some beetles play a critical role in the decomposition of terrestrial organism's post-mortem (Misof et al., 2014, Matthews and Matthews, 2009, Foottit and Adler, 2009, Labandeira and Sepkoski, 1993, Gullan and Cranston, 2010, Capinera, 2008).

Historically it has proven difficult to morphologically map the phylogenetic inter-relationships between genera or families within *Insecta*. The difficulties largely relate to the limited nature of the body fossil records for key groups or during key time intervals. However, recent advancements in genetics and gene sequencing work have provided a new method to map evolutionary interrelationships as well as to establish estimated dates for the origins and divergence of key groups within this taxonomic class. (Misof et al., 2014, Labandeira and Eble, 2005, Labandeira and Sepkoski, 1993) However, physical morphological change only represents one component in evolutionary history. Understanding key behavioural innovations is of equal importance (Wilf et al., 2006, Sohn et al., 2015, Labandeira et al., 2002, Labandeira, 2006, Labandeira, 2005).

Scientists often draw on proxy data to map behavioural innovations, an example of which, would be the study of bio erosional traces / taphonomic modifications to bones produced by insects in a terrestrial environment. Osteophagia amongst insects has thus attracted the attention of various groups of scientists from entomologists, forensic pathologists to archaeologists and palaeontologists (Xing et al., 2015, Neto et al., 2016, Backwell et al., 2012, Pokines and Symes, 2013, Byrd and Castner, 2010, Wylie et al., 1987, Huchet et al., 2009, Pittoni, 2009, Huchet et al.,

2013, Pirrone et al., 2014b, Saneyoshi et al., 2011, Kirkland and Bader, 2010, Bader et al., 2009, Queiroz et al., 2017, Plug, 2004, Van Schalkwyk and Moifatswane, 2000, Plug and Pistorius, 1999, Pistorius and Steyn, 1995, Plug, 1993). Therefore, several scientific disciplines have either directly or indirectly generated data relating to the way in which specific groups of insects modify bone. Due largely to the temporal and geographic longevity and distribution of this unique behaviour three distinctive bodies of knowledge have been created and can thus be drawn upon to better understand and interpret traces or taphonomic signatures identified in the palaeontological and archaeological records. These bodies are knowledge are associated to the following branches of scientific investigation; taphonomy, ichnology and neo-taphonomic experimentation. The objectives and focus of these complementary but distinctive branches of science are often disparate, and thus research within these branches often develop in isolation to one another. (Gianechini and de Valais, 2015, Pirrone et al., 2014b, Saneyoshi et al., 2011, Cabral et al., 2011, Kirkland and Bader, 2010, Bader et al., 2009, Britt et al., 2008, Roberts et al., 2007, Csiki, 2006, Hasiotis, 2004, Paik, 2000, Rogers, 1992, Havlik et al., 2014, Backwell et al., 2012, Pomi and Tonni, 2011, Pittoni, 2009, Fejfar and Kaiser, 2005, Laudet and Antoine, 2004, Kaiser, 2000, Martin and West, 1995, Kitching, 1980, Tobien, 1965, Zanetti et al., 2015, Zanetti et al., 2014, Parkinson, 2013, Holden et al., 2013, Abdel-Maksoud et al., 2011, Fernández-Jalvo and Monfort, 2008, Freymann et al., 2007, Deyrup et al., 2005, Roberts et al., 2003, Watson and Abbey, 1986)

Taphonomic investigations have tended to focus on causal agent identification to reconstruct the sequence of taphonomic events which resulted in the formation of the deposit. Causal agent identification in relation to insect bone damage has specifically been suggested to inform on environmental or climatic conditions (Backwell et al., 2012, Fejfar and Kaiser, 2005, Kaiser, 2000, Martin and West, 1995). However, more broadly speaking other taphonomic branches of investigation (e.g. bone weathering, fluvial transportation, etc) have been utilised across both archaeological and palaeontological studies. However, research in relation to insect damage to bone often develops within these branches of science in relative isolation.

In contrast to taphonomic focus, ichnologists tend to actively discourage discussions of causal agency but rather focus discussions on how the traces can inform upon ancient behaviour (Seilacher, 2007, Genise and Chubut, Buatois et al., 1998, Bertling et al., 2006, Pirrone et al., 2014a). In fact, ichnological investigations are well suited to identifying key evolutionary developments and behavioural innovations which have taken place in the past, evidence of which

may otherwise remain elusive in the fossil record (Wilf et al., 2006, Sohn et al., 2015, Labandeira and Eble, 2005, Labandeira, 2005, Buatois and Mángano, 2018, Zherikhin, 2003, Seilacher, 1967). Bone is by no means the only substrate on which traces have been studied to understand the palaeobiology of ancient insects. Comprehensive studies of insect-plant interactions (particularly insect trace damage to leaves) has contributed substantially to understanding of niche partitioning, niche specialisation, changes in functional feeding groups, as well as the impact of large extinction events on insect diversity in the past (Gastaldo et al., 2005, Labandeira, 1997, Labandeira, 2005, Labandeira, 2006, Labandeira et al., 2002, Labandeira and Sepkoski, 1993, Wilf et al., 2006).

An extensive body of published data exists in relation to the ways in which terrestrial invertebrates, particularly insects, modify hard tissue or bone. Most studies where motivated by the desire to describe and interpret anomalous taphonomic signatures or bioerosional traces identified on skeletal remains from diverse contexts. The impact insects have on skeletal remains was first reported over a century ago in association with mummified human remains from Egypt (Derry, 1911) but it was only during the 1970's and 1980's that descriptions of this nature became more frequent. During this time reports were largely restricted to archaeological sites (Haglund-Calley, 1976, Wood, 1976, Watson and Abbey, 1986) and Cenozoic aged deposits (Kubiak and Zakrzewska, 1974, Kitching, 1980, Denys, 1986, Brink, 1987, Gentry, 1987, Hill et al., 1987, Thenius, 1988) but by the 90's reports began to surface of insect damage to bone from continental deposits dating from Iron Age deposits of Southern Africa and extending into Mesozoic aged deposits (Rogers, 1992, Jerzykiewicz et al., 1993, Fastovsky et al., 1997, Hasiotis et al., 1999, Schwanke and Kellner, 1999, Plug, 2004, Van Schalkwyk and Moifatswane, 2000, Plug and Pistorius, 1999, Pistorius and Steyn, 1995, Plug, 1993). Early studies adopted a taphonomic approach and largely focused on causal agent identification with the aims of inferring palaeoclimatic and palaeoenvironmental conditions based on the identification of the causal agent.

A seminal study adopting a neo-taphonomic approach was undertaken by Watson & Abbey (1986). The aims of the study were to use controlled actualistic experiments to document the taphonomic signatures of various species of Australian termites and the ways in which they modify bone. The ultimate objective of this study was to identify the causal agent of bone damage observed in aboriginal cave burials (Watson and Abbey, 1986). In the proceeding decades an increasing number of neo-taphonomic / neo-ichnological studies have been conducted to expand

on this body of knowledge (Watson and Abbey, 1986, Freymann et al., 2007, Fernández-Jalvo and Monfort, 2008, Abdel-Maksoud et al., 2011, Backwell et al., 2012, Holden et al., 2013, Zanetti et al., 2014, Zanetti et al., 2015). The vast majority of work has largely adopted taphonomic methods and objectives but since the turn of the millennium a number of studies have moved away from a taphonomic approach but rather adopted an ichnological approach which involves the formal diagnosis of ichnotaxa, as well as reconstructing the behaviour of the associated organism (Pirrone et al., 2014b, Serrano-Brañas et al., 2018, Roberts et al., 2007, Xing et al., 2015, Parkinson, 2016, Neto et al., 2016).

1.3 Ichnotaxonomy

Ichnotaxonomy is used to identify and describe morphologically distinctive trace fossils. Traces are diagnosed independently of any assumed or associate biological species. Ichnotaxa are given a genus and species rank by ichno-taxonomists. Thus, ichnotaxonomy develops independently of an association to a biological species as described with in an alpha-taxonomic framework. Alpha-taxonomy seeks to identify and describe new biologically distinctive species. Whilst Beta-taxonomy deals with the inter-relatedness of genera and species and seeks to map the evolutionary inter-relationships of biological organisms. (Genise and Edwards, 2003, Lockley, 2007, Minter et al., 2007, Kim et al., 2008, Bromley, 2012, Hopner and Bertling, 2017)

1.4 The Publications Contributing to this Thesis

The candidate's research in this field, as highlighted in the multi-disciplinary papers presented in this thesis, thus seeks to draw from ichnological, taphonomic and experimental data to better understand and interpret damage to bones produced by insects in the terrestrial environments. It also involves itself with the science of ichnotaxonomy in describing newly observed traces.

This body of work submitted for examination for the fulfilment of a Doctor of Philosophy by publication at the University of the Witwatersrand, makes a significant and ongoing contribution to the field of bone modification by insects, insect related bioerosion in bone and adds to the general knowledge of science by assisting in the prevention of the misidentification and misattribution of bone modification in the fossil and modern record by offering clear descriptions and visualisation of ichnotaxa and insect related bioerosion on bone. Materials and methods are described within each publication.

The five published papers and one submitted paper presented below present a significant addition to the science of ichnology in two broad temporal periods that cover the Mesozoic and the Pliocene to the Quaternary. They include the following contributions of particular significance:

- Describes four novel ichnotaxa (Xing et al., 2016; Parkinson, 2016), two of which are described in the sole-authored paper Parkinson (2016).
- Identifies and describes two previously unreported geographical and temporal instances of ichnotaxa in the Mesozoic record (Neto et al, 2016).
- Describes evidence for the oldest feeding traces by insects in the fossil record (Xing et al., 2016).
- Describes the first insect frass and insect coprolites on fossil bone in the Plio-Pleistocene record of Africa (Parkinson, 2016).
- Identifies and describes the first ichno trace fossils on hominid remains from the important hominid-bearing fossil site of Sterkfontein in South Africa (Odes et al., 2016)
- Ensured ichno trace fossils were not mis-identified as other types of bone modification (Odes et al, 2016 and Xing et al., 2018).
- Set a standard for the identification of *Dermestus maculatus* damage through extended experimental work (Parkinson, submitted).
- Provides a basic set of criteria to differentiate between Dermestid and Termite bone modifications in the fossil record (Parkinson, submitted).
- Demonstrates the benefits of classifying bone modifications according to their usefulness in identification and differentiation. Thus, establishing the conceptual framework for the UID index system.

1.4.1 – Paper 1:

XING, L., PARKINSON, A. H., RAN, H., PIRRONE, C. A., ROBERTS, E. M., ZHANG, J., BURNS, M. E., WANG, T. & CHOINIERE, J. 2015. The earliest fossil evidence of bone boring by terrestrial invertebrates, examples from China and South Africa. *Historical Biology*, 1-10.

Candidate author position – 2nd author

Candidate contribution – Dr Xing approached the candidate in 2014 and provided him with photos of traces identified on the skeletal remains of a *Chuanjiesaurus sp.* dinosaur from the Middle Jurassic, Chuanjie Formation, Yunnan Province, China. The candidate formulated the data collection methodology to enable the description and analysis of a potentially new ichnospecies. The candidate was assisted by Dr Ran who took photographs of the specimens as well as provided the measurement data of the traces. Dr Xing provided the geological context for the Chinese specimen. The candidate spent several weeks examining similarly aged fossil specimens held at the Evolutionary Studies Institute at the University of Witwatersrand to find traces of a similar age in a South African context. The candidate found an isolated prosauropodamorph fibula from the Upper Jurassic Elliot Formation, Karoo Basin South Africa. Dr Choiniere provided the geological context for the South African specimen. The South African specimen underwent a test scan using MicroCT technologies, but unfortunately the density between bone and the sedimentary infill of the traces was insufficiently distinct to enable worthwhile 3D rendering and volumetric analysis. Therefore, the specimen was mechanically prepared used an air scribe. The candidate invited Dr Roberts onto the project as a matter of courtesy as he was responsible for the description of the first ichnospecies of the genus *Cubiculum*. The candidate also needed to be conscious of other traces being described and studied at the time to ensure the taxonomic and descriptive independence of work being done on the Chinese specimens. These concurrent studies involved specimens received by the candidate from Mr Neto in Brazil, as well as independent work being conducted by Dr Pirrone on a new species of *Cubiculum* from Argentina. The candidate was solely tasked with compiling the first draft of the manuscript as well as providing the full descriptions, analysis, and interpretation of the traces from both South African and Chinese. The candidate wrote the entire manuscript except for section 2 and was thus given the second author position. It was always known to the candidate that Dr Xing would be the

primary author for providing the specimens. Dr Zhang, Dr Burns, Dr Wang were all invited onto the manuscript by Dr Xing as a matter of courtesy as they had been involved in the excavation and curation of the Chinese specimens included in this study. The candidate handled the submission and review process.

Journal impact factor – 2.023

Citations - 17

Paper significance and impact - Xing et al., 2015 summarises both the temporal and geographic distribution of reports of insect bone interactions during the Mesozoic. It presents the oldest evidence of such interactions from both the African and Asian continents. It provides a systematic ichnological description of a new trace species, *Cubiculum inornatus* isp. nov. as well as a description of other more generalised traces which remain unassigned to existing ichnotaxa. It motivates the establishment of a new ichnospecies due to the unique morphological characteristics of the traces. Morphological differences between the associated traces are explained in terms of incipient stages of production. *Cubiculum inornatus* is associated to the production of pupation chambers by an unknown terrestrial insect. It briefly discusses the potential bias to interpretation because of taphonomic processes. It also highlights how morphological features can inform on behaviour as well as the nature of the mouth part of the causal agent. The paper briefly discusses the palaeo-geographic implications of the findings. It also highlights how both discussions and conclusions can be limited by small sample sizes as well as by morphologically simplistic traces.

1.4.2 – Paper 2:

Neto, V. D. P., Parkinson, A. H., Pretto, F. A., Soares, M. B., Schwanke, C., Schultz, C. L., & Kellner, A. W. (2016). Oldest evidence of osteophagic behavior by insects from the Triassic of Brazil. *Palaeogeography, Palaeoclimatology, Palaeoecology*, *453*, 30-41.

Candidate author position – 2nd author

Candidate contribution – In 2014 Dr Pretto & Dr Soares contacted the candidate in relation to traces discovered by one of their honours students (Mr Neto) on dinosaur bone found in a continental setting from the Middle and Upper Triassic Santa Maria Supersequence, Paraná Basin, Brazil. Due to the candidates' previous and ongoing research on trace fossils from the Mesozoic and given the limited work at that time on trace fossils of such great age, the candidates previous experience and expertise was recognised as critical to the analyses of these ancient traces. The methods the candidate had developed for the Chinese studies for documenting, recording and then analysing the trace fossils was applied to the traces described in this study. The candidate provided the method and data collection framework which enabled Mr Neto to provide the candidate with the needed information and photographic evidence to take the study forward and determine whether the establishment of new ichnospecies were in fact warranted. Therefore, the candidate was heavily involved in the advancement of these methods and was then tasked with conducting both the documentation and comparative analyses of the traces which formed the backbone of the results presented in this important paper. However, the candidate's contributions were not only limited to the technical development and improvement of the analytical techniques, but he was also solely responsible for the ichnotaxonomic diagnosis, interpretation of the results of these analyses and formulation of the discussion. In recognition of this contribution the candidate was made second author on the paper. Mr Neto created a very basic draft of the manuscript and thus given the first author position. Dr Schwanke, Dr Schultz and Dr Kellner were invited onto the paper as a matter of courtesy by Mr Neto's supervisors, but these authors only provided comments on the completed manuscript prior to submission. It was agreed that Mr Neto would handle the submission process, whilst the candidate facilitated any changes suggested post review.

Journal impact factor – 3.22

Citations - 19

Paper significance and impact - Neto et al. 2016 summarises the 22 existing ichno-taxa identified in bone and breaks down the nature of the occurrences in terms of ecological setting (i.e. marine or terrestrial) and associated mechanism of creation (i.e. vertebrate tooth marks or insect damage). It also breaks down the ichno-taxa specifically attributed to terrestrial insects and the associated distribution of the traces across different substrate (i.e. bone or

wood). This study represents the first ichnological study to use non-destructive 3D imaging and visualisation software to provide descriptions of traces which go beyond the external bone surface. It describes seven trace morphologies identified on bones from the Middle to Upper Triassic of Brazil. This study documents previously unreported instances of both *Cubiculum inornatus* and *Osteocallis mandibulus*. Thus, extending both the temporal and geographic ranges of these ichnotaxa into the Upper Triassic of Brazil. Importantly, the study provided a systematic ichnological description of a new ichno-taxa *Osteocallis infestans* isp. nov. which is identified as a feeding trace. The new evidence presented once again pushes back the temporal range of this unique behaviour amongst terrestrial invertebrates. It also brings into questions whether discussions relating to the most inferred causal agents (Dermestid beetles and termites) are justified. The trace's reported in this study clearly pre-date the accepted origins of both groups of insects. Therefore, no attempt is made to infer a causal agent. Rather discussions focus on ethological interpretations as well as reconstruction of post-mortem processes gleaned from the limited taphonomic evidence available. The study highlights once again how limited data samples and morphological simplicity of traces can limit inference.

1.4.3 – Paper 3

Parkinson, Alexander H. "Traces of insect activity at Cooper's D fossil site (Cradle of Humankind, South Africa)." *Ichnos* 23, no. 3-4 (2016): 322-339.

Candidate author position – Sole Author

Candidate contribution – As the candidate's expertise evolved based on work on much earlier ichno traces from the Mesozoic, it became of interest to apply the same methods to the study of traces he observed on fossils from much younger Plio-Pleistocene aged deposit at the Coopers site in South Africa. While methods applied to earlier trace fossils from the Plio-Pleistocene challenges were faced in that the preservation of the bones studied was substantially different from those found in the Mesozoic. Therefore, the methods had to be modified and developed by the candidate to suit this study. Additionally, the candidate developed several new methods of visual presentation of the finds. In addition, the candidate applied for the first time to Plio-Pleistocene aged traces from South African sites distribution models that added to the taphonomic understanding of site formation. While many of the

methods applied in this paper were not “new” to the field, they were novel in their application under these circumstances and thus were significant in advancing the science.

Journal impact factor – 1.469

Citations - 11

Paper significance and impact - Parkinson 2016 summarises the previously reported traces/taphonomic signatures on bone attributed to insects from terrestrial deposits in Southern and Eastern Africa over the last 3 million years. More specifically it describes a total of nine morphologically disparate traces from the Pleistocene of South Africa. It provides systematic ichnological descriptions for two new ichno-taxa namely; *Munitusichnus pascens* nov. isp. & *Cubiculum cooperi* nov. isp. Additionally, this study recorded the first instance of preserved insect frass and insect coprolite on bone. This study uses several novel methods to neo-taphonomic ichnological studies which visually present the information to the reader. First, the distribution of the modifications across a generalised quadruped skeleton giving a visual indication of the limited nature of their distribution and indicating how the distribution relates to bone density. Furthermore, the spatial distribution of the modifications across the fossil site was also presented and this was used to formulate a new and testable hypothesis pertaining to the site formation processes. The research discusses the benefits and/or limitations of inferring a specific causal agent/s. Lastly, it makes several ethological inferences based on the data. One of these inferences is that *Munitusichnus pascens* is believed not to represent a single behaviour but rather a portion of the life cycle of an unknown beetle. In summary this research has adopted a multi-disciplinary approach to the interpretation of the traces identified at Copper’s D. The research harnesses experience drawn from three distinctive bodies of knowledge; taphonomy, ichnology and neo-taphonomic experimentation. In doing so it provides the most comprehensive description and interpretation of traces in bone attributes to insects from terrestrial deposits.

1.4.4 – Paper 4

ODES, E., PARKINSON, A. H., RANDOLPH-QUINNEY, P. S., BERGER, L. R. & ZIPFEL, B. 2016. Osteopathology and insect modifications in the *Australopithecus africanus* StW 431 skeleton using micro-computed tomography South African Journal of Science.

Candidate author position – 2nd author

Candidate contribution – This paper began initially as a collaborative effort to study what was thought to be solely osteopathologies on the fossilised bones of an important hominid skeleton from Sterkfontein, South Africa as part of the thesis work on osteopathologies of the lead author. The candidate was asked to participate in this project due to his expertise on trace fossil morphologies when trace markings were unidentifiable by the primary research team and the candidate was able to identify them as ichno trace fossils. This work was clearly secondary to the primary aim of the paper but formed an important contribution to not only this study, but the novel identification of clear ichno traces on hominid remains from Sterkfontein. It also demonstrated the importance of the candidate's expertise in this area and prevented the misidentification of ichno traces as pathologies. The candidate also assisted in the application of recording and describing not only these traces, but his methods were of recording, describing and visualising trace fossils contributed to the visualisation and description of the pathologies also found on the skeleton. The candidate contributed further in writing the ichno trace aspects of the discussion and of general editing of the remainder of the manuscript. Important discussions relating to the ways in which gross general morphology either contributes to, or inhibits, ichno-taxonomic description, taphonomic reconstruction or the identification of the associated causal agent are made in this paper by the candidate that are separate to the contribution of Odes and the other authors whose expertise related to Osteopathology and general taphonomy. This paper highlights the importance of a multi-disciplinary approach to the study of fossils where it was demonstrated that two separate fields of study – that of Osteopathology and Ichnology can together contribute to a better and more complete scientific outcome.

Journal impact factor – 2.197

Citations - 4

Paper significance and impact – Odes *et al.* 2016 presents a palaeo-pathological description, but more relevant to this thesis, it presents a taphonomic description of post-mortem damage to StW431 because of an unknown insect. StW431 comprises a partial skeleton attributed to *Australopithecus africanus* recovered from the Sterkfontein Cave system during the late 1980's. Three-dimensional non-destructive imaging and visualisation methods were applied to the skeleton to differentiate between pathological and post-mortem modifications of hard tissue. Two disparate trace morphologies attributed to insect damage are described and were identified by the candidate. These traces represent the first ichno trace fossils noted on hominid remains from Sterkfontein. As noted, important discussions relating to the ways in which gross general morphology either contributes to, or inhibits, ichno-taxonomic description, taphonomic reconstruction or the identification of the associated causal agent are made in this paper.

1.4.5 – Paper 5

Xing, Lida, Bruce M. Rothschild, Patrick S. Randolph-Quinney, Yi Wang, Alexander H. Parkinson, and Hao Ran. "Possible bite-induced abscess and osteomyelitis in Lufengosaurus (Dinosauria: sauropodomorph) from the Lower Jurassic of the Yimen Basin, China." Scientific reports 8, no. 1 (2018): 1-8.

Candidate author position – 5th author

Candidate contribution – The candidate was approached by Dr Xing in 2016 in relation to bone damage identified on a Lufengosaurus huenei dinosaur and was invited to undertake the analysis. Initial analysis suggested the damage was not related to post-mortem insect activities but rather ante- or peri- mortem pathology. It was thus proposed the specimen be analysed by means of high-resolution imaging using micro-computed tomography (Micro-CT). The candidate then assisted in reconstruction and interpretation of the micro CT volume, in generating the 2D orthoslices as well as the 3D surface reconstruction which enabled the differential diagnosis to be undertaken. The candidate undertook histological feature

identification from the micro CT data in collaboration with co-author Randolph-Quinney, and assisted in the differential diagnosis. The candidate was solely responsible for the production of the composite plates from photographic, orthoslices and three-dimensional volume data. Lastly, the candidate assisted in the analysis and final editing of the manuscript pre- and post-review.

Journal impact factor – 4.379

Citations - 12

Paper significance and impact – Paper significance and impact – Xing et al. 2018 describes pathological lesions on the rib of a Jurassic sauropod dinosaur, Lufengosaurus huenei, investigated using micro-CT scans. By application of adapted clinical and palaeopathological criteria, the lesion was shown to have formed and partially healed in the lifetime of the animal. The analysis concluded that a bone infection (osteomyelitis) was the most likely cause of the lesion. This is the second instance of osteomyelitis being recognised in a sauropodomorph dinosaur.

Xing et al. 2018 use the above determinations to assess the possibility that the lesion was caused by a puncture or bite caused by tooth or claw, and further examine contemporaneous theropods to try to identify possible attackers (concluding that Sinosaurus sp. was a possibility, but also noting that exact identification of the attacker is unnecessary). This culminates in an enhanced understanding of the complexities of dinosaur palaeo-immunology, behavioural and life history information for dinosaurs, as well as ecology from a taphonomic assessment of bone damage.

1.4.6 – Paper 6

Modern bone modification by *Dermestes maculatus* and criteria for the recognition of dermestid traces in the fossil record. *Historical Biology*, (2022), 1-13.

Candidate author position – Sole Author

Candidate contribution – The experimental trials were conducted as part of the candidates MSc research. However, the proposed system of classifying modifications in terms of their "usefulness in identification and differentiation" UID index is a novel concept which was conceptualised during the candidates PhD research. The manuscript suggests that all insect related modifications should be classified into three broad categories, UID 1 to UID3. An UID 1 rating should only be given to modifications which are sufficiently distinctive to facilitate identification of the trace maker and enable differentiation from other trace types, or traces made by other insects. Whilst UID 2 ratings should be given to traces which when found in isolation are not sufficiently distinctive but when found to co-occur with UID 1 modification types can be referred to a potential causal agent. Lastly modifications attributed to UID 3 suggests that the modification have a limited scope in their application in identification or differentiation of an associated causal agent to the genus level. This system has wide scope of application not only for insect damage to bones but potentially across many subdisciplines of taphonomy. The manuscript also provides a basic summary of differences between Dermestid versus Termite damage to bone. Providing criteria for differentiation in the fossil record. The establishment of the UID rating system is a culmination of the years of research in this field, and provides a precautionary model for interpreting insect modifications and exactly what inferences should be drawn from such discoveries.

Journal impact factor – 2.023

Citations - n/a

Paper significance and impact – This manuscript is the culmination of the candidates focused independent work on attempting to further define the trace evidence of the important contributor to the ichno record by *Dermestes*. *Dermestes* are one of the most inferred agents of bone damage in the fossil record due to their known involvement in the carrion reduction process. They also have a widespread geographical distribution and have been used for decades in laboratory work for defleshing corpses. This paper provides detailed descriptions of the bone modifications produced by Dermestids under experimental conditions. The manuscript briefly compares dermestid modifications to those of termites, and provides a basic set of criteria to differentiate between these the modifications in the fossil record. The manuscript also highlights the disparity between modifications attributed to dermestids in the fossil record and modifications produced by dermestids under experimental conditions. The

candidate suggests that more focused research is desperately needed, but that ultimately a reinterpretation of the fossil record is advisable.

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CHAPTER 2 (PAPER 1)

THE EARLIEST FOSSIL EVIDENCE OF BONE BORING BY TERRESTRIAL INVERTEBRATES, EXAMPLES FROM CHINA AND SOUTH AFRICA.

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The earliest fossil evidence of bone boring by terrestrial invertebrates, examples from China and South Africa

Lida Xing[a], Alexander H. Parkinson[b,c], Hao Ran[d], Cecilia A. Pirrone[e], Eric M. Roberts[f], Jianping Zhang[a], Michael E. Burns[g], Tao Wang[h] and Jonah Choiniere[c]

[a]School of the Earth Sciences and Resources, China University of Geosciences, Beijing, China; [b]School of Geosciences, University of the Witwatersrand, Johannesburg, South Africa; [c]Evolutionary Studies Institute and DST-NRF Centre of Excellence in Palaeosciences, University of the Witwatersrand, Johannesburg, South Africa; [d]Key Laboratory of Ecology of Rare and Endangered Species and Environmental Protection, Guangxi Normal University, Ministry of Education, Guilin, China; [e]IANIGLA – CCT – CONICET – Mendoza, Mendoza, Argentina; [f]Department of Earth and Oceans, James Cook University, Townsville, Australia; [g]Department of Biological Sciences, University of Alberta, Edmonton, Canada; [h]Lufeng Land and Resources Bureau, Lufeng, China

ABSTRACT

We report the oldest fossil evidence of osteophagia by terrestrial invertebrates on both the Asian and African continents. Bones attributable to the Middle Jurassic dinosaur *Chuanjiesaurus* (Dinosauria: Sauropoda) were found with post-mortem insect modification in the Chuanjie Formation, Yunnan Province, China. The morphology of the borings closely matches the ichnogenus *Cubiculum*. Based on the lack of bioglyphs observed in *Cubiculum ornatus,* a new ichnospecies is proposed here. The new trace fossil, *Cubiculum inornatus* isp. nov., is interpreted to have been constructed for pupation by an unknown taxon of insect. Additionally, we report even older borings from Early Jurassic dinosaur bones of the Elliott Formation in the Karoo Basin, which represent the second oldest occurrence of insect traces in bone from continental settings. Both trace fossils sites have palaeogeographic implications for the origins and dispersal of osteophagia amongst terrestrial invertebrates during the Mesozoic. These discoveries push back the antiquity of pupation in animal bones by more than 100 million years to the Middle Jurassic, indicating that this behaviour, and osteophagy more generally, originated early in the Mesozoic, roughly comparable with the origination of insect pupation in woody substrates (Late Triassic).

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1. Introduction

Insects are a highly diverse group of organisms that originated during the Early Ordovician roughly 479 million years ago (Misof et al. 2014). Yet, because of their generally limited fossil record, information about the evolution of insects and their behaviour remains limited. Recently though, significant breakthroughs in understanding the timing and relationships among, the certain insect clades have been made due to advances in molecular phylogenetics and dating (Misof et al. 2014). However, capturing evidence of the timing of major behavioural innovations and advances is more difficult. Here, the fossil and rock record plays an important role in understanding and reconstructing the timing of development of certain behaviours. In particular, the study of borings in fossil bones has proven to be a valuable new avenue of research in paleobiology; both in marine and continental ecosystems. The wealth of readily accessible vertebrate fossils housed in museum collections provides an important archive of information for investigating insect behaviours. In a similar vein study of traces of plant–insect interactions (particular trace damage to leaves) has proven to be a valuable proxy for understanding insect evolution and behaviour. Such studies have greatly expanded our understanding of functional feeding groups, niche portioning, and insect diversity across major extinction events (Labandeira 1997, 2005, 2006; Labandeira et al. 2002; Wilf et al. 2006). Thus, comprehensive comparative studies of insect–bone interactions can substantially contribute to our understanding of the carrion-dependent invertebrate communities though time.

Insects are the most regularly inferred macro-bioeroders of bone in continental settings. Osteophagia by modern insects was reported in the early twentieth century (Derry 1911), but this behaviour is by no means modern, as putative insect traces have been reported in bones from as far back as the Triassic (Schwanke and Kellner 1999). However, there appears to be a significant increase in the abundance of bone borings reported in fossil bones from the Late Jurassic (Hasiotis et al. 1999; Chin and Bishop 2006; Britt et al. 2008; Bader et al. 2009) and Cretaceous (Rogers 1992; Paik 2000; Nolte et al. 2004; Roberts et al. 2007; Kirkland and Bader 2010; Cabral et al. 2011; Saneyoshi et al. 2011; Pirrone, Buatois and González Riga 2014b; Gianechini and De Valais 2015).

CONTACT Alexander H. Parkinson palaeoparky@gmail.com

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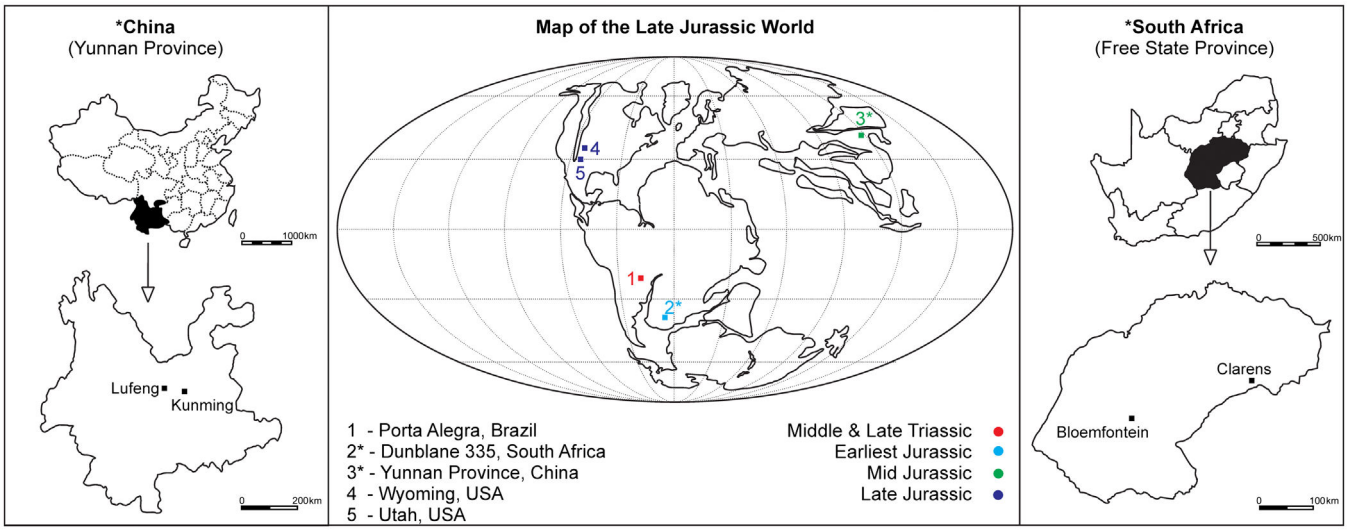

Figure 1. Geographic map showing the locations of the fossil localities described in this study, as well as a global distribution map indicating the location of reports of osteophagia by terrestrial invertebrates by the end of the Jurassic Period.

Reports from Asia are restricted to Cretaceous-aged deposits in Korea (Paik 2000) and Mongolia (Kirkland and Bader 2010; Saneyoshi et al. 2011; Fanti et al. 2012). However, Xing et al. (2013) recently reported insect-generated traces associated with dinosaur bones from the Lower Jurassic of China. Interestingly, they report only minor surface damage to the bones which appears to be incidental, associated with the construction of shelter tubes around a dinosaur carcass. Reports of insect damage to bone on the African continent are restricted to fossil localities dating from the last 3 million years (Kitching 1980; Newman 1993; Kaiser 2000; Val et al. 2015). A number of reports come from hominin-bearing sites within the Cradle of Humankind, namely Malapa Cave (Val et al. 2015), Swartkrans (Newman 1993) and Sterkfontein (Pickering 1999), whilst others from South Africa are attributed to either Late Stone Age or Middle Stone Age sites (Backwell et al. 2012) as well as the fossil sites of Florisbad and Makapansgat Member 3 (Kitching 1980, Brink 1987). A single report comes from Laetoli (Tanzania) (Kaiser 2000) and the last from Porc Epic Cave (Ethiopia) (Backwell et al. 2012).

Traces in bone produced by insects primarily fall into eight general morphological categories including grooves, striae, pits, bores, tubes, chambers, furrows or channels (Pirrone, Buatois and Bromley 2014a). An increasing number of empirical studies reveal a diversity of modern insects that modify bone including dermestids (Fernández-Jalvo and Monfort 2008; Zanetti et al. 2014), tenebrionids (Holden et al. 2013), clerids (Zanetti et al. 2015) and termites (Watson and Abbey 1986; Backwell et al. 2012). However, in a palaeontological context, the identification of the responsible agent is difficult to ascertain. Neoichnological research is critical to the identification of particular causal agents, which can provide valuable palaeoecologic, palaeoenvironment and palaeoclimatic insights (Genise et al. 2004; Seilacher 2007; Backwell et al. 2012; Huchet et al. 2013). However, beyond documenting fossil trace markers, trace fossils represent a key for establishing the timing of certain behaviours in ancient organisms. Thus, the study of bone modifications by insects has developed into an important branch of ichnology. The establishment of ichnotaxa for these traces is a relatively recent endeavour and to date only three ichnospecies having been diagnosed – *Osteocallis mandibulus* (Roberts et al. 2007), *Cubiculum ornatus* (Roberts et al. 2007) and *Cubiculum levis* (Pirrone, Buatois and González Riga 2014b). These ichnotaxa are interpreted as being representative of either, feeding traces (*O. mandibulus*) or pupation chambers (*C. ornatus* & *C. levis*). An examination of the geographic distribution of reports of bone borings by terrestrial invertebrates suggests that by the Late Jurassic this behaviour was relatively wide spread (Figure 1). Here, we extend the temporal range of this behaviour by reporting the oldest evidence of insects pupating in bone from the Asian continent, and in doing so push back the range of *Cubiculum* to the Jurassic of China. Additionally, we provide the oldest reported evidence of insect–bone interactions from Africa.

2. Geologic settings and vertebrate fauna

2.1. Chuanjie Formation, Yunnan, China

The Red Beds of the Lufeng Series, in the Lufeng Basin, are approximately 750 m thick, and are conventionally divided into upper and lower units (Bien 1941). An Early Jurassic age was proposed for the Lower Lufeng Formation and a Middle Jurassic age for the Upper Lufeng Formation (Sheng et al. 1962). Fang et al. (2000) assigned strata that had at various times been included in the Upper Lufeng Formation to the Chuanjie, Laoluocun, Madishan and Anning formations (Fang et al. 2000). Based on stratigraphic correlation and invertebrate fossils, such as charophyta, ostracodes and bivalves, the Chuanjie Formation can be attributed to lacustrine deposition (Cheng et al. 2004). The climate of Middle Jurassic of the Sichuan–Yunnan region is estimated as subtropical warm-moist to semiarid (Wang et al. 2008; Li et al. 2011; Sekiya 2011). In the Chuanjie Formation, the Chuanjie bone bed is the only horizon that has produced skeletal material of dinosaurs. The bone bed has yielded material from at least four individuals of the large sauropod *Chuanjiesaurus anaensis* (Fang et al. 2000; Sekiya 2011), the theropod *Shidaisaurus jinae* (Currie et al. 2009) and a variety of turtles such as *Xinjiangchelys* (Tong et al. 2015).

2.2. Elliot Formation, Karoo Basin, South Africa

The Elliot Formation is a package of predominantly fluvially derived sedimentary rocks that crop out in Lesotho, Free State and Eastern Cape Provinces of South Africa (Haughton 1921; Kitching and Raath 1984; Smith and Kitching 1997; Bordy et al. 2004). The Elliot Formation was deposited during the last interval of development of the Karoo Basin (Catuneanu et al. 1998; Catuneanu et al. 2005), and estimates of its age range between latest Triassic and Early Jurassic (Kitching and Raath 1984; Olsen and Galton 1984; Smith and Kitching 1997). Aeolian sandstones and lacustrine shales can also be found within these typical terrestrial red beds, which are renowned for their preservation of dinosaur fossils, as well as other Mesozoic terrestrial vertebrates (Haughton 1921; Olsen and Galton 1984; Yates and Kitching 2003; Yates 2005; Yates et al. 2010). The 'middle Elliot Formation' does not have a formal lithostratigraphic definition, but it was recognised by Kitching & Raath (1984) as being deposited in a fluvial channel and floodplain environment, with the upper portions of the section having extensive pedogenic horizons with occasional subaerial depositional environments. The middle Elliot was extensively reviewed by Smith and Kitching (1997), who found widespread pedogenic nodule conglomerates in middle Elliot Strata, which they interpreted as evidence for a large-scale deflation surface in the north-western part of the Karoo Basin in the Early Jurassic. They reported a rich fauna of vertebrates from these middle Elliot layers, typified by the derived therapsid *Tritylodon*, and they proposed a formally defined '*Tritylodon* Acme Zone' to biostratigraphically recognise the specificity of the middle Elliot Fauna. However, Bordy et al. (2004) was unable to find widespread evidence of a lithologically characteristic *Tritylodon* Acme Zone in their work in the southern part of the basin and in Lesotho, and they considered the middle Elliot strata to be part of the upper Elliot Formation.

3. Materials and methods

Institutional abbreviations and acronyms. ZLJ = Lufeng Dinosaur Museum of World Dinosaur Valley Park, China. BPI = Bernhard Price Institute for Palaeontological Research now referred to as the ESI (Evolutionary Studies Institute, University of the Witwatersrand, South Africa).

In 1995, the skeleton ZLJ0121 was discovered and collected by Lufeng Dinosaur Museum from the Chuanjie Formation in Lufeng County (GPS: 24°58′20.84″N, 102° 4′34.31″E), Yunnan Province, China (Figure 1). ZLJ0121 belongs to *Chuanjiesaurus anaensis* (Fang et al. 2000; Sekiya 2011). *Chuanjiesaurus anaensis* was originally considered a member of Cetiosauridae (Fang et al. 2000), but was later moved into Mamenchisauridea (Sekiya 2011). The ZLJ0121 materials included six articulated caudal vertebrae, catalogued as ZLJ0121-1-6. All the caudal vertebrae of ZLJ0121 are procoelous. There are no pleurocoels in the proximal caudal centra. The ventral surface of the proximal caudal centra is convex transversely. Their morphology is consistent with the description for the first to tenth caudal centra of *C. anaensis* (Sekiya 2011). Therefore, the ZLJ0121 materials are interpreted as belonging to the anterior caudal vertebrae. The caudal vertebrae (ZLJ0121) do not display pathology. Traces were only recorded on two of the six caudal vertebrae (ZLJ0121-3 and ZLJ0121-4) (Figure 2).

BPI-1-4268 is an isolated prosauropodamorph fibula collected in 1971 by James Kitching in the Caledon Park area, Dunblane 335. Dunblane 335 is approximately 1–2 km SW of the town of Clarens in the Eastern Free State, South Africa (Figure 1). The farm consists mainly of Drakensberg volcanic or Clarens sandstone, with only a thin strip of Elliot Formation in the southern half of the farm. The collections record for BPI-1-4268 states that the specimen is from the 'Middle Elliot Formation'. Thus BPI-1-4268 is associated to the Triassic-Jurassic boundary and is Rhaetian-Hettangian in age (Olsen and Galton 1984).

The following measurements were taken for all traces described in this study: length, width and depth. Length and width measurements were obtained using calipers, whilst depth was measured from the deepest to most superficial point on the cast of the traces also using digital caliper. All measurements are presented in mm.

4. Results

4.1. Systematic ichnology

Ichnogenus *Cubiculum* Roberts et al. 2007.

Type Ichnospecies: Cubiculum ornatus Roberts et al. 2007.

Emended diagnosis: Discrete ellipsoidal borings in bone. Hollow, oval borings bored into inner spongy and outer cortical bone surfaces. Boring length two to five times greater than its diameter. Structures may be isolated but may form dense, locally overlapping concentrations.

Remarks: Emendation of the ichnogeneric diagnosis is considered necessary to extend the size range whilst disassociating the wall (flank) profiles from the ichnogenus. The proportions of length two to five times greater than its diameter will allow for broader inclusion of chamber-shaped morphologies. Wall (flank) profiles are considered an ichnotaxobase at the ichnospecies level. The occurrence of bioglyphs as well as their pattern of occurrence (Pirrone, Buatois and Bromley 2014a) are also considered an ichnotaxobase at an ichnospecies level.

Cubiculum inornatus isp nov.

Holotype: Specimen ZLJ0121-T1 on the caudal vertebrae ZLJ0121-4 of *Chuanjiesaurus anaensis* from Middle Jurassic Chuanjie Formation of Yunnan, China (Figure 3(A)).

Referred specimens: One complete (T1) and three partially completed (T2, T3, T4) specimens (ZLJ0121-T-1-4) on two caudal vertebrae (ZLJ0121-3 and ZLJ0121-4) of *Chuanjiesaurus anaensis* from Middle Jurassic Chuanjie Formation of Yunnan, China (Figure 3(A)–(C)).

Etymology: Latin '*in*' meaning 'not', '*ornatus*' meaning 'decorated'.

Diagnosis: Ellipsoidal borings embedded in cortical bone penetrating into trabecular bone. The borings display a length width ratio ranging from 2:1 and 5:1 and slightly tapers towards one end. The walls run perpendicular to the bone surface, whilst the base of the boring is rounded. No filling or bioglyphs are present.

Description: All of the specimens are borings embedded in cortical and trabecular bone with a length 2–5 times that of its associated width. All the chambers have a rounded bottom (not flat), and the interior walls of the trace are smooth and do not record bioglyphs. The holotype (T1) measures 47.6 mm long and is 9.6 mm wide and displays a near perfect ellipsoidal morphology.

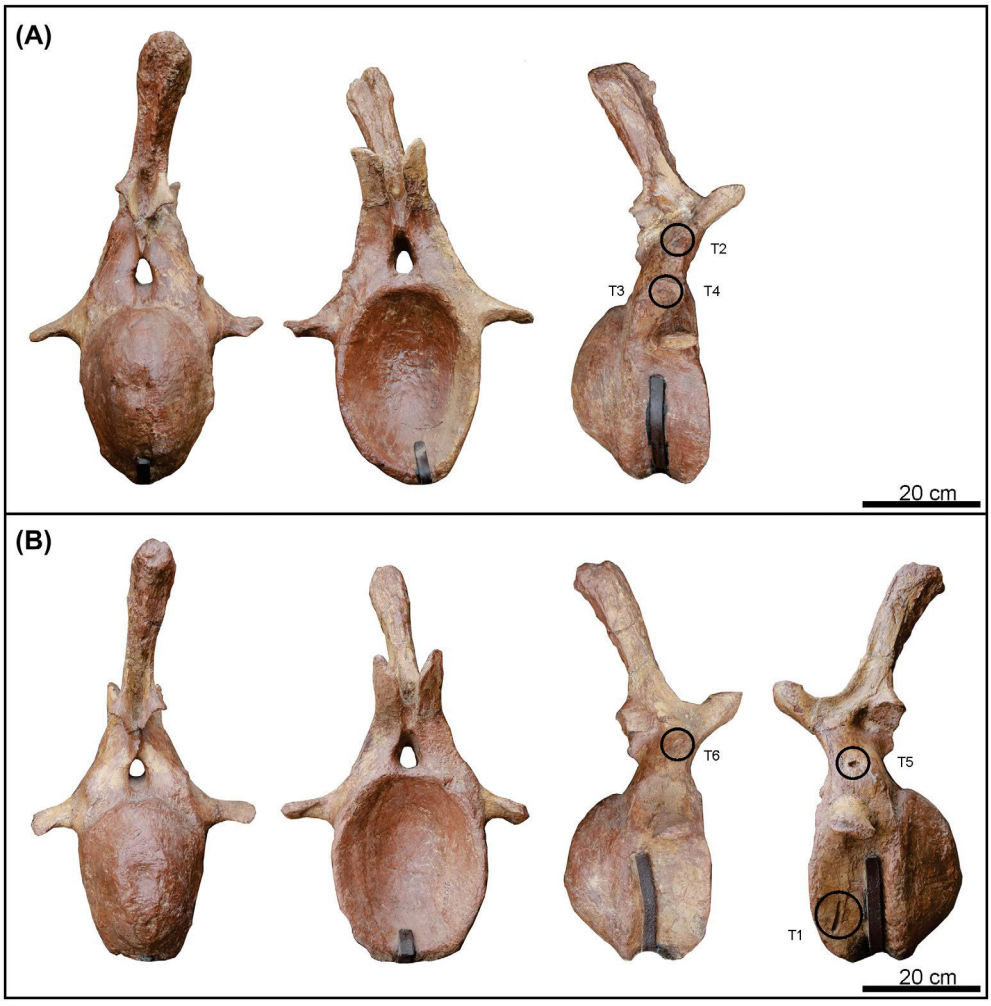

Figure 2. Photographs of two caudal vertebrae (ZLJ0121-3 and ZLJ0121-4) of *Chuanjiesaurus anaensis* recovered from World Dinosaur Valley Park, Chuanjie Formation, Yunnan, China. Circles indicate the position of the traces identified on the two vertebra. T1 is the holotype of *Cubiculum inornatus* isp. nov. T2, T3, T4 incomplete specimens of *C. inornatus*, and T5, T6 are interpreted as feeding traces.

Table 1. Measurements of insect traces from Chuanjie bone bed, Yunnan Province, China (ZLJ) and Elliot Formation, South Africa (BPI).

Trace type	Specimen number	Maximum diameter (mm)	Minimum diameter (mm)	Maximum depth (mm)	Figure reference
Cubiculum inornatus	ZLJ0121-T1	47.6	9.6	7.4	3A
	ZLJ0121-T2	29.1	7.8	8.2	3B
	ZLJ0121-T3	12	4.9	7.2	3C
	ZLJ0121-T4	16.4	5.7	7.2	3C
Hole	ZLJ0121-T5	15.1	12.1	9.2	3D
Hole	ZLJ0121-T6	20.3	8.8	7.5	3E
Hole	BPI-1-4268-T1	13.12	11.45	12.5	4B
Hole	BPI-1-4268-T2	10.6	–	–	4B
Tube – entrance	BPI-1-4268-T3.1	6.7	7.1	12.8	4C
Tube – exit	BPI-1-4268-T3.2	9.8	9.3	19.4	4C

Trace 2 (Figure 3(B)) measures 29.1 mm long and 7.8 mm wide, Trace 3 measures 12 mm long and 4.9 mm wide. Lastly, Trace 4 measures (Figure 3(C)) 16.4 mm long and 5.7 mm wide (Table 1). Their extremes intersect, so their major axis form a 45° angle.

4.2. Other trace descriptions

4.2.1. China

Two other traces (T5 and T6) were identified in association with *Cubiculum inornatus* but their unique morphology enables differentiation from *C. inornatus*. The maximum and minimum diameters of T5 (Figure 3(D)) are similar but the resultant morphology is irregular, whilst the minimum diameter of the trace well exceeds the minimum diameter of *C. inornatus*. Neither the walls nor the bottom of this trace are smooth (unlike that of *C. inornatus*). This trace can be differentiated from other Mesozoic- and Cenozoic-aged bite traces as reported in the literature (Mikuláš et al. 2006; Hone and Tanke 2015). Trace 6 has a lens-shaped morphology, marked constriction on both ends and a maximum diameter of close to 9 mm at its centre (Figure 3(E)). Lastly, the base of T6 is flat and runs near parallel to the bone surface which is unlike the well-rounded bottom of *C. inornatus*.

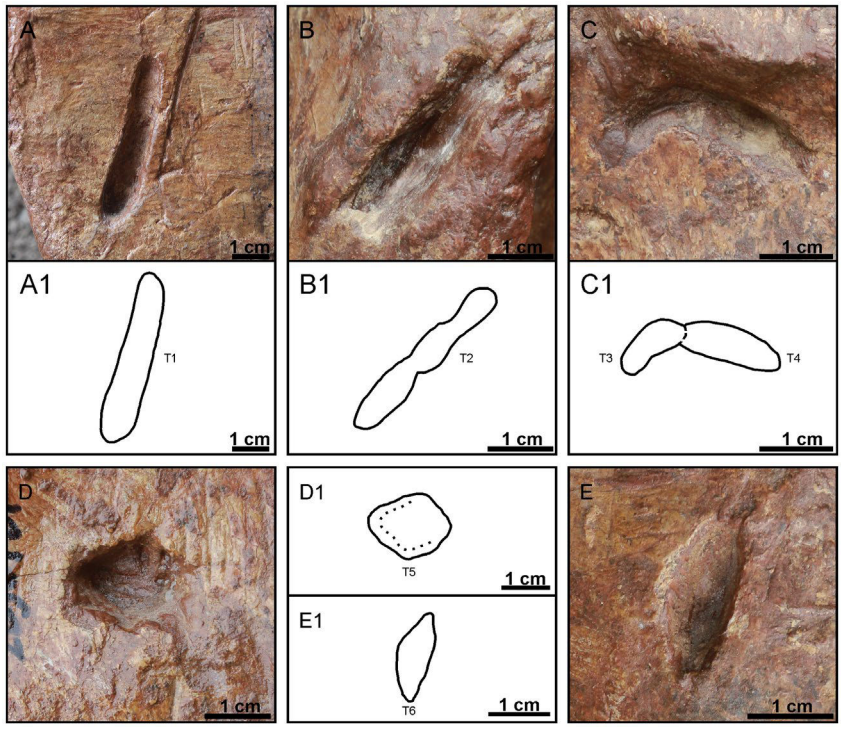

Figure 3. Photographs and schematics of invertebrate traces from World Dinosaur Valley Park, Chuanjie Formation, Yunnan Province, China (A and A1) Holotype of *Cubiculum inornatus* isp. nov. (B, B1, C, and C1) incomplete specimens of *C. inornatus* isp. nov. (D, D1, E, and E1) Feedings traces found in association with *C. inornatus* isp. nov.

4.2.2. South Africa

BPI-1-4268 is an isolated fibula displaying damage on both the proximal and distal ends (Figure 4(A)). Two borings have been excavated into the proximal end of the long bone (Figure 4(B)). The first boring (T1) has a maximum diameter of 13 mm with a minimum diameter of 11.45 mm. In surface view, the hole has an almost circular morphology and penetrates the bone at a slightly oblique angle relative to the bone surface, to a depth of 12.5 mm. The walls of the boring are smooth and straight, whilst the base is slightly rounded. The boring has been extended by excavating a channel in the wall of the boring towards the most distal margin of the fibula. The boring does not penetrate through the bone. The second boring (T2) on the proximal end of the fibula penetrates the bone (Figure 4(B)), has a maximum diameter of 10.6 mm and a near circular morphology. The most proximal margin of the boring has not been preserved and during preparation a small amount of infill was left *in situ*. The distal portion of the fibula shows a higher degree of modification in comparison with the proximal end. A tube entrance visible on the bone surface (Figure 4(C), T3.2), travels into the bone for close to 20 mm, then changes direction and travels a further 13 mm, then terminates at the contact region with the area where the bone has been completely destroyed (Figure 4(C), T3.1). A schematic drawing of this tube is presented in Figure 4(C)1. Measurement data is presented in Table 1.

5. Discussion

Cubiculum inornatus displays marked differences when compared to all other diagnosed *Cubiculum* ichnospecies including *C. ornatus* (Roberts et al. 2007) and *C. levis* (Pirrone, Buatois and González Riga 2014b): *Cubiculum inornatus* may fall within the reported size range of *C. ornatus* (Roberts et al. 2007); however, the absence of concave walls (flanks) and bioglyphs is what differentiates the species. Similarly, *Cubiculum inornatus* is distinguishable from *C. levis* (Pirrone, Buatois and González Riga 2014b) due to the lack of pronounced concavity of the walls (flanks) and constriction of the entrance which gives this species its characteristic bowl-shaped morphology.

The four specimens of *C. inornatus* display both size and minor morphological variation which is common for other *Cubiculum* ichnospecies (Roberts et al. 2007; Pirrone, Buatois and González Riga, 2014b). However, all specimens of *C. inornatus* maintain a gross ellipsoidal morphology. Generally, size variation is most regularly attributed to the variable presence of soft/desiccated tissue during construction (Martin and West 1995; Hasiotis et al. 1999; Bader et al. 2009; Kirkland and Bader 2010; Huchet et al. 2013), but morphological variation has previously been explained in terms of incipient stages of construction (Britt et al. 2008). We propose that *C. inornatus* holotype (Figure 3(A)) is reflective of the final stage of construction, whilst the remaining specimens (Figure 3(B)–(C)) are merely representative of incipient stages of construction. Minimal exposure to the trace makers would account for the limited number of traces on the two vertebra and the absence of traces on the four remaining vertebrae. The vertebrae were isolated but articulated when recovered from the lacustrine sediments (Cheng et al. 2004) of the Chuanjie Formation. It is hypothesised that the *Chuanjiesaurus anaensis* skeleton had reached the early dry stage of decomposition and that subsequent transportation is the most likely process which resulted in the disassociation of the rest of the skeleton from the recovered vertebrae. However, remnant ligaments and desiccated

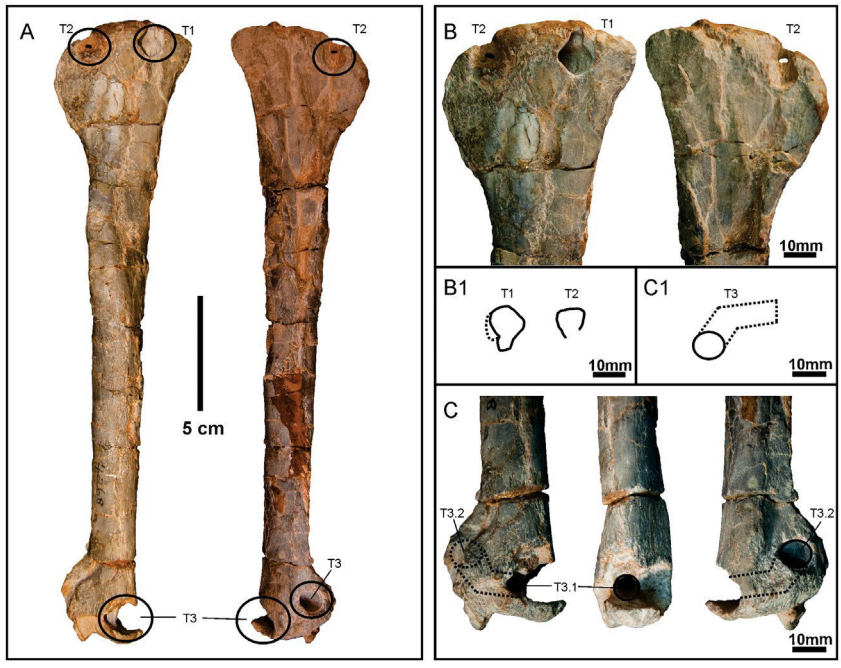

Figure 4. Photographs and schematics of the traces identified on the fibula of a prosauropodamorph from the Elliot Formation of South Africa.
Note: (A) Macro view of the fibula indicating the position of the traces (B and B1) Holes identified on the proximal end (C and C1) Tube identified on the distal end.

tissue kept the six vertebrae in articulation prior to burial. The *C. anaensis* could have either been washed down stream then deposited in the paleo-lake, else may have died along the lake shore. Unfortunately, there is insufficient evidence to support either hypothesis.

The gross morphology of *C. inornatus* is consistent with other traces that have been interpreted as pupal chambers (Laudet and Antoine 2004; Roberts et al. 2007; Britt et al. 2008; Bader et al. 2009; Huchet et al. 2013; Xing et al. 2013; Pirrone, Buatois and González Riga, 2014b). *Cubiculum inornatus* is also interpreted as pupal chambers and thus its inclusion in the *Cubiculum* ichnogenus. The description of *C. inornatus* from the Middle Jurassic of Asia represents the oldest evidence of this behaviour by terrestrial invertebrates suggesting that this behaviour first originated very early in the evolutionary history of insects and possibly in Asia. Currently, all the representatives of *Cubiculum* fall to traces in bone produced by terrestrial invertebrates.

The disassociation of bioglyphs from the diagnosis for the ichnogenus is well motivated as the presence of bioglyphs and their associated pattern of occurrence may be a key diagnostic feature which could enable differentiation of *Cubiculum* ichnospecies and/or trace markers. It has been suggested that mouth part morphology is relatively consistent within the same species of insects at the varying stages of development (Labandeira 1997; Grimaldi and Engel 2005; Britt et al. 2008), with some exceptions (Eggleton 2011; Gotoh et al. 2011), and that the boring behaviour is consistent between multiple individual of the same species (Matthews and Matthews 2009). Therefore, consistency in behaviour and mouth part morphology could then be used as a basis for differentiation of different species or groups of trace makers. Additionally, the pattern of occurrence and placement of bioglyphs could further elucidate the behavioural process of construction. For example, the presence of bioglyphs only at the bottom of a chamber would suggest a vertical approach to excavation, whilst bioglyphs on both the side walls and bottom would suggest a vertical approach followed by a horizontal expansion of the chamber. In the case of *C. inornatus,* the absence of bioglyphs is equally as informative as this suggests that the mouth parts of the agent where capable of excavating bone, but perhaps not hard enough to leave sufficiently deep bioglyphs to enable their preservation. However other factors such as the nature of the bone as well as preservation bias also need to be considered, as experimentation has shown that bioglyphs are infrequently discernable when insects modify cancellous bone (Backwell et al. 2012; Parkinson 2013) and shallow bioglyphs could easily be destroyed by a diversity of other taphonomic processes, such as sedimentary abrasion, or natural bone deterioration due to burial conditions.

The traces identified on the South Africa specimen are interpreted as feeding traces, which is the behaviour best supported by experimental data (Watson and Abbey 1986; Fernández-Jalvo and Monfort 2008; Backwell et al. 2012; Holden et al. 2013). On the distal portion of the fibula, it is unclear which modification event took place first, but the entrance of the tunnel in the area of bone destruction is well preserved, which could suggest that bone removal took place first, followed by the invertebrate boring into the bone. The abrupt change in direction of the tube supports the involvement of a biological agent as this would not be expected if the damage was pathological. Additionally, it is unclear whether the tube changes direction or records false branching as a result of accidental intersection (D'alessandro and Bromley 1987; Pirrone, Buatois and Bromley 2014a). However, the involvement of different individuals is consistent with the size difference between the two entrances. The entrance on the bone

surface is the largest measuring 9.8 × 9.3 mm, whilst the entrance inside the area of destroyed bone measures only 6.7 × 7.1 mm. These traces appear broadly similar to those described from the Two Medicines Formation from the Upper Cretaceous deposits in north-western Montana (Rogers 1992). However, the South African borings have smaller diameters in comparison, whilst the small sample sizes from both localities and the morphological simplicity of the traces limit comprehensive comparison.

5.1. Potential causal agents

At present, known insect–bone modifiers include termites (Isoptera), some species of ants (Hymenoptera), tineid moths (Lepidoptera), beetles of the families Dermestidae and Cleridae (Coleoptera) and mayflies (Ephemeroptera) (Watson and Abbey 1986; Deyrup et al. 2005; Freymann et al. 2007; Fernández-Jalvo and Monfort 2008; Abdel-Maksoud et al. 2011; Backwell et al. 2012; Holden et al. 2013; Parkinson 2013; Zanetti et al. 2014; Zanetti et al. 2015). Ants and termites are social insects that construct complex nest systems without isolated pupal chambers (Wilson 1971; Ran 2014), whilst may fly larvae live in water and create U-shaped borings (Britt et al. 2008; Xing et al. 2013). Tineid moths utilise the keratin sheath of horns for the purposes of pupation, thus associated bone damage is primarily restricted to the inner boney horn core (Mccorquodale 1898; Walsingham 1898; Hill 1975; Hill et al. 1987; Deyrup et al. 2005). None of these match the morphology of the traces described in this study.

The most likely trace maker would be a member of the order of Coleoptera. The most common beetle trace maker proposed is members of the dermestidae (Rogers 1992; Chin and Bishop 2006; Roberts et al. 2007; Britt et al. 2008; Bader et al. 2009). However, this inference is always approached with a degree of caution, with only one report proposing a particular species (*Dermestes maculatus*) (Britt et al. 2008). Dermestids body fossils have been reported from the Late Triassic (Dunstan 1923), but their dating is controversial (Kiselyova and Mchugh 2006; Kadej and Háva 2011). It is more widely accepted that the earliest body fossil of a basal dermestid appears only during the Late Cretaceous. In light of the body fossil record, it is thus unlikely that a member of the dermestidae family is responsible for the modifications identified in the Jurassic of China and South Africa. Furthermore, there is a paucity of experimental/observational data which would support dermestids as a potential agent of pupation chambers in bone (Haynes 1993; Fernández-Jalvo and Monfort 2008; Holden et al. 2013; Parkinson 2013).

The trace maker would have been long and thin based on the morphology and size of the chamber (Huchet et al. 2013). The occurrence of the traces clearly indicates that the trace maker was able to modify bone, but the lack of bioglyphs may suggest an inability to producing sufficiently deep bioglyphs which would enable their preservation. In a modern context, many scavenger insects are involved at the onset of decomposition of a carcass, but they are less likely to pupate in or on the dead body due to other insects preying on them during this stage (Kulshrestha and Satpathy 2001; Amendt et al. 2010; Byrd and Castner 2010). During the later stages of decomposition, the abundance of scavenger beetles greatly diminishes (Kulshrestha and Satpathy 2001; Amendt et al. 2010; Byrd and Castner 2010), which may then prompt insects to pupate on the remaining carcass and possibly excavate bone. A lack of associated feeding traces suggests that the agent was not feeding on the bone but merely explored the bone as a protective pupation substrate (Haynes 1993; Holden et al. 2013).

Unfortunately, due to the sample size and morphological simplicity of the traces on the South African specimen, any attempt to infer a causal agent would merely be speculative. Nonetheless, what is clear is that in Southern Africa by the earliest Jurassic, an invertebrate agent had developed mouth parts which were capable of modifying bone. The restriction of modifications to the proximal and distal ends of the long bones suggests that the agent was able to modify less dense areas of long bones but was perhaps unable to modify thicker cortical bone, but this requires further testing.

5.2. Palaeogeographic implications

The earliest report of osteophagia by insects in a continental setting date from the Middle and Upper Triassic aged deposits of Brazil (Schwanke and Kellner 1999), whilst previous to this study other Mesozoic reports were restricted to the Upper Jurassic deposits of North America (Hasiotis et al. 1999; Britt et al. 2008; Bader et al. 2009) and by the Cretaceous reach a more global distribution (Rogers 1992; Paik 2000; Kirkland and Bader 2010; Cabral et al. 2011; Fanti et al. 2012; Pirrone, Buatois and González Riga, 2014b; Gianechini and De Valais, 2015). South Africa and China now have the second and third oldest reported cases of osteophagia by terrestrial invertebrates. Evidence indicates that this behavioural tendency had already evolved in South Africa by the Earliest Jurassic and in Asia by the Middle Jurassic (Figure 1). The description of *Cubiculum inornatus* demonstrates that the behavioural tendency of constructing pupal chambers in bone possibly first evolved in Asia but was more widespread by the end of the Cretaceous as *Cubiculum* sp. have been described from Western Argentina (Pirrone, Buatois and González Riga, 2014b), Utah, and Madagascar (Roberts et al. 2007). The morphological disparity across the described species of *Cubiculum* likely demonstrates convergent evolution of the necessary mechanisms to facilitate bone modification, as well as the associated behaviour of pupating in bone. Unfortunately, little is known about the carrion dependency of invertebrate communities during the Mesozoic and evidence of this dynamic niche specialisation will likely remain elusive.

6. Conclusions

Cubiculum inornatus represents the oldest evidence of pupation in bone by insects in a continental setting and represents some of the oldest examples of osteophagy from the fossil record. The traces identified on the South African specimen shows that insect–bone interactions on the modern African continent are not restricted to the last 4 million years, but in fact have ancient roots dating back some 190 million years. Our research demonstrates that insects were key players in carrion-dependent invertebrate communities during the Mesozoic, and that within a relatively short period of time after the origins of this behaviour, this niche was occupied by multiple species across several continents. The associated ages of the modifications draws into

question which particular insect produced the traces, as the most regularly inferred agents in existing literature only appear during the Late Cretaceous. To elucidate the origins of bone boring, a systematic approach to examining fossil collections is necessary to further our understanding of the origins and dispersal patterns of this behaviour, and the number of causal agents associated with decomposition during the Mesozoic.

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ORCID

Alexander H. Parkinson http://orcid.org/0000-0001-9067-7266

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CHAPTER 3 (PAPER 2)

OLDEST EVIDENCE OF OSTEOPHAGIC BEHAVIOUR BY INSECTS FROM THE TRIASSIC OF BRAZIL

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Oldest evidence of osteophagic behavior by insects from the Triassic of Brazil

Voltaire D. Paes Neto [a,*], Alexander H. Parkinson [b,c], Flávio A. Pretto [a], Marina B. Soares [a], Cibele Schwanke [d], Cesar L. Schultz [a], Alexander W. Kellner [e]

[a] Instituto de Geociências, Universidade Federal do Rio Grande do Sul, Porto Alegre, Rio Grande do Sul State, Brazil
[b] School of Geosciences, University of the Witwatersrand, Private Bag 3, Wits, 2050, South Africa
[c] Evolutionary Studies Institute and DST-NRF Centre of Excellence in Palaeosciences, University of the Witwatersrand, Private Bag 3, Wits, 2050, South Africa
[d] Instituto Federal de Educação, Ciência e Tecnologia do Rio Grande do Sul, Campus Porto Alegre, Porto Alegre, Rio Grande do Sul State, Brazil
[e] Laboratory of Systematics and Taphonomy of Fossil Vertebrates, Department of Geology and Paleontology, Museu Nacional, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil.

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ABSTRACT

This study reports the earliest known evidence of osteophagy by invertebrates in a continental setting from the Middle and Upper Triassic Santa Maria Supersequence, Paraná Basin, Brazil. Samples from the *Dinodontosaurus* Assemblage Zone (Pinheiros-Chiniquá Sequence – Middle Triassic) and the *Hyperodapedon* Assemblage Zone (Candelária Sequence – Upper Triassic) were analyzed and a number of trace morphologies were found, including tubes, holes and channels. We report the occurrence of *Cubiculum inornatus* from the Middle Triassic and *Osteocallis mandibulus* from the Upper Triassic and diagnose a new ichnospecies *O. infestans* isp. nov. The occurrence of these trace morphologies suggests that insect bone-modification behavior originated in Triassic Gondwanic environments, and dispersed during the Mesozoic, achieving a more cosmopolitan distribution during the Cretaceous.

1. Introduction

Bioerosion on bone tissue produced by terrestrial invertebrates appears to have originated in the Triassic (Schwanke and Kellner, 1999; Raugust et al., 2013; Müller et al., 2015), before increasing in frequency during the Jurassic (Hasiotis et al., 1999; Hasiotis, 2004; Dangerfield et al., 2005; Chin and Bishop, 2006; Britt et al., 2008; Bader et al., 2009; Carpenter, 2013; Xing et al., 2015) and Cretaceous (Rogers, 1992; Jerzykiewicz et al., 1993; Paik, 2000; Getty et al., 2003; Nolte et al., 2004; Makovicky et al., 2005; Roberts et al., 2007; Kirkland and Bader, 2010; Cabral et al., 2011; Saneyoshi et al., 2011; de Valais et al., 2012; Carpenter, 2013; Pirrone et al., 2014b). The first report of osteophagy by terrestrial invertebrates from the Middle Triassic was briefly mentioned by Schwanke and Kellner (1999), followed by few reports that have also alluded to this behavior in the Late Triassic (Raugust et al., 2013; Müller et al., 2015). Although these studies hint at a rich record of insect trace fossils on bones at the beginning of the Mesozoic, ichnological descriptions of Triassic trace fossils are scarce.

Ichnological research on bone-modification in general is still quite new (e.g. Pirrone et al., 2014a), especially within a continental setting. There are currently 22 recognized ichnotaxa based on trace fossils on bones, nine of which are related to teeth traces (Cruickshank, 1986; Mikuláš et al., 2006; Rinehart et al., 2006; Jacobsen and Bromley, 2009) and six are associated with invertebrate bioerosion in marine settings (Muñiz et al., 2010; Higgs et al., 2011; Belaústegui et al., 2012; Cione et al., 2012). The seven remaining ones are attributed to terrestrial invertebrates (Thenius, 1988; Roberts et al., 2007; Pirrone et al., 2014b, Xing et al., 2015). The first ichnogenera assigned to traces produced by insects was *Asthenopodichnium ossibiontum*, found on Miocene bone elements (Thenius, 1988). Nextly, *Cubiculum ornatus* and *Osteocallis mandibulus* were erected, representing bioerosion on bones from the Upper Cretaceous also attributed to insects (Roberts et al., 2007). Subsequently, the ichnogenus *Cubiculum* was re-diagnosed to allow the inclusion of a number of ichnotaxa: *C. inornatus* from the Middle Jurassic of China (Xing et al., 2015), *C. levis* from the Upper Cretaceous of Argentina (Pirrone et al., 2014b), and lastly a new *Cubiculum* ichnospecies from the Pleistocene of South Africa (Parkinson, in press) that co-occurs with another new insect related trace fossil (Parkinson, in press). The current diagnoses for these ichnotaxa are summarized in Table 1 and their general aspects are shown in Fig. 1.

The aims of this study are to report the oldest evidence for osteophagy among terrestrial invertebrates, to revise the ichnogenus *Osteocallis* and to erect a new ichnospecies for this ichnogenus, *O. infestans*. The present study provides the first conclusive evidence of osteophagy among Middle Triassic terrestrial insects and expands

* Corresponding author.
E-mail address: voltairearts@gmail.com (V.D. Paes Neto).

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Table 1
Taxonomic diagnosis and description of the seven ichnotaxa found on bone elements from continental settings attributed to terrestrial invertebrates. Ichnogenera are displayed in blue, and ichnospecies schematic drawings are depicted in Fig. 1.

Ichnotaxon	Diagnosis/Description
Asthenopodichnium Thenius 1979	Small, U-shaped *spreiten* or pouch-like structures in wooden, organic-rich or bone substrates (after Uchman et al., 2007).
A. ossibiontum Thenius, 1988 (Fig. 1A)	U-shaped buildings with a tube diameter of 3–5 mm, with a notch length of an average of 10–15 mm. Depth of the underground buildings up to 7 mm (Thenius, 1988)
Osteocallis Roberts et al., 2007	Shallow, meandering trail of arcuate grooves (apparently paired) bored into external (cortical) bone surfaces. May consist of a single trail or a network of randomly overlapping trails (Roberts et al., 2007).
O. mandibulus Roberts et al., 2007 (Fig. 1B)	A shallow trail of grooves or scratches bored into outer surface of bone, from 1.1 to 12.9 mm wide and may extend for many tens of mm in length. Trail width between 1.0 and 3.5 mm most commonly. Penetration of the borings extremely shallow, from 0.5 to 1.9 mm deep, and composed of commonly paired, arcuate scratches <1 mm wide. Locally develops into deeper excavations, pits, or furrows (Roberts et al., 2007).
Cubiculum Roberts et al., 2007	Discrete ellipsoidal borings in bone. Hollow, oval borings bored into inner spongy and outer cortical bone surfaces. Boring length two to five times greater than its diameter. Structures may be isolated but may form dense, locally overlapping concentrations (after Xing et al., 2015).
C. ornatus Roberts et al., 2007 (Fig. 1C)	Borings occurring in isolated, dense or overlapping concentrations. Walls roughened commonly composed of shallow, arcuate (apparently paired) grooves (after Pirrone et al., 2014b).
C. levis Pirrone et al., 2014b (Fig. 1D)	Discrete, bowl-shaped borings in bone. Borings display marked concavity of flanks and bottom and marked constriction of walls in the upper area. Boring boundaries, walls and bottom are sharp and smooth; no bioglyphs are observed. No filling is present. Placed in cortical and cancellous bone. Borings are arranged in groups of three to five and parallel to each other, according to its major axis (Pirrone et al., 2014b).
C. inornatus Xing et al., 2015 (Fig. 1E)	Ellipsoidal borings embedded in cortical bone penetrating into the trabecular bone. The borings display a length width ratio ranging from between 2:1 to 5:1 and slightly tapers towards one end. The walls run perpendicular to the bone surface, whilst the base of the boring is rounded. No filling or bioglyphs are present (Xing et al., 2015)
Cubiculum new ichnospecies Parkinson (Fig. 1F)	Discrete oval borings embedded in bone with straight walls running perpendicular to the bone surface and bottoms are flat and run parallel to the bone surface. The contact between the walls and the bottom are predominately sharp, but may be slightly rounded. The length is roughly double the maximum width. The trace either occurs individually or in groups of two to four borings. In groups traces infrequently intersect but in general show no consistent orientation to one another.
New ichnogenera Parkinson, In press (Fig. 1G)	A circular boring penetrating cortical bone at an acute oblique angle relative to the bone surface, with a crescent shaped excavation that partly surrounds the boring.

our understanding of Late Triassic occurrences by providing a comprehensive description of the Santa Maria Supersequence trace fossils.

2. Geological Context

The Paraná Basin extends over at least 1. 700. 000 km² through Argentina, Brazil, Paraguay and Uruguay (Fig. 2A). The basin contains deposits dating from the Late Ordovician to Late Cretaceous (Milani and Ramos, 1998; Horn et al., 2014). Middle and Upper Triassic beds are mostly restricted to the southernmost portion of the basin, in the State of Rio Grande do Sul, with restricted exposure (Fig. 2B). These beds comprise the Middle–Upper Triassic Santa Maria Supersequence, which crops out in a narrow E–W belt in the center of the state (Horn et al., 2014). The deposits of this Supersequence consist of a series of fluvial and lacustrine red beds. Recent reassessments of the stratigraphic framework of the Santa Maria Supersequence (Horn et al., 2014) have recognized four third-order sequences (Fig. 2D), which are, from bottom to top: the Pinheiros-Chiniquá Sequence, the Santa Cruz Sequence, the Candelária Sequence, and the Mata Sequence. The first three of these sequences are known to yield vertebrate fossils (Horn et al., 2014). According to Zerfass et al. (2003) and Horn et al. (2014) the Pinheiros-Chiniquá Sequence comprises clast-supported conglomerates and cross-bedded sandstones overlain by laminated mudstones, reflecting a fluvial deposit that changes to shallow lacustrine deposits. The Santa Cruz Sequence is also characterized by red mudstones, overlain by sandstones and conglomerates interpreted as alluvial deposits. The Candelária Sequence is represented by high-sinuosity fluvial and overbank deposits consisting of medium-to fine-grained, cross-bedded sandstones and mudstones lenses at the base, grading to thick mudstones in the middle part. In its top portion, a lacustrine deltaic system is defined with a succession of siltstone-mudstone layers intercalated with lenses of fine-grained, cross-bedded or climbing cross-laminated sandstones (Zerfass et al., 2003).

The vertebrate fossils of these three Sequences are biostratigraphically arranged into four Assemblage Zones (AZs) (Langer et al., 2007; Soares et al., 2011; Horn et al., 2014) (Fig. 2D).

3. Materials and Methods

Our material consists of several trace fossils found on bone elements of four vertebrate specimens, two (MCT 430-R and MCT 1584-R) assigned to the Middle Triassic *Dinodontosaurus* AZ and two (UFRGS-PV-1099-T and UFRGS-PV-1177-T) to the Late Triassic *Hyperodapedon* AZ. Reference numbers of each trace (Fig. 1 of the Supplementary Material), and their descriptions, are listed in Tables 1 and 2 of the Supplementary Material. MCT 430-R represents a semi-articulated skeleton with three trace-bearing bones (i.e. ulna, ilium and tibia). MCT 1584-R consists of an isolated bioeroded humerus. Both vertebrate specimens are ascribed to a dicynodont cf. *Dinodontosaurus* and were collected in the vicinity of the municipality of Candelária (Fig. 2C), at the Sanga do Ribeiro Site and Sanga do Moreira Site, respectively. UFRGS-PV-1099-T consists of a semi-articulated skeleton of a yet indeterminate sauropodomorph dinosaur, bearing bone alterations on one femur and two vertebrae. UFRGS-PV-1177-T is a partial skull and mandible of *Exaeretodon riograndensis*, with a trace-bearing dentary. UFRGS-PV-1099-T and UFRGS-PV-1177-T were both collected at the Janner Site, Agudo municipality (Fig. 2C). Vertebrate specimens MCT 430-R and MCT 1584-R are housed in the vertebrate collection of the Museu de Ciências da Terra (MCT), Serviço Geológico do Brasil. Vertebrate specimens UFRGS-PV-1099-T and UFRGS-PV-1177-T are deposited in the vertebrate collection of the Laboratório de Paleontologia de Vertebrados, Departamento de Paleontologia e Estratigrafia, Instituto de Geociências (UFRGS-PV), Universidade Federal do Rio Grande do Sul. MCT and UFRGS-PV are both public domain permanent collections, and both allowed us to describe the previously mentioned vertebrate specimens.

All trace fossils were originally filled with sedimentary matrix and underwent mechanical preparation. No inclusions or fill patterns were observed during the mechanical preparation, nor were bioglyphs associated with any trace. Bioglyphs consist of distinct engravings in the walls of borings or other traces, produced by the activity of an animal (*sensu* Ekdale and de Gibert, 2010), and are considered important ichnotaxobases for borings on bones (Pirrone et al., 2014a). The trace fossils were subsequently observed using a Zeiss Stemi DV4

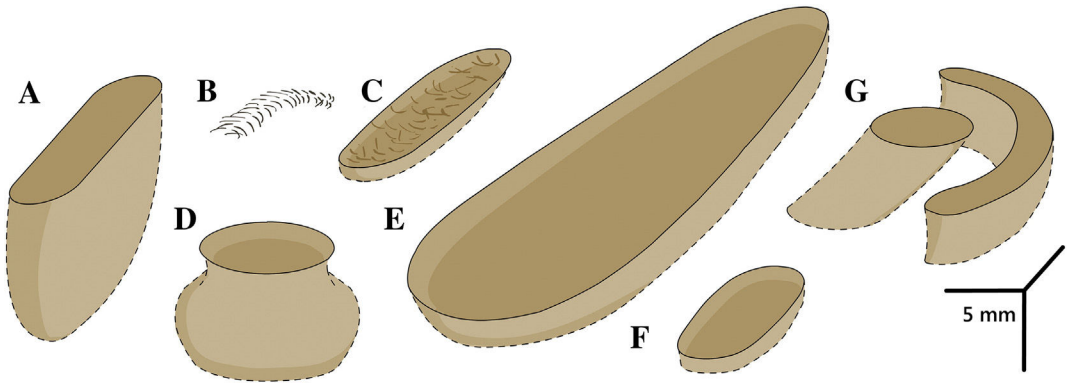

Fig. 1. Schematic drawing of the purported ichnotaxa attributed to terrestrial invertebrates occurring on the surface of bones found in continental deposits. Scale bar = 5 mm.(A) *Asthenopodichnium ossibiontum* Thenius, 1988. (B) *Osteocallis mandibulus* Roberts et al., 2007. (C) *Cubiculum ornatus* Roberts et al., 2007. (D) *C. levis* Pirrone et al., 2014b. (E) *C. inornatus* Xing et al., 2015. (F) New *Cubiculum* ichnospecies Parkinson., in press. (G) New south african ichnotaxon Parkinson, in press.

stereoscopic microscope at magnifications from 4 to 25x. The traces were described in terms of proposed ichnotaxobases for bioerosional traces in bone following Pirrone et al. (2014a). The following taphonomic variables were recorded for each of the vertebrate studied: degree of articulation with other elements (isolated or articulated), physical element integrity (complete when more than 90% of skeletal element is preserved, or partially preserved when less than <90% of skeletal element is preserved) and weathering stage (*sensu* Behrensmeyer, 1978).

The vertebrate specimens from the Pinheiros-Chiniquá Sequence (MCT 430-R and MCT 1584-R) have suffered significant diagenetic alteration, being covered by concretionary calcitic material that precludes a precise evaluation of their weathering stages. All observations are summarized in Table 2. UFRGS-PV-1099-T (vertebrae and femur) was scanned using medical-grade computed tomography using a Phillips© Brilliance CT 16 slice model (Hospital de Clínicas de Porto Alegre - HCPA), whilst digital segmentation and visual rendering were done using Amira 5 and Mimics 10.01 software. Detailed images of a trail of grooves (UFRGS-PV-1099-T) were acquired using a Nikon AZ100M with Z-stack (Departamento de Zoologia of the Instituto de Biociências, UFRGS). Lastly, silicon casts were constructed over the trail of UFRGS-PV-1177-T.

Trace morphologies were measured using a digital caliper, and digitally confirmed using ImageJ©. Diameter measurements were taken on circular traces; for ovoid traces the larger and smaller axis were measured. Length was only recorded for grooves, chambers and channels. Depth was measured perpendicular to the bone surface for all traces. Descriptive statistics of the measurements were obtained using IBM SPSS version 20.

4. Results

A total of seven trace morphotypes were recorded on the Middle–Late Triassic bones (Fig. 3). Traces are primarily concentrated on or near the articular regions, and they display relatively smooth walls with no observable evidence of bioglyphs. Tubes perforating the bone and surface channels occur on bones from both Assemblage Zones (see Table 2). Compiled taphonomic attributes of the vertebrate skeletons and summarized information about the trace fossils are shown on Table 2.

Chambers of the ichnospecies *Cubiculum inornatus* were found on Middle Triassic bones (see section 4.1). Trails of grooves were exclusively recognized on the Late Triassic bones (see section 4.2), and among these, we identified the ichnotaxon *Osteocallis mandibulus* and also a new ichnospecies belonging to this ichnogenus, which is described in section 4.2.3.2.

4.1. Middle Triassic Traces (Dinodontosaurus Assemblage Zone)

Holes and tubes are recurrent traces recorded on bones from this assemblage zone (descriptive statistics in Table 3). Trace morphologies are mostly circular, but occasionally ovoid. These bone alterations are primarily concentrated on articular regions, epiphyses or close to the epiphysis–diaphysis junctions, most likely due to the softer bone tissue of these regions.

4.1.1. Traces on the humerus of cf. Dinodontosaurus MCT 1584-R

This element (Fig. 4A) was not found in direct association with other skeletal elements. It bears a total of five tubes, one hole, one chamber and one channel-like trace. Three of those tubes (Fig. 4B) are clustered in the ectepicondylar region, generally showing a circular entrance morphology with a diameter of approximately 5 mm, and a depth of less than 25 mm. The other two are embedded in a sinuous channel-like trace located on the proximal epiphysis, and have a 4 mm diameter and measure less than 20 mm deep (Fig. 2 of the Supplementary Material). The sinuous channel-like trace (Fig. 4D) is 6 mm wide, 30 mm long and ranges from 2- to 7 mm in depth. The hole (Fig. 3 of the Supplementary Material) is ovoid with a larger axis of 6.5 mm and is located on the anterior distal portion of the bone, in close proximity to an ellipsoidal chamber (Fig. 4C) which measures 3.8 mm wide, 10 mm long and 3 mm deep. The chamber, assigned to *Cubiculum inornatus* (see section 4.1.3), has slightly concave walls, and is located in the cortical bone.

4.1.2. Traces on the ulna, tibia and ilium of cf. Dinodontosaurus MCT 430-R

The vertebrate specimen consists of an articulated partial postcranium of a mature dicynodont of which the left ilium (Fig. 4E), one ulna (Fig. 4H) and a tibia bear bioerosional traces. On the lateral surface of the ilium, an individual *Cubiculum inornatus* (Fig. 4F), measuring 29.7 mm long, 5 mm wide and 3.5 mm deep, was recorded, along with three oblique holes and two holes near the articular surface (Fig. 4G). These holes are generally 6 mm wide, and the oblique ones present a depth of 14.4 to 18.7 mm and are all embedded in the upper cortical surface of the bone, but do not penetrate into the lower trabecular bone. Another *C. inornatus* (Fig. 4I) measuring 23.1 mm in length and 3.85 mm in width was recorded on the proximal articulation of the ulna along with two other holes (Fig. 4H; Figs. 4 and 5 of the Supplementary Material). Lastly, a single hole was recorded on the posterior rim of the distal articulation of the tibia (Fig. 6 of the Supplementary Material). None of the holes recorded in MCT 430-R penetrate the bone, unlike some of the borings in MCT 1584-R.

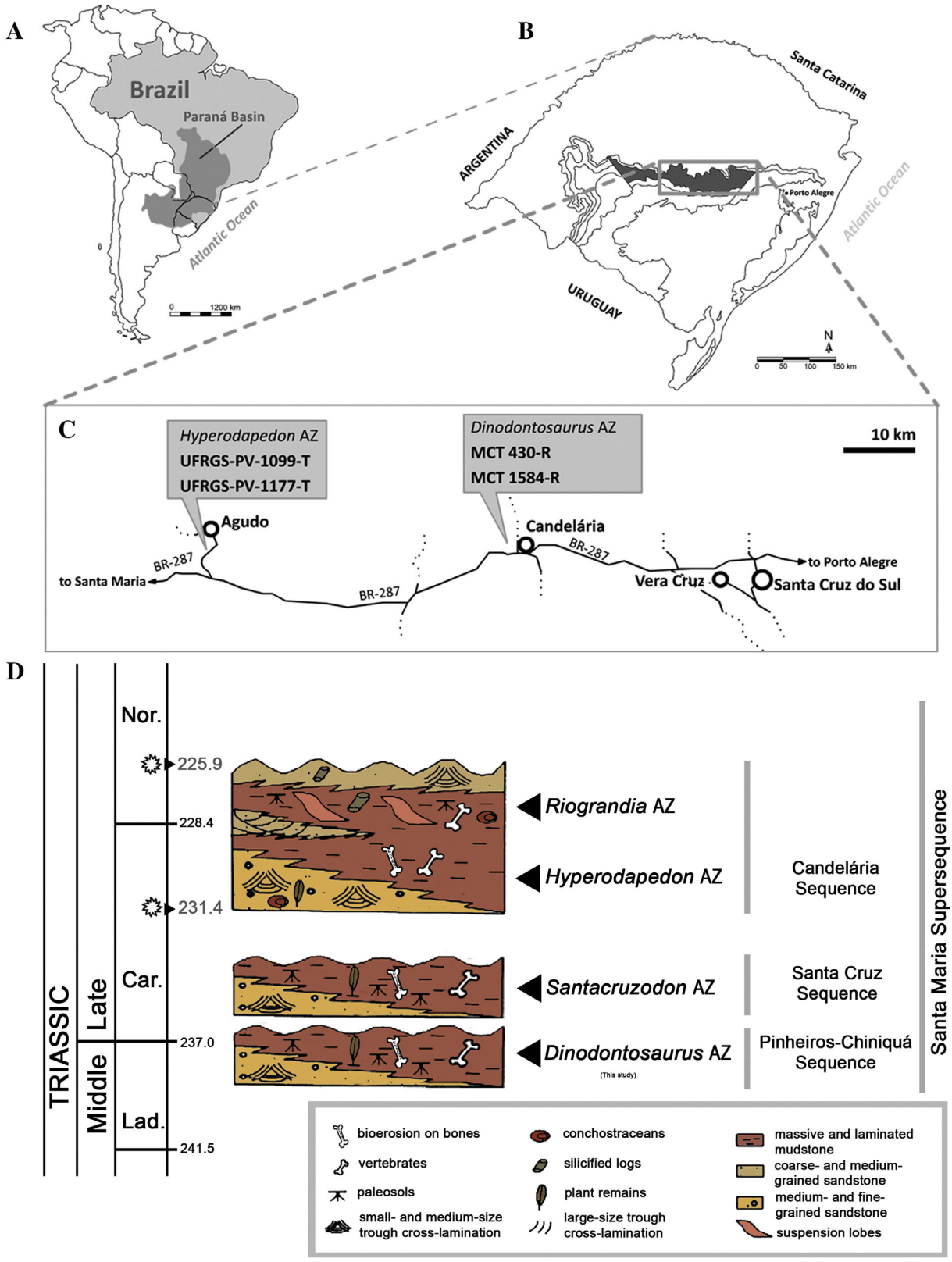

Fig 2. Geological and geographic context. (A) Location map of the Paraná Basin in South America. (B) Outcropping limits of the Santa Maria Supersequence in Rio Grande do Sul State, Brazil. (C) Geographical provenance of the studied specimens. (D) Chrono- and biostratigraphy of southern Brazilian Triassic units modified from Horn et al. (2014). Trace fossil on bones are found in the *Dinodontosaurus* AZ (this study), *Santacruzodon* AZ (Raugust et al., 2013) and *Hyperodapedon* AZ (Müller et al., 2015; this study). Ages (in millions of years) following Gradstein et al. (2012). The absolute date of 231.4 Ma is related to the base of the Ischigualasto Formation and the 225.9 Ma date is related to the base of the Los Colorados Formation, both geological units from Argentina (Martínez et al., 2012). The local faunas from these formations are, respectively, correlated with the Brazilian *Hyperodapedon* and *Riograndia* AZs (Langer et al., 2007; Soares et al., 2011). The youngest Mata Sequence, devoid of fossil bones, is not shown on this figure. Abbreviations: AZ = Assemblage Zone; Lad = Ladinian; Car = Carnian; Nor = Norian.

Table 2
Vertebrate specimen overview with summarized taphonomic and trace fossil information. Physical element integrity was considered complete for all available bone elements.

AZ	Vertebrate Specimen	Modified Bone Elements (number of traces)	Weathering stages	Type of Traces
Dinodontosaurus AZ Middle Triassic	MCT 1584-R cf. *Dinodontosaurus* (isolated humerus)	Humerus (8)	?	Hole (n = 1), tubes (n = 5), *Cubiculum inornatus* (n = 1) and a sinuous channel (n = 1)
	MCT 430-R cf. *Dinodontosaurus* (semi-articulated skeleton)	Ulna (3) Ilium (4) Tibia (1)	?	Holes (n = 8) and *Cubiculum inornatus* (n = 2)
Hyperodapedon AZ Late Triassic	UFRGS-PV-1099-T Undescribed dinosaur (semi-articulated skeleton)	Femur (4) Vertebra (1) Vertebra (1)	1	*Osteocallis mandibulus* (n = 1), tubes (n = 4) and channel (n = 1)
	UFRGS-PV-1177-T *Exaeretodon riograndensis* (partially preserved articulated skull)	Dentary (1)	0	*Osteocallis* isp. nov. (n = 1) and isolated grooves

4.1.3. Systematic Ichnology

4.1.3.1. Ichnospecies. Cubiculum inornatus Xing et al., 2015

4.1.3.1.1. Diagnosis. Ellipsoidal borings embedded in cortical bone penetrating into the trabecular bone. The borings display a length width ratio ranging from between 2:1 to 5:1 and slightly tapers towards one end. The walls run perpendicular to the bone surface, whilst the base of the boring is rounded. No filling or bioglyphs are present.

4.1.3.1.2. Material. Ellipsoid chambers MCT 1584-R E (on the isolated humerus MCT 1584-R, Fig. 4C), MCT 430-R C and MCT 430-R J (on the ilium and on the ulnae of MCT 430-R, Fig. 4F and I, respectively).

4.1.3.1.3. Comments. The three chambers found on the Middle Triassic bones can be assigned to *C. inornatus* based on the morphology of the walls, those being almost perpendicular to the bone surface, rounded at the base and lacking bioglyphs and filling. Also, one of their ends is tapered, a feature shared with the *C. inornatus* type material. Notwithstanding, the two chambers found on MCT 430-R (Fig. 4F and I) fall out of the expected length:width ratio that diagnoses the ichnospecies (2:1 to 5:1), reaching a ratio of up to 6:1. We suggest that this difference may be a local variation, and is not sufficient to exclude the Middle Triassic chambers from the *C. inornatus* ichnospecies.

4.2. Late Triassic Traces (Hyperodapedon Assemblage Zone)

Circular to ovoid tubes and channels are among the patterns observed in the two evaluated vertebrate specimens of this assemblage zone. Both present trails of grooves related to the ichnogenus *Osteocallis*, a feature not observed on the Middle Triassic sample.

4.2.1. Traces on the femur and two vertebrae of an indeterminate dinosaur UFRGS-PV-1099-T

The semi-articulated dinosaur skeleton shows three trace-bearing bones. Four tubes were found on the distal articulation area of the right femur (Figs. 5A to C and 5E), with a circular opening of approximately 5 mm of diameter. Three of these trace fossils have external openings, one in close proximity to a channel (Fig. 5C), 6 mm wide and 30 mm long. The fourth tube is located internally with an opening to the medullar cavity (Fig. 5E). They were found in association with a single trail of grooves assigned to *Osteocallis mandibulus* (Fig. 5D) (see section 4.2.3). The proximal and distal portions of the femur are damaged and this could potentially be related to biogenic bone-modification. Two vertebrae found associated with the femur also present some trace fossils; one records a single hole (Fig. 5F and G) on the ventral portion of the centrum. A CT-scanned image of this single hole-bearing vertebrae revealed that this hole was actually the external opening of a tube that runs through the vertebral centrum, connecting to a second opening close to the neurocentral junction (Fig. 5G). The other vertebra also presents a short tube with a 3 mm diameter (shown in Fig. 5H, with the vertebra in lateral view) in direct association with a tube-like structure on the surrounding matrix (Fig. 5H). This structure emerges from the short tube, situated on the neurocentral junction, and passes through the neural canal. Almost 4 mm in diameter, this burrowing-structure has a different sedimentary composition to that of the rock, resembling a calcrete-filled burrow.

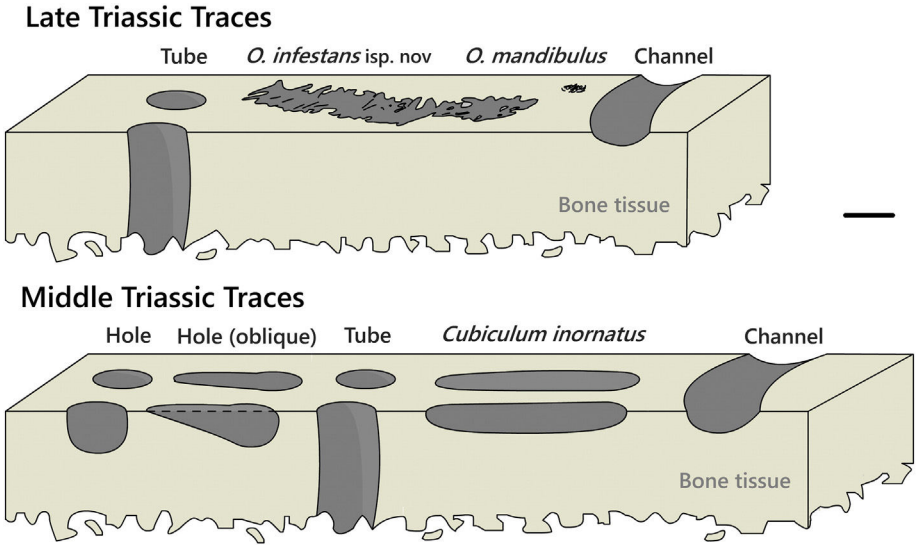

Fig. 3. Overview of trace morphologies from bones of the Middle Triassic *Dinodontosaurus* Assemblage Zone and Late Triassic *Hyperodapedon* Assemblage Zone. Scale bar = 5 mm.

Table 3
Descriptive statistics obtained from the Middle Triassic traces

	Trace morphology	Lesser diameter	Greater diameter	Depth
MCT 1584-R	Borings (hole and tubes) (n = 6)	Min: 3.0 mm Max: 4.8 mm Mean: 4.2 mm Std. Deviation: 0.5 mm	Min: 3.8 mm Max: 6.3 mm Mean: 5.1 mm Std. Deviation: 0.7 mm	Min: 15.0 mm Max: 20.0 mm Mean: 17.6 mm Std. Deviation: 2.2 mm
MCT 430-R	Holes (n = 7)	Min: 2.0 mm Max: 6.0 mm Mean: 3.9 mm Std. Deviation: 1.5 mm	Min: 5.7 mm Max: 8.0 mm Mean: 6.7 mm Std. Deviation: 0.8 mm	Min: 2.0 mm Max: 8.5 mm Mean: 4.4 mm Std. Deviation: 2.4 mm

Min = Minimum; Max = Maximum;

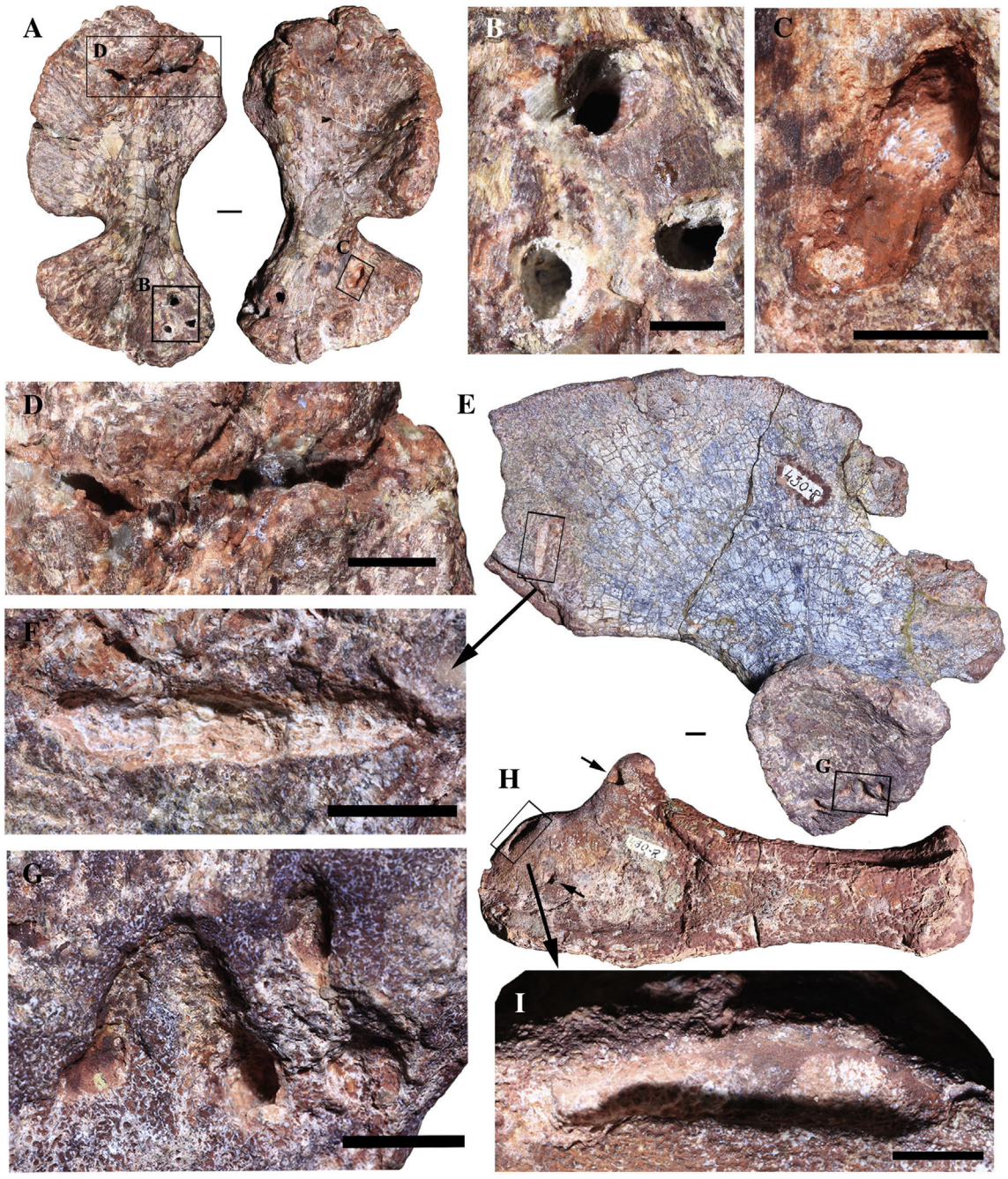

Fig. 4. Trace morphologies from the Middle Triassic *Dinodontosaurus* Assemblage Zone. (A) The isolated trace-bearing humerus MCT 1584-R on which tubes (B), *Cubiculum inornatus* (C) and a channel (D) were identified. (E) Lateral view of the left ilium MCT 430-R on which *C. inornatus* and holes were identified. (F) Detail of the *C. inornatus* found on MCT 430-R left ilium. (G) Detail of the distinct holes found on the pubis articulation area of the MCT 430-R left ilium. (H) General view of MCT 430-R ulna, arrows indicate holes. (I) Detail of *C. inornatus* found on the proximal portion of the ulna of MCT 430-R. Scale bar in A, D–H = 10 mm and B, C and I = 5 mm.

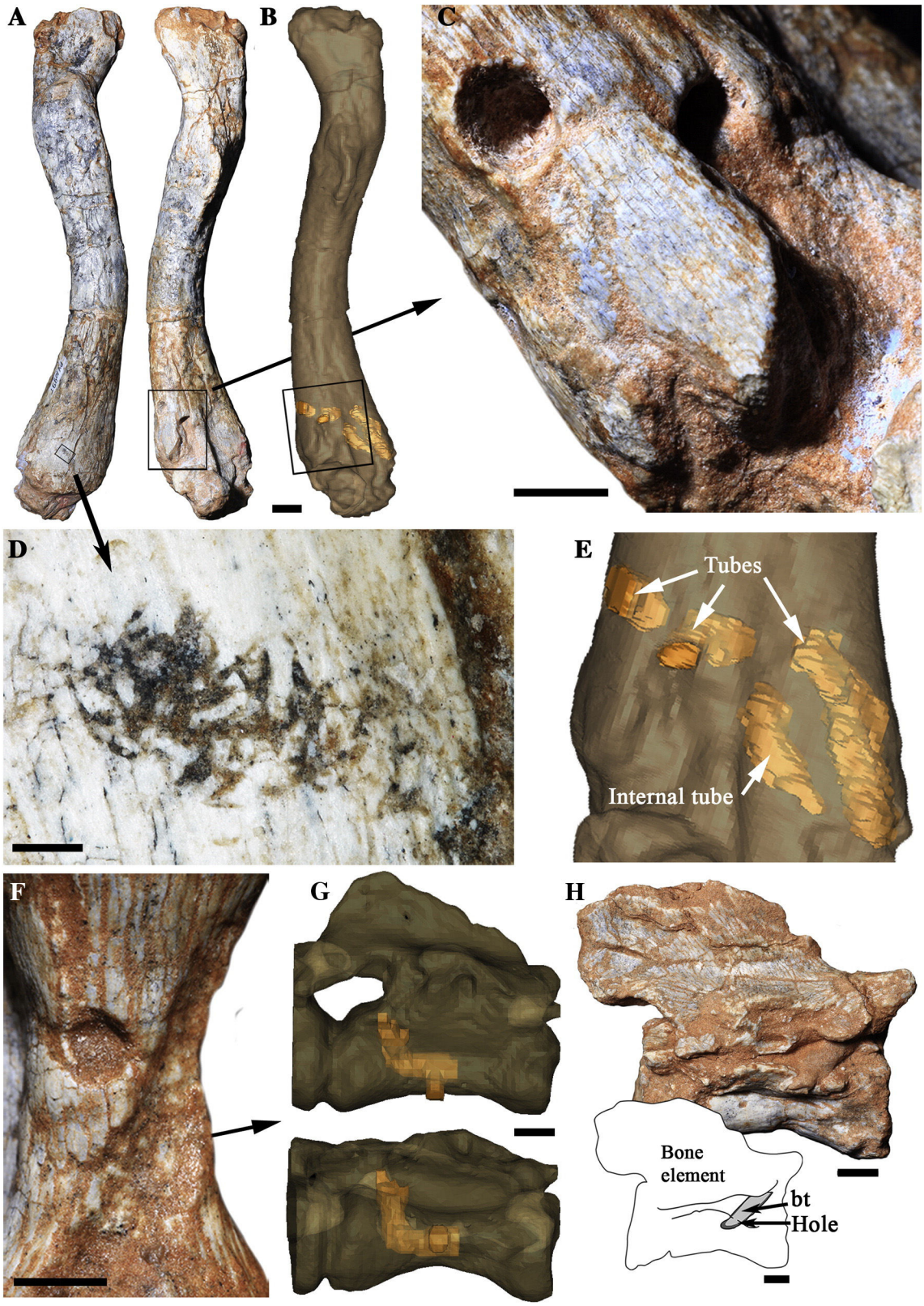

Fig. 5. Trace morphology found in UFRGS-PV-1099-T from the Upper Triassic *Hyperodapedon* Assemblage Zone. (A) Overview of the right femur. (B) CT-scan image of the same femur showing digital casts of the tubes. (C) External tubes. (D) *Osteocallis mandibulus*. (E) Detail of the morphology of the tubes shown in (B). (F) Ventral view of the centrum of a trunk vertebra showing a matrix-filled tube. (G) CT-scan image showing the internal structure of the tube shown in (F). (H) Photograph and schematic drawing of another trunk vertebra showing the burrow-like tube (bt), that trespasses the bone, through a hole on the border of the neurocntral suture. Scale bar in A = 10 mm, B and C = 5 mm, D = 1 mm, F-– H = 5 mm. Abbreviations bt = burrow-like tube.

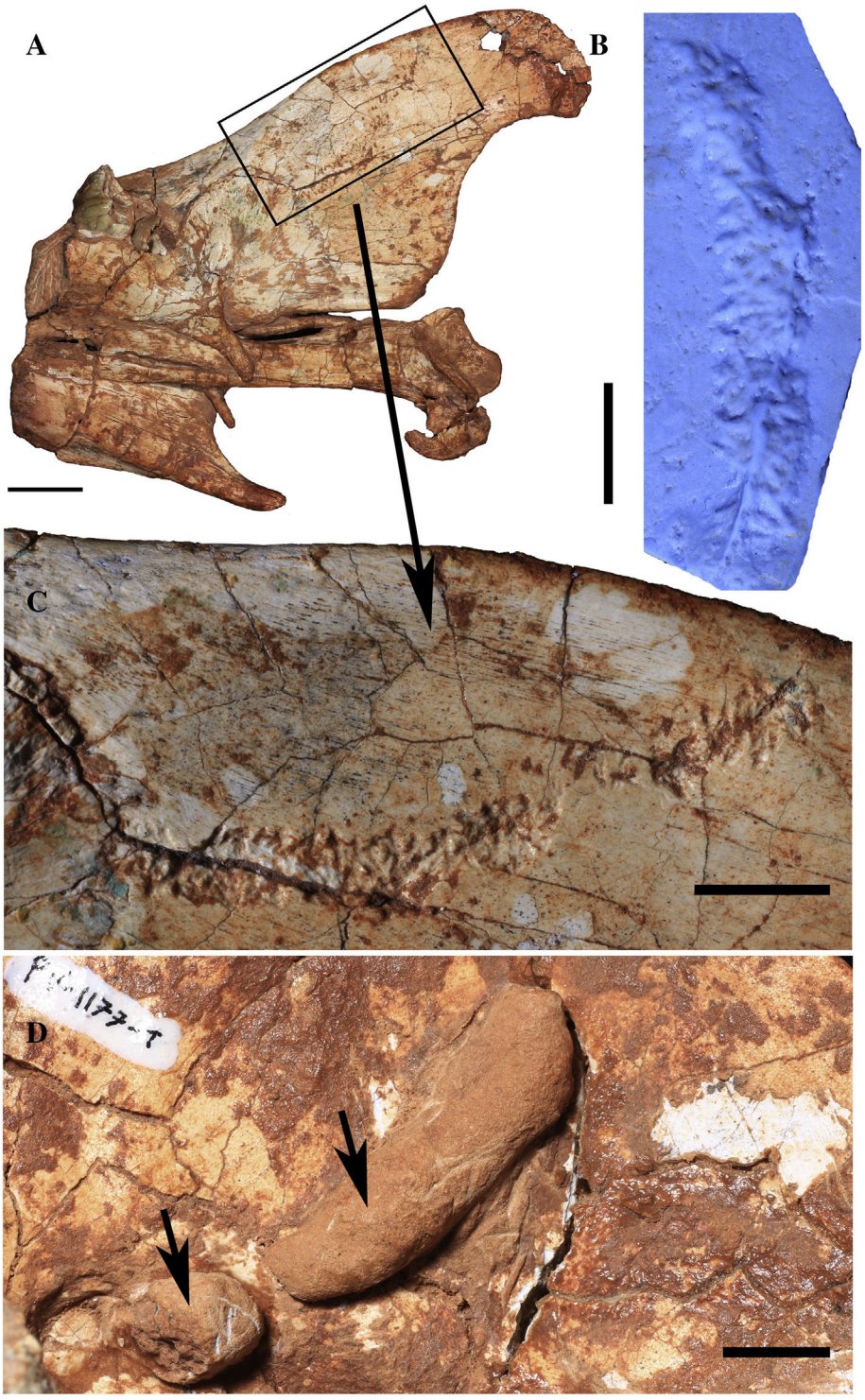

Fig. 6. *Osteocallis infestans* isp. nov and burrowing traces found on UFRGS-PV-1177-T from the Late Triassic *Hyperodapedon* Assemblage Zone. (A) Lingual view of the mandible. (B) Silicon cast of the larger trail of *O. infestans* isp. nov. showing details of the groove pattern. (C) Detail of the dentary showing the holotype of *O. infestans* isp. nov. (D) Detail of the burrow structures found adjacent to the skull surface. Scale bar on A = 20 mm, B = 5 mm, C and D = 10 mm.

4.2.2. Traces on the dentary of Exaeretodon riograndensis UFRGS-PV-1177-T

The vertebrate specimen consists of the posterior portion of the skull of a cynodont, with the right mandibular ramus in full articulation (Fig. 6A) bearing a traced trail (Fig. 6B and C), which was previously mentioned by Liparini et al. (2013). Situated on the medial portion of the coronoid process of the dentary, the trail comprises expressively etched grooves (Fig. 6B and C). The distinctive morphology of this trace has prompted the establishment of a new ichnospecies belonging to the ichnogenus *Osteocallis* (see section 4.2.3). Similar to what occurs with UFRGS-PV-1099-T, tube-like structures were observed in the sediment adjacent to the bone surface (Fig. 6D).

4.2.3. Systematic Ichnology

Ichnogenus: *Osteocallis* Roberts et al., 2007

Type ichnospecies: *Osteocallis mandibulus* Roberts et al., 2007

Type horizon and locality: Maevarano Formation, Upper Cretaceous of Madagascar.

Emended diagnosis: Shallow trail of mandibular grooves bored into external (cortical) bone surfaces. It may present as a single trail or a network of randomly overlapping trails (modified from Roberts et al., 2007).

Remarks: We dissociate the meandering nature and arcuate shape of the isolated grooves, as well as the apparently paired nature of the grooves from the diagnosis of the ichnogenus, and refer to these as particular features of the type ichnospecies *O. mandibulus*. Thus, the ichnogenus *Osteocallis* can properly accommodate the new trace described below (UFRGS-PV-1177-T). We suggest the general use of this ichnogenus to accommodate all shallow trails of grooves in cortical bone.

4.2.3.1. Osteocallis mandibulus Roberts et al., 2007

4.2.3.1.1. Material. A small trail UFRGS-PV-1099-T G (Fig. 5D) on the anterior distal portion of a dinosaur right femur (UFRGS-PV-1099-T).

4.2.3.1.2. Description. A small trail 4 mm in length formed by successive small (Fig. 5D), shallow and mainly paired grooves (single scratch mean length of less than 0.5 mm) on the bone surface. Forming a small cluster on one extremity, the trail does not exceed 2 mm in width.

4.2.3.1.3. Comments. Despite been extremely small, this trace fossil falls within the range observed by Roberts et al. (2007), and, along with a trail reported by Müller et al. (2015), represents the earliest known occurrence of *O. mandibulus* (pushing back its occurrence by more than 150 Ma). In the type material from the Maevarano Formation, Late Cretaceous of Madagascar, *O. mandibulus* was found associated with *C. ornatus* (chamber) and tubes. No chamber-like traces were observed during this study on any Late Triassic bone elements, but tubes and channels were indeed recorded on skeletal elements associated with the femur which recorded *O. mandibulus*. No burrows were recorded on the sediment close to *O. mandibulus*, but a burrow/bioerosion association is present on a trunk vertebra from the same vertebrate specimen (Fig. 5H).

4.2.3.2. Osteocallis infestans new ichnospecies

4.2.3.2.1. Holotype. A trail of grooves UFRGS-PV-1177-T A (Fig. 6C) located on the right dentary of a traversodontid cynodont *Exaeretodon riograndensis* (UFRGS-PV-1177-T).

4.2.3.2.2. Type locality and stratigraphic level. Janner outcrop, Agudo municipality, Rio Grande do Sul, Brazil, Upper Triassic (Carnian), Candelária Sequence of the Santa Maria Supersequence.

4.2.3.2.3. Etymology. Variation of *infestantibus* (Latin), meaning aggressive.

4.2.3.2.4. Diagnosis. Shallow to moderately deep, meandering trail of generally straight overlapping thick grooves excavated into the cortical bone surfaces. Grooves are randomly orientated, often overlapping other grooves which are either perpendicular or parallel to one another. The intensity of the grooves culminates in an irregular furrow-like morphology for the trail.

4.2.3.2.5. Description. The recorded trace fossil represents an expressively excavated trail of straight grooves situated over the lingual side of the coronoid process of the dentary (Fig. 6A). The trail is divided by a non-excavated region that only presents isolated grooves. The short continuous section measures 13 mm long and 3.6 mm wide, whilst the longest section of the trail measures 31 mm long and almost 6 mm wide (Fig. 6B and C). The individual grooves have a size range of 2 and 5 mm long, with the width of the grooves ranging from 0.55 to 0.65 mm. The longer trail is deeper than the shorter one, and displays an irregular furrow-like morphology, which is up to 1.2 mm deep. The excavation pattern is more evident on the shorter trail, which does not show marked overlapping of the individual grooves. The two trails are separated by a less modified region which only displays three small striae (~2 mm in length).

4.2.3.2.6. Comments. O. infestans isp. nov. differs from *O. mandibulus* in its roughly etched furrow-like morphology, with no regular separation between isolated grooves. The type ichnospecies (*O. mandibulus*) displays a serial, regular and consistent distribution of paired grooves. Thus, the morphology of *O. infestans* is differentiated from *O. mandibulus* by the occurrence of non-paired, non-arcuate grooves which display randomly orientated and overlapping grooves. The highly overlapping and deeper groove pattern reinforces the validity of *O. infestans* isp. nov. as a new ichnospecies and suggests it was produced by a more aggressive mandible apparatus or by a more aggressive agent. The previously mentioned vertebrate specimen (UFRGS-PV-1099-T) which recorded the presence of *O. mandibulus* was found on the same outcrop as the vertebrate specimen displaying *O. infestans* isp. nov. (UFRGS-PV-1177-T). This suggests that it is possible in the future to find *O. infestans* in association with other trace morphologies including tubes, holes and channels. Burrowing structures were found adjacent to the bone tissue of UFRGS-PV-1177-T, but located closer to the skull and not in direct association with *O. infestans* isp. nov. These burrowing traces are larger than the burrows recorded in UFRGS-PV-1099-T, and present two different morphotypes: ellipsoid and rod-shaped burrows (Fig. 6D).

5. Discussion

All the trace fossils described in this contribution indicate that the osteophagic behavior of invertebrates in continental environments originated before the end of the Ladinian (242 – 235 Ma). This raises the question of which invertebrate group was the trace maker. The most commonly inferred extant causal agents in the literature are either dermestid beetles (e.g. *Dermestes*) or termites (e.g. Termitidae) (e.g. Bader et al., 2009; Britt et al., 2008; Dangerfield et al., 2005; Hasiotis et al., 1999; Hasiotis, 2004; Huchet et al., 2011; Huchet et al., 2013).

The oldest supposedly recognized dermestid comes from the Late Triassic of Australia (Dunstan, 1923), but this assignment is a matter of contentious debate (Kiselyova and McHugh, 2006; Kadej and Háva, 2011). The oldest undisputed dermestids are known from the Cretaceous Burmese ambers (Kiselyova and McHugh, 2006). The earliest records of termites also come from the Cretaceous (Krishna et al., 2013) and are phylogenetically related to the wood-feeding cockroach genus *Cryptocercus* (Lo et al., 2000; Inward et al., 2007; Legendre et al., 2008). Therefore, the most commonly inferred trace makers postdate the invertebrate osteophagic behavior reported here. However, recent gene-sequence work (e.g. Misof et al., 2014) may provide a key resource which could be used to identify the trace maker in the Middle and Late Triassic ichnofossils. Molecular data point out that many groups of insects seem to have radiated during the beginning of the Triassic, including stem-termite lineages, which were recently indicated as probable producers of some new Jurassic trace fossils (Xing et al., 2013).

Insects persisted as a diverse group during the major ecological changes that permeate the Triassic (Fraser and Sues, 2011), and are good candidates to be the producers of these traces. Nonetheless, in the following section we do not attempt to attribute a causal agent; we focus our discussion on the taphonomical, ethological and paleoecological interpretations of these trace fossils.

5.1. Taphonomy and Ethology of Middle Triassic Traces

Trace morphologies such as holes, tubes and *Cubiculum inornatus* were found in both Middle Triassic samples MCT 430-R and 1584-R (Figs. 3 and 4). A non-parametric Wilcoxon signed rank test was conducted using the greater and lesser diameter measurements of the borings (holes and tubes). The results ($P = 0.184$) show that there is no statistical difference in the size of the boring entrance preserved on both vertebrate specimens, which suggests that the same producer may have been responsible for all these trace fossils. The primary

difference between the borings in the two vertebrate specimens is the lack of tubes in MCT 430-R. This may be related to differences in *post-mortem* bone-modification conditions, like the degree of subaerial exposure and humidity, but these are difficult to infer due to the lack of weathering stage information for the samples.

It is difficult to attribute the origin of the channel (Fig. 4D) on MCT 1548-R to insect activity. However, the occurrence of two tubes at the base of the channel (Fig. 02 of the Supplementary Material) suggests that the modifications are contemporaneous, alluding to a feeding trace interpretation (Praedichnia *sensu* Vallon et al., 2016). Although experimental observations (e.g. Backwell et al., 2012) support tubes (and holes) as feeding traces (e.g. seeking lipid-rich bone marrow), they are regularly interpreted as pupation chambers (e.g. Huchet et al., 2013). The morphological simplicity of these traces, as well as the limited sample size, limits the interpretation of this behavior, and both scenarios are equally parsimonious. However, the association of channels, tubes and holes suggest that all these traces may have been produced by a carrion-eating insect during feeding over a subaerially exposed vertebrate carcass.

Another possibility remains that the trace fossils may have been produced post burial. The degree of articulation of the bone-bearing elements of MCT 430-R (Fig. E, H and I) with other elements suggests rapid burial and also that cartilage and ligaments were in place at the point of deposition and burial (*sensu* Lyman, 1996). Traces that damage bone as a result of the activities of a post-burial insect producer may be incidental and have been proposed for similar cases (e.g. Kirkland and Bader, 2010; Huchet et al., 2011). Nonetheless, considering that tubes are concentrated on the less dense regions of the bone (e.g. epiphyses), in association with other different trace fossils, the total evidence makes incidental modification unlikely.

Cubiculum inornatus was interpreted by Xing et al. (2015) as a pupation chamber trace (Pupichinia); likewise, other *Cubiculum* ichnospecies (Roberts et al., 2007; Pirrone et al., 2014b) are probably related to insect activity. The three studied availed *C. inornatus* (Fig. C, F and I) were found in association with putative feeding traces (i.e. tubes, holes and channels). Thus, we consider plausible the possibility that the same osteophagic group may have bioeroded the bone tissue for both feeding and pupation purposes.

5.2. Taphonomy and Ethology of Late Triassic Traces

The analyzed skeletal elements (UFRGS-PV-1099-T and UFRGS-1177-T) display low weathering-stage values, indicating that the carcasses must have been exposed to weathering processes (assumingly subaerial conditions) for a short period of time. This is also supported because ligaments must have still been present to allow the bones to remain in articulation when buried. The short period of subaerial exposure prior to burial may explain why both vertebrate specimens present a low frequency of modifications per element. Tubes and channels (Fig. 5C, E to H) are consistent with morphologies found on the Middle Triassic bones, and may also be interpreted as feeding traces (Praedichnia *sensu* Vallon et al., 2016). The most significant traces identified in this sample are those referred to *Osteocallis* (Figs. 5D, 6B and C).

Concerning *Osteocallis mandibulus* temporal and biogeographical occurrence, it is possible that its restriction to Upper Triassic assemblages from Rio Grande do Sul State, Brazil, may be an artifact of preservation rather than a precise record of its behavior in the Mesozoic. It is plausible to assume that delicate traces such as these would be destroyed during fossil diagenesis, as could have happened with the diagenesis that affected the vertebrate specimens from the Middle Triassic.

O. infestans isp. nov. was found in isolation, but there were burrowing structures in the sedimentary matrix around the skull UFRGS-PV-1177-T. The burrowing structures such as the tube-like structure in UFRGS-PV-1099-T (Fig. 5H) and the above mentioned on UFRGS-PV-1177-T (Fig. 6D) launches questions about whether the associated trace makers responsible for the bioerosional traces were soil-burying insects, like some extant beetles from the families Staphilinidae, Silphidae and Histeridae (Gaudry, 2010), pointing to an underground scenario for the trace production. Tube-like burrowing structures shown here are unlikely to be related to root erosion, because their small size and regular shape differ from the "rhyzolithes" (Pretto et al., 2015) found in the same outcrop as both vertebrate specimens (Janner Site), but also from the expected pattern of root etching (e.g. Saul and Saul, 2001; Pokines and Baker, 2013; but see Schultz, 1996). A similar direct association of burrowing and boring traces was documented on dinosaur bones from the Jurassic (Xing et al., 2013) and Cretaceous (Saneyoshi et al., 2011; Pirrone et al., 2014b), as well as other cases where tunneling structures were found in the vicinities of Cretaceous (Kirkland and Bader, 2010; Paik, 2000) and Holocene (Huchet et al., 2011) age bored bones. Due to uncertainty, we consider this association in the Upper Triassic sample to be coincidental, but further research may reveal the true relationship of the burrowing traces and bone borings.

5.3. Paleoecological Approaches and Final Remarks

The traces recovered on bones from the Middle and Upper Triassic deposits of Brazil represent, for the time being, the oldest record of osteophagy by insects in a continental setting. This implies that the niche of osteophagy was already occupied at the end of the Ladinian (242–235 Ma) in Gondwanic terrestrial ecosystems. The traces relation to feeding (tubes, holes, channels and *Osteocallis* ichnospecies) and pupation (*Cubiculum inornatus*) strategies means that by the Middle Triassic insects had developed mouth parts capable of modifying the less dense regions of bones (based on the distribution of bone modifications). The presence of pupation chambers in the Middle Triassic also suggests that carcass usage by insects was also as complex as most of the other younger Mesozoic occurrences. Moreover, the identification and description of a new ichnospecies of *Osteocallis* highlights the growing diversification of osteophagic behavior by insects during the Late Triassic; this implies that by the Late Triassic more than one agent was able to modify bones using their mandibles.

Our study provides evidence that insects became a major contender in the carrion reduction process at that time. The beginning of the Mesozoic is distinctive in its great ecological changes (Fraser and Sues, 2011) that may have influenced the evolution and the exploration of this niche by some insect groups that only reached global distribution later. It may be of interest in the future to examine the Triassic-aged deposits of the Karoo Super group of South Africa, or other Mesozoic deposits of India, Antarctica and Australia to further evaluate the biogeographic history of *Osteocallis*.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.palaeo.2016.03.026.

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CHAPTER 4 (PAPER 3)

TRACES OF INSECT ACTIVITIES AT COOPER'S D FOSSIL SITE (CRADLE OF HUMANKIND, SOUTH AFRICA)

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Traces of Insect Activity at Cooper's D Fossil Site (Cradle of Humankind, South Africa)

Alexander H. Parkinson

School of Geosciences & Evolutionary Studies Institute, University of the Witwatersrand, Johannesburg, South Africa

ABSTRACT

This study constitutes the first comprehensive description and analysis of trace fossils produced by insects on bone from a faunal assemblage in the Cradle of Humankind, South Africa. A total of 9,500 bone fragments from Cooper's D were visually inspected for evidence of insect trace fossils. Fifty-three specimens with insect traces were identified and analysed using optical microscopy at low magnifications. Analysis revealed frass and coprolites preserved on some bones, and nine morphologically distinctive traces. Circular boreholes associated with a crescent-shaped excavation are attributed to a new ichnogenus *Munitusichnus pascens*, while ellipsoid surface borings (pupation chambers) are assigned to a new ichnospecies *Cubiculum cooperi*. Evidence suggests that traces were produced by more than one agent at different stages during the taphonomic process. Modification either took place whilst bones were sub-aerially exposed, at the point of final deposition, or post-burial of the remains. *Munitusichnus pascens* and *Cubiculum cooperi* represent the first ichnotaxa in bone from the Cenozoic of African.

KEYWORDS

Pleistocene; Ichnology; Bone modification; Terrestrial invertebrates

Introduction

The objectives of this study are to comprehensively describe the diversity of trace fossils identified on faunal remains recovered from the Cooper's D fossil locality, and consequently to diagnose the first ichnotaxa in bone attributed to insects from a Cenozoic aged deposit in Africa. To date, no ichnotaxa in bone attributed to terrestrial insects have been formally diagnosed from the Cenozoic of Africa, and only four ichnotaxa have been diagnosed in bones from the Mesozoic aged deposits in Madagascar, Utah, Argentina, and China, which include *Osteocallis mandibulus, Cubiculum ornatus* (Roberts et al., 2007), *Cubiculum levis* (Pirrone et al. 2014), and *Cubiculum inornatus* (Xing et al., 2015). Reports of insect trace fossils in bone from Africa come primarily from South Africa (Kitching, 1980; Brain, 1981; Brink, 1987; Newman, 1993; Pickering, 1999; Backwell et al., 2012; Dirks et al., 2015; Val et al., 2015), Tanzania (Kaiser, 2000; Fejfar and Kaiser, 2005), and Ethiopia (Backwell et al., 2012) and are restricted to the late Cenozoic aged deposits. South African instances are mostly restricted to localities within the Cradle of Humankind, including Malapa Cave (Val et al., 2015), Swartkrans (Newman, 1993; Pickering et al., 2005), Sterkfontein (Brain, 1981; Pickering, 1999; Val and Stratford, 2015), Kromdraai (Brain, 1981), Plovers Lake (Backwell et al., 2012), Luleche (Adams et al., 2007), and Dinaledi chamber (Dirks et al., 2015). Further afield insect trace fossils have been reported from Makapansgat (Kitching, 1980), Florisbad (Brink, 1987), Jubilee shelter, and Sibudu Cave (Backwell et al., 2012). Lastly, traces have recently been described on the fibula of a prosauropodamorph dinosaur from the Late Triassic/Earliest Jurassic, Elliot Formation of South Africa (Xing et al., 2015).

Published data from Malapa Cave shows a diverse range of trace morphologies including, intersecting grooves, parallel grooves, pits with bioglyphs, star shaped pits and borings (Val et al., 2015). However, these trace types have not been formally described. Insect borings from Swartkrans are reported to range from two to five millimetres in diameter, penetrate up to 10 mm deep and are orientated either perpendicular or obliquely to bone surface. The smaller borings were attributed to termites, but larger borings were associated to an unknown beetle or moth larvae (Newman, 1993). Borings have been alluded to from Sterkfontein member 5, Sterkfontein Name Chamber, Kromdraai A, Luleche, and Florisbad, but no formal descriptions have been published (Brain, 1981; Adams et al., 2007; Val and Stratford, 2015). Borings from Makapansgat member 3 are reported to penetrate the bone surface at variables angles and range from 4–5 mm in diameter, and were attributed to a member of

CONTACT Alexander H. Parkinson palaeoparky@gmail.com School of Geosciences, University of the Witwatersrand, Private Bag 3, Wits, 2050, South Africa.

the Dermestidae (Kitching, 1980). Insect traces from Sibudu Cave include borings, grooves and star-shaped marks. Grooves and star-shaped marks are also reported from Plovers Lake Cave and Jubilee Shelter Cave (Backwell et al., 2012). Unfortunately, no size data was provided for modifications from these particular sites, but neoichnological experiments suggested termites as the most likely causal agent (Backwell et al., 2012). Similarly, star-shaped marks have also been reports from a site in Ethiopia (Proc Epic Cave) and Tanzania (Laetoli) which were also attributed to termites (Kaiser, 2000; Fejfar and Kaiser, 2005; Backwell et al., 2012). Insect damage to bone from Laetoli include grooves, star shaped marks and subcortical cavities (Kaiser, 2000).

Contextual information

Cooper's D is geographically located in a UNESCO world heritage site aptly named the Cradle of Humankind, Gauteng Province, South Africa (Fig. 1A). Cooper's D represents a cave-fill deposit hosted in a stromatolite-rich dolomite sequence deposited near the Archaean Paleoproterozoic boundary. Cooper's D is stratigraphically positioned in the Monte Cristo Formation of the Malmani Subgroup, Transvaal Supergroup (Eriksson and Truswell, 1974; Eriksson et al., 2006; Martini, 2006). The site has been radiometrically dated at 1.4–1.5 Ma (de Ruiter et al., 2009). Cooper's D was once a 3 × 20 m cave that has been subsequently de-roofed and is now represented by an east-west trending channel (Fig. 1B) (de Ruiter et al., 2009). The decalcified sediments in the channel have been extensively excavated since 2001, with roughly 60 m^2 of sediment removed. Roughly 55,000 bone fragments have been removed and are heavily encrusted in manganese dioxide, which is common for the majority of sites in the region (Cukrowska et al., 2005). The site has been divided into two sections: Cooper's D east and Cooper's D west. Three geological facies have been described from the site: facies A, B, and C (Berger et al., 2003; de Ruiter et al., 2009). Facies A occurs on the eastern side of the deposit where the majority of bones described in this study were recovered from. Only three bones are described from the western side of the deposit in this study and are associated to either Facies B or C. Lithological evidence suggests that the cave was once a closed system and a roof collapse separated the eastern and western sides of the deposit which were subsequently infilled from two separate cave entrances. The cave is believed to have gone through different periods of deposition and nondeposition. The western side of the deposit was dated at 1.4–1.5 Ma, but this date is believed to be the approximate age of the eastern deposits as well. The presence of a talus cone on the eastern side of the deposit is supported by the orientation of the clasts in facies A. Vertical entrances above both sides of the deposit is supported by abundance of taxa with good climbing abilities (primates and carnivores), and an overhanging roof is supported by the abundance of microfauna typically accumulated by owls (Berger et al., 2003; Val et al., 2014). A previous taphonomic analysis of the deposit examined 8,488 bones, which suggested 1% of the sample showed carnivore damage and only 0.3% had gastric acid etching (de Ruiter et al., 2009). No other taphonomic damage was reported. Carnivore to ungulate ratios were previously used to suggest hyena as the predominant bone accumulating agent (de Ruiter et al., 2009), but a more recent taphonomic analysis suggests alternating occupation by large bodied baboons and a diversity of carnivores (Val et al., 2014). A number of hominin remains attributed to *Australopithecus robustus* have been reported from the Cooper's D deposit, including isolated teeth, two juvenile mandibular fragments, an adult thoracic vertebra and a partial cranium (Shaw, 1939; Berger et al., 1995; Steininger et al., 2008; de Ruiter et al., 2009). Insect traces in bones have not been reported from Cooper's D until now.

Materials and methods

All faunal specimens larger than 20 mm from the Cooper's D collection were inspected (roughly 9,500 bone fragments) for macroscopic traces of insect activity, and 53 bone specimens were identified as such. All fragments were identified in terms of skeletal element, then to at least taxonomic family group, and where possible, bovids were identified to a size class sensu Brain (1974) (Table 1). Each modification type was spatially mapped on a generalized quadruped skeleton to show their associated skeletal distribution. This was done using ArchGIS 10.3 sensu Orton (2010). The weathering stage was recorded for each bone specimen sensu Behrensmeyer (1978). All specimens were coated to variable degrees in manganese dioxide, and in some instances, the manganese had been removed prior to this study sensu Cukrowska et al. (2005). All specimens were analyzed using an Olympus SZX 16 Multifocus microscope fitted with a digital camera at magnifications between 7 and 115x. Modifications were categorized and recorded in a table. Terminologies used to describe the morphology of traces is based on a recent summary of general morphologies of bioerosional traces in bone (Pirrone et al., 2014), but the terms striae and grooves as well as bore and tubes have been synonymized. Variables chosen for consideration in this study are drawn from the recently proposed ichnotaxobases for such traces, including presence or absence of bioglyphs or filling, site of emplacement, nature of branching, and the pattern of occurrence (Pirrone et al., 2014). Length and width measurements were taken using Stream Essentials © image processing software linked to the Olympus SZX Multifocus microscope.

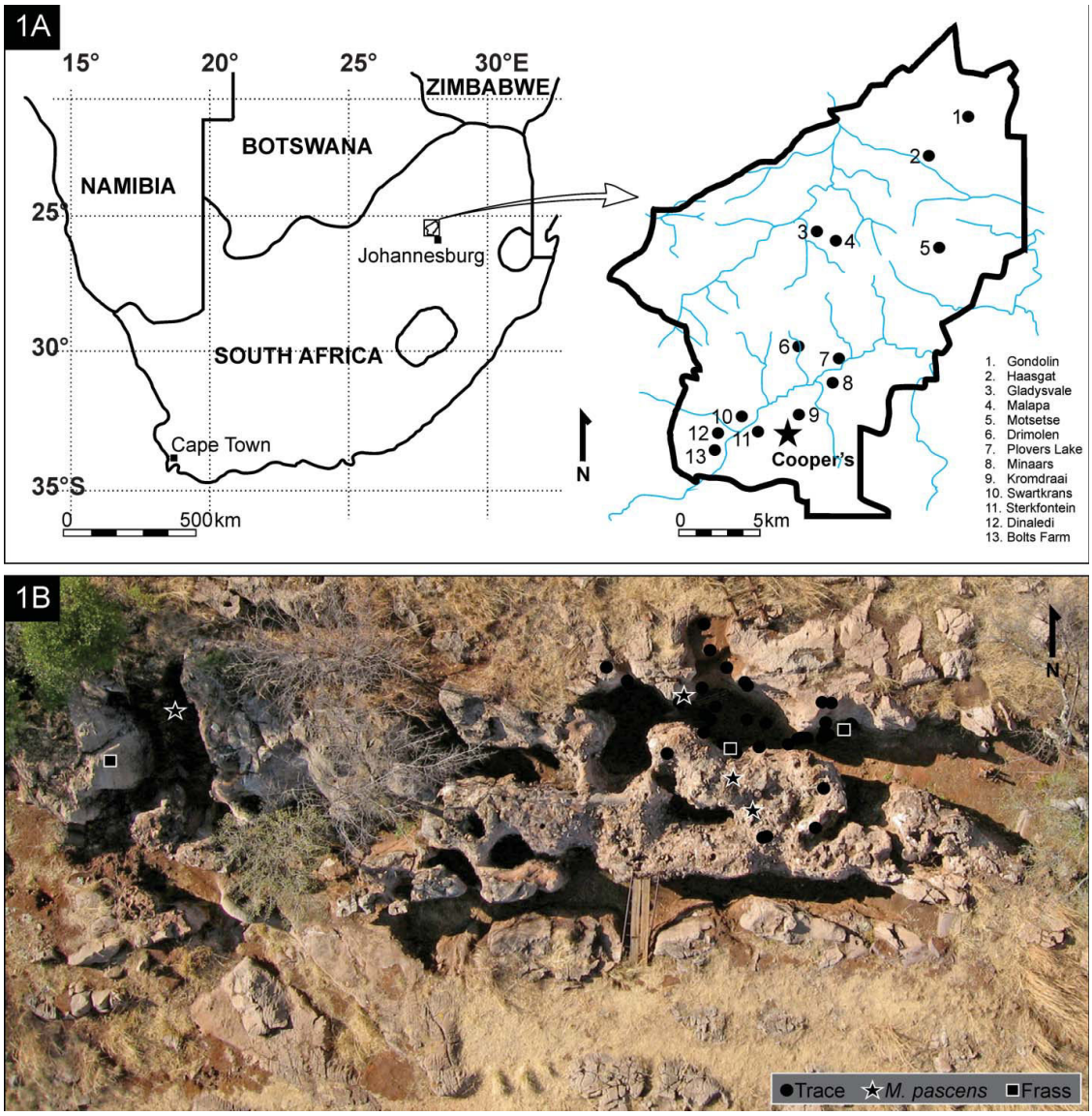

Figure 1. A. Map of South Africa indicating the geographic position of the Cradle of Humankind (COH) and a map of the COH indicating the position of Cooper's D and other fossil sites in the area. B. Aerial photograph of Cooper's D showing the spatial distribution of the bone specimens discussed in this study.

Depth measurements were obtained using digital callipers. Measurements are in millimetres (mm) unless the measurement is less than 1 mm; then measurements are presented as micrometres (μm). Descriptive statistics were calculated in IBM SPSS version 20. The spatial distribution of bone specimens was mapped in ArcGIS 10.3 and then overlaid on a photograph of the deposit. Modifications recorded in the assemblage were compared with those found at other Plio-Pleistocene cave sites in the region and with available experimental data gleaned from the literature. "Clustered" refers to traces which are in direct contact with one another, whilst "associated" refers to instances in which multiple traces are found on a single skeletal element but are not in direct contact with one another. All specimens reported in this study are curated at the Evolutionary Studies Institute, University of the Witwatersrand, Johannesburg, South Africa. All instances of the diagnosed trace taxa reported in this study have been allocated accession numbers starting at BP/1/705.

Results

A total of 53 bones recorded traces of insect activities, and their spatial distribution across the site is indicated in Fig. 1B. The plan and cross-sectional views of the trace morphologies observed are presented in Fig. 2. One trace morphology was sufficiently distinctive to diagnose a new ichnotaxon: *Munitusichnus pascens* (Figs. 2 and 3). The most frequently recorded chamber morphology (Figs. 2B, 4B, 4C) is diagnosed as a new ichnospecies belonging to

Table 1. Summary table of specimens and traces identified on faunal remains from Cooper's D.

Accession Number*	Family	Element	Weathering Stage	*M. pascens* (n = 4)	*C. cooperii* (n = 18)	Chambers (n = 4)	Tubes (n = 67)	Holes (n = 21)	Furrows (n = 5)	Striae (n = 10)		Figure References
										Type 1	Type 2	
18	Bovidae	Proximal Epiphysis Radius	0	—	—	—	1	—	—	—	—	
10084	Indeterminate	Rib Fragment	0	—	—	—	1	—	—	—	—	
10813	Indeterminate	Distal Humerus	0	—	—	—	2	—	—	—	—	
10819	Bovidae	Proximal Epiphysis Femur	0	—	—	—	2	1	—	—	—	
1327	Indeterminate	Proximal Epiphysis Femur	0	—	—	Oblique Oval	2	—	—	—	—	9B
13294	Bovidae	Proximal Radius	0	—	—	—	1	—	—	—	—	
1431	Bovidae	Distal Phalanx	0	—	—	—	1	1	—	—	—	
1570	Suidae	Pelvis Fragment (3 pieces)	0	—	—	—	2	2	1	—	—	5C, 6C, 7B, 9C, 9D
1628	Suidae	Proximal Femur	0	—	2	—	6	—	1	2	—	5B, 8C
164	Indeterminate	Proximal Phalanx	0	—	—	—	1	—	—	—	—	
1641	Indeterminate	Proximal Femur	0	—	2	—	5	—	—	—	—	4B
16699	Indeterminate	Vertebra (thoracic)	0	—	—	—	1	—	—	—	—	
20658	Suidae	Vertebrae (cervical)	0	1	—	—	2	—	—	—	—	3D
2112	Indeterminate	Carpal	0	—	—	—	1	—	—	—	—	
2859	Indeterminate	Distal Epiphysis Femur	0	1	—	—	—	—	—	—	—	
3130	Bovid II	Sacrum	0	1	—	—	4	1	—	—	—	3C
3664	Suidae	Proximal Ulna (epiphyseal plate)	0	—	—	—	1	—	—	—	—	
3711	Bovid II	Distal Femur	0	1	—	—	—	—	1	—	—	3B
3921	Indeterminate	Vertebra (thoracic)	0	—	—	—	—	1	—	—	—	
3994	Indeterminate	Distal Phalanx	0	—	—	—	1	—	—	—	—	
5658	Indeterminate	Proximal Epiphysis Humerus	0	—	—	—	2	—	—	—	—	
5799	Indeterminate	Indeterminate	0	—	—	—	1	—	—	—	—	
5880	Bovidae	Carpal	0	—	1	—	1	—	—	—	—	
6004	Indeterminate	Vertebra (thoracic)	0	—	—	—	1	—	—	—	—	
6062	Bovid III	Distal Epiphysis Femur	0	—	—	—	2	4	—	—	—	
6080	Indeterminate	Vertebra (thoracic)	0	—	1	—	—	—	—	—	—	
6139	Suidae	Astragulas	0	—	—	—	2	—	—	—	—	
762	Felidae	Distal Tibia	0	—	—	—	1	—	—	—	—	
7710	Indeterminate	Carpal	0	—	1	—	4	—	—	—	—	
7887	Suidae	Humerus, Shaft Fragment	0	—	—	—	—	—	1	—	—	
7894	Bovidae	Distal Femur	0	—	—	—	1	—	—	—	1	8D
7904	Indeterminate	Distal Phalanx	0	—	—	—	1	—	—	—	—	
7905	Bovidae	Distal Metapodial	0	—	—	—	1	—	—	1	—	
8487	Bovidae	Tarsal (navicular)	0	—	—	—	1	—	—	—	—	
8546	Bovid II	Distal Metatarsal	0	—	—	—	—	—	—	—	1	
9930	Bovidae	Astragulas	0	—	1	—	—	—	—	—	1	
1367	Bovidae	Calcaneus	1	—	1	—	2	—	—	—	—	4C
189	Bovidae	Astragulas	1	—	—	—	1	—	—	1	—	
2794	Bovidae	Proximal Radius	1	—	—	—	2	—	—	—	—	
2870	Suidae	Calcaneus	1	—	—	—	2	4	—	—	—	5D, 6B
3477	Indeterminate	Proximal Phalanx	1	—	—	—	2	—	—	—	—	
3787	Indeterminate	Radius Shaft Fragment	1	—	—	Elongated	—	—	—	—	—	4E
5269	Bovidae	Distal Humerus	1	—	—	—	1	—	—	—	—	
613	Suidae	Proximal Phalanx	1	—	3	—	1	—	—	1	—	
6743	Bovid III	Distal metatarsal	1	—	—	Ovoid	—	—	—	—	—	4D
7779	Bovidae	Proximal Radius	1	—	—	—	1	—	—	—	—	
853	Indeterminate	Long Bone Shaft Fragment	1	—	—	—	—	4	—	—	—	6D
8566	Bovid II	Distal Humerus	1	—	—	Oblique Oval	—	—	—	—	—	4F, 9A
17339	Indeterminate	Proximal Phalanx	2	—	1	—	—	—	1	1	—	
859	Bovidae	Horn core	—	—	—	—	1	—	—	—	—	
10870	Bovidae	Distal Metatarsal	1 (An), 3 (Pt)	—	—	—	1	—	—	—	—	
6092	Bovidae	Proximal Phalanx	1 (Dr), 3 (Vt)	—	3	—	—	—	—	1	—	8B
9627	Bovidae	Distal Tibia	1 (Dr), 3 (Vt)	—	2	—	1	3	—	—	—	

*Accession number for fossils skeletal remains prefix is U.W.27 -; Weathering stages following (Behrensmeyer, 1978).
An – Anterior surface, Pt - Posterior Surface, Dr - Dorsal Surface, Vn - Ventral Surface.

the ichnogenus *Cubiculum* (Roberts et al., 2007). Three other chamber morphologies were also identified (Figs. 2C, 4D–F). A number of other general trace morphologies were identified, including bores (Figs. 2D, 5), holes (Fig. 2E, 6), furrows (Figs. 2F, 7), and grooves (Figs. 2G, 8). Frass and coprolites were also identified in association with selected traces (Fig. 9).

Systematic ichnology

Ichnogenus *Munitusichnus* New Ichnogenus

Type ichnospecies: Munitusichnus pascens n. isp.

Etymology: Munitus (Latin) meaning fortified, protected, defended, or sheltered. Refers to the inferred behavior associated to the trace.

Diagnosis: A main circular boring penetrating cortical bone at an acute oblique angle relative to the bone surface, with a lateral crescent shaped boring that partly surrounds the main boring (Figs. 2A, 3B–D).

Munitusichnus pascens isp. nov.

Type material: The holotype (BP/1/705, Fig. 3B) occurs on a distal femur of a bovid size class II (U.W.27-3711), the paratype (BP/1/706, Fig. 3C) occurs on the partial sacrum of bovid size class II (U.W.27-3130). All specimens are curated at the Evolutionary Studies Institute, University of the Witwatersrand, South Africa.

Referred specimens: Four bone specimens each record a single occurrence of the ichnospecies (U.W.27-3711, U.W.27-3130, U.W.27-2859, U.W.27-20658). See Table 1 for associated taxa and skeletal elements.

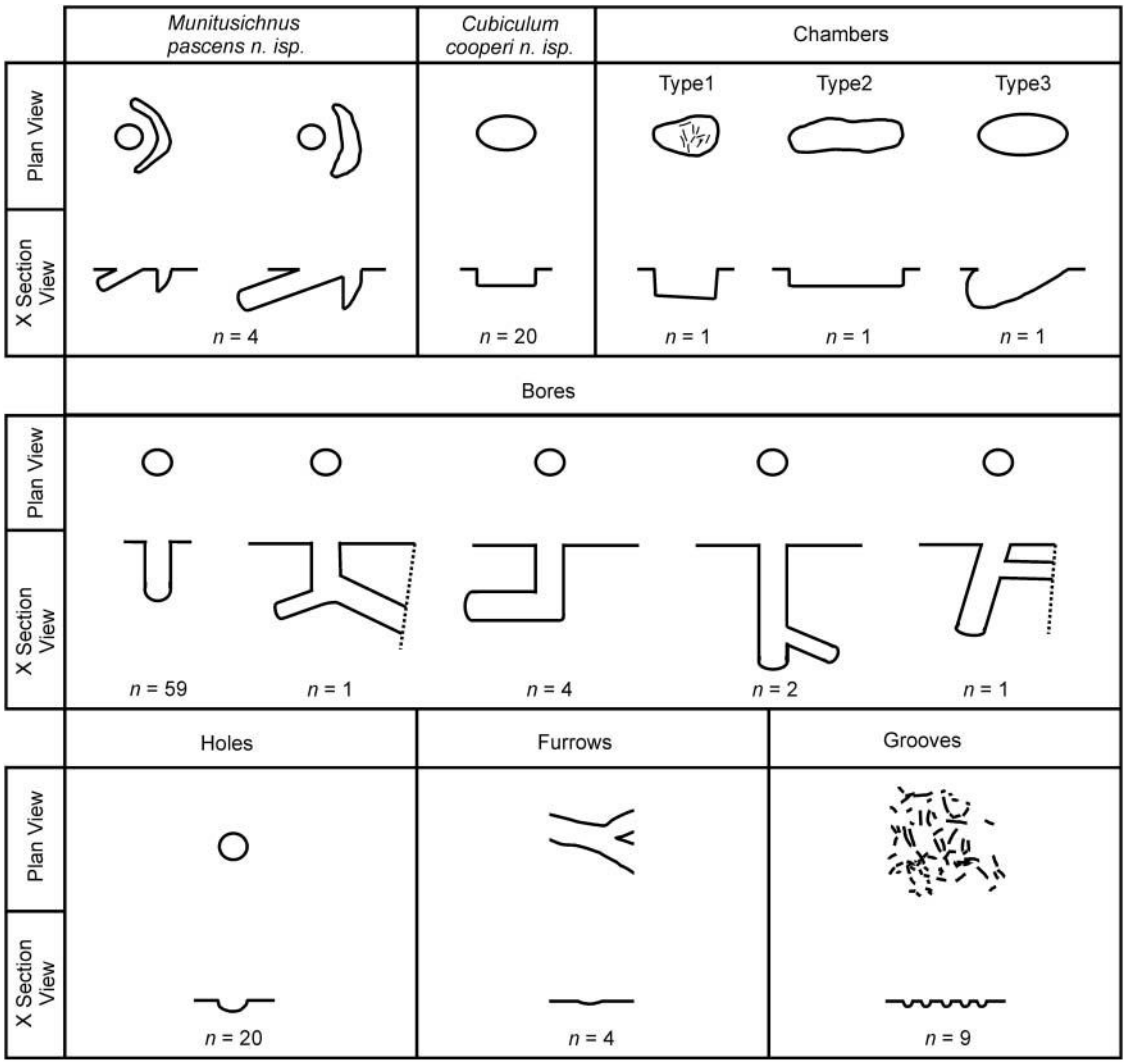

Figure 2. Plan and cross-section view of the trace morphologies identified at Cooper's D, following general morphologies recently summarized by Pirrone et al. (2014).

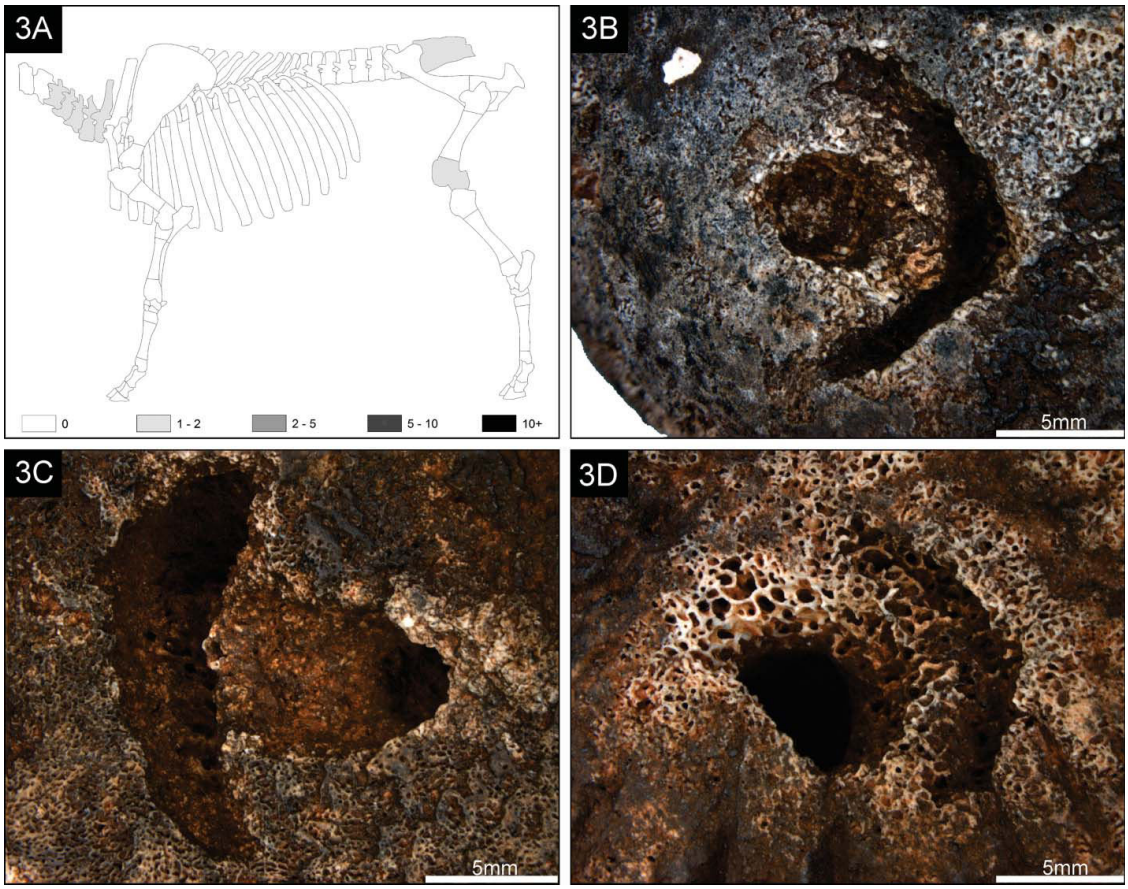

Figure 3. A. Spatial distribution of *Munitusichnus pascens* across a generalized quadruped skeleton. B. Holotype of *Munitusichnus pascens* BP/1/705. C. Paratype of *Munitusichnus pascens* BP/1/706 both recovered from Cooper's D eastern deposit. D. *Munitusichnus pascens* on U.W.27-20658 recovered from Cooper's D western deposit.

Type locality: Specimens were all recovered from the decalcified sediments of Cooper's D fossil locality within the Cradle of Humankind, South Africa.

Etymology: pascens (Latin) meaning feeding.

Diagnosis: Only known ichnospecies, same as for the ichnogenus.

Description: *Munitusichnus pascens* occurs as an individual trace and never clustered, excavated through the cortical lamellae, and terminates in trabecular bone, with no evidence of filling or associated bioglyphs. The trace is made up of two component morphologies, a main boring as well as a crescent shaped boring (Figs. 2A, 3B–D). The main boring has an average diameter of 5 mm with a depth range of 7–12 mm, whilst the crescent has a maximum width of 2.5 mm and average depth of 6 mm. The crescent wall closest to the main boring is orientated perpendicular to the bone surface, whilst the opposite wall curves outwards. The holotype (BP/1/705, Fig. 3B) is the best preserved specimen and shows that both the boring and crescent are separated by a wall of bone which is connected to the bone surface. However, on all other referred specimens (BP/1/706 - BP/1/708) the interconnecting wall does not remain connected to the bone surface and has either been removed by the trace maker, or eroded as a result of another bioerosional process. These two component morphologies (boring and crescent) are interpreted as being representative of a single trace due to the following; all other borings (except one) identified at Cooper's are excavated perpendicular to the bone surface (Fig. 2D) and not at an acute oblique angle as is the case for *Munitusichnus pascens*, and no other isolated crescent shaped excavations have been identified at Cooper's D. The absence of the individual components of *M. pascens* (i.e., main obliquely orientated boring, and the crescent shaped boring) occurring in isolation, supports the interpretation that these two components constitute a single trace. The trace was found to co-occur with other modification morphologies described later in this study including; bores, furrows and holes. The spatial distribution of *M. pascens* across a generalized quadruped skeleton (Fig. 3A) shows that the trace is restricted to the less dense skeletal elements (i.e., epiphysis, sacrum or vertebra).

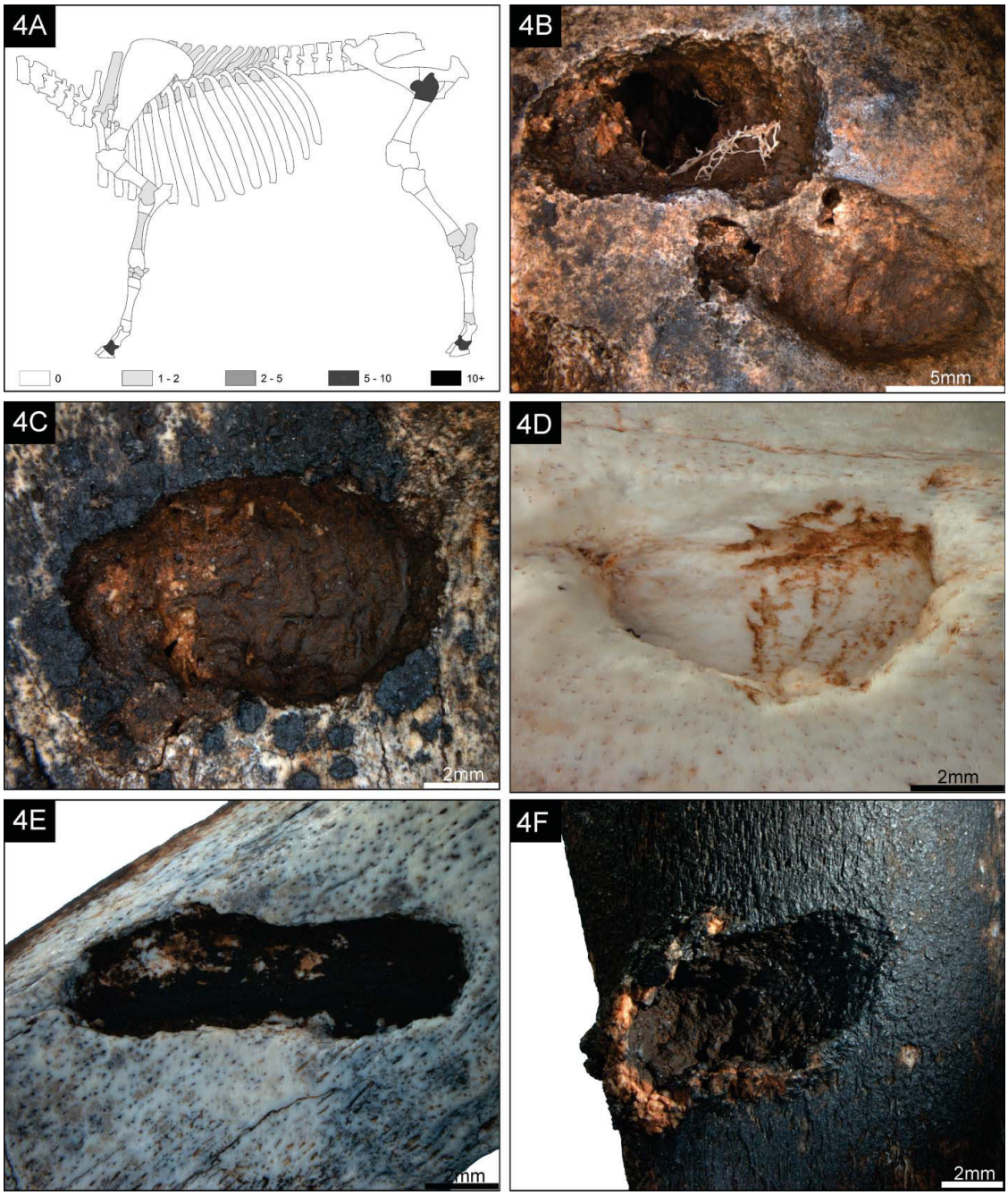

Figure 4. A. Spatial distribution of *Cubiculum cooperi* across a generalized quadruped skeleton. B. Holotype (BP/1/709) of *C. cooperi* on U.W.27-1641. C. *C. cooperi* on U.W.27-1367. D. Ovoid chamber (Type 1) recorded on U.W.27-6743. E. Elongated chamber (Type 2) recorded on U.W.27-3787. F. Obliquely orientated oval chamber (Type 3) and frass identified on U.W.27-8566.

Ichnogenus *Cubiculum* (Roberts et al., 2007; Pirrone et al., 2014; Xing et al., 2015)

Type *Ichnospecies: Cubiculum ornatus* (Roberts et al., 2007)

Diagnosis (Xing et al., 2015): Discrete ellipsoidal borings in bone. Hollow, oval borings bored into inner spongy and outer cortical bone surfaces. Boring length two to six times greater than its diameter. Structures may be isolated but more commonly form dense, locally overlapping concentrations.

Remarks: The recent emendation of the ichnogeneric diagnosis by Xing et al. (2015) is suitable to allow for the incorporation of *Cubiculum cooperi*. Thus no further emendation is proposed.

Cubiculum cooperi isp. nov.

Type material: Holotype (BP/1/709, Fig. 4B) is one of two locally overlapping structures recorded on the proximal femur of a juvenile vertebrate of an indeterminate taxon (U.W.27-1641). All specimens are curated at the

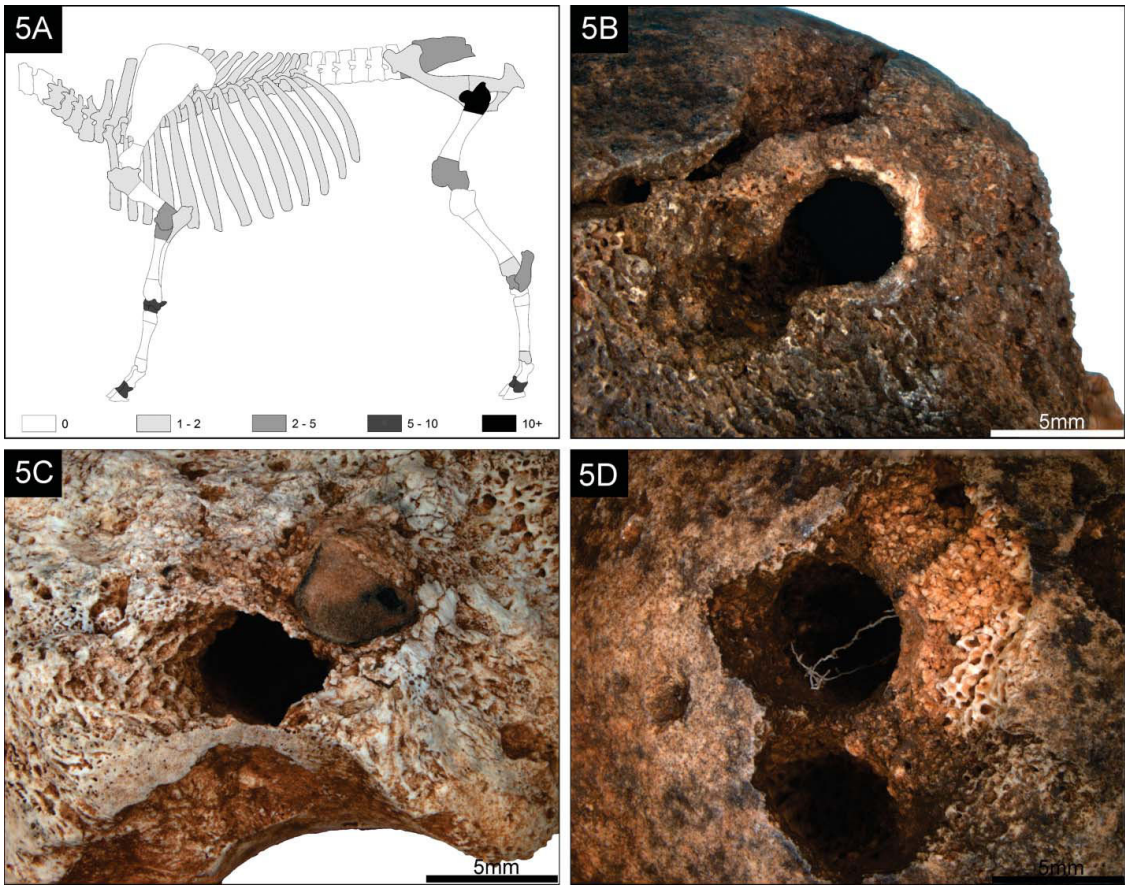

Figure 5. A. Spatial distribution of bores across a generalized quadruped skeleton. B, C. Individual bores recorded on U.W.27-853 and U.W.27-1570, respectively. D. The only occurrence of two clustered bores on U.W.27-2870.

Evolutionary Studies Institute, University of the Witwatersrand, South Africa.

Referred specimens: A total of 11 bone specimens record either an individual structure (U.W.27-1367, U.W.27-17339, U.W.27-5880, U.W.27-6080, U.W.27-7710, U.W.27-9930) or a cluster of structure (U.W.27-1628, U.W.27-1641, U.W.27-6092, U.W.27-613, U.W.27-9727) of this ichnospecies. See Table 1 for associated taxa and skeletal elements.

Type locality: Specimens were all recovered in the decalcified sediments from the Cooper's D fossil locality within the Cradle of Humankind, South Africa.

Etymology: "Cooper" derived from the site name of Cooper's Cave from which the type ichnospecies was discovered.

Diagnosis: Discrete oval borings embedded in bone with straight walls running perpendicular to the bone surface. Bottoms are flat and run parallel to the bone surface (Figs. 2B, 4B, 4C). The contact between the walls and the bottom are predominately sharp but may be slightly rounded. The length is roughly double the maximum width. The trace either occurs individually (Fig. 4C) or in clusters (Fig. 4B) of two to four borings. In clusters, traces infrequently intersect (Fig. 4B) but in general show no consistent orientation to one another.

Description: Neither filling nor bioglyphs are associated with this trace. Descriptive statistics for *Cubiculum cooperi* is presented in Table 2. *Cubiculum cooperi* is distinguishable from *Cubiculum ornatus* (Roberts et al., 2007) based on the lack of the following features: bioglyphs, concave flanks and the length being three to four times that of the diameter. *Cubiculum cooperi* is also distinguishable from *Cubiculum levis* (Pirrone et al., 2014) due to the lack of pronounced concavity of the flanks, constriction of the entrance which both culminate in a general bowl shaped morphology. *Cubiculum inornatus* records a rounded base and has a high width to length ratio, neither of which are evident in *C. cooperi* (Xing et al., 2015). *Cubiculum cooperi* was found to co-occur with a number of other trace morphologies on individual bones including bores, furrows, holes and grooves (Table 1). All associated trace morphologies are described in the proceeding sections, but *C. cooperi* was not found to co-occur with *Munitusichnus pascens*. The spatial distribution of this new ichnospecies across a generalized quadruped skeleton (Fig. 4A) shows that it is most frequently recorded on phalanges, and an epiphysis

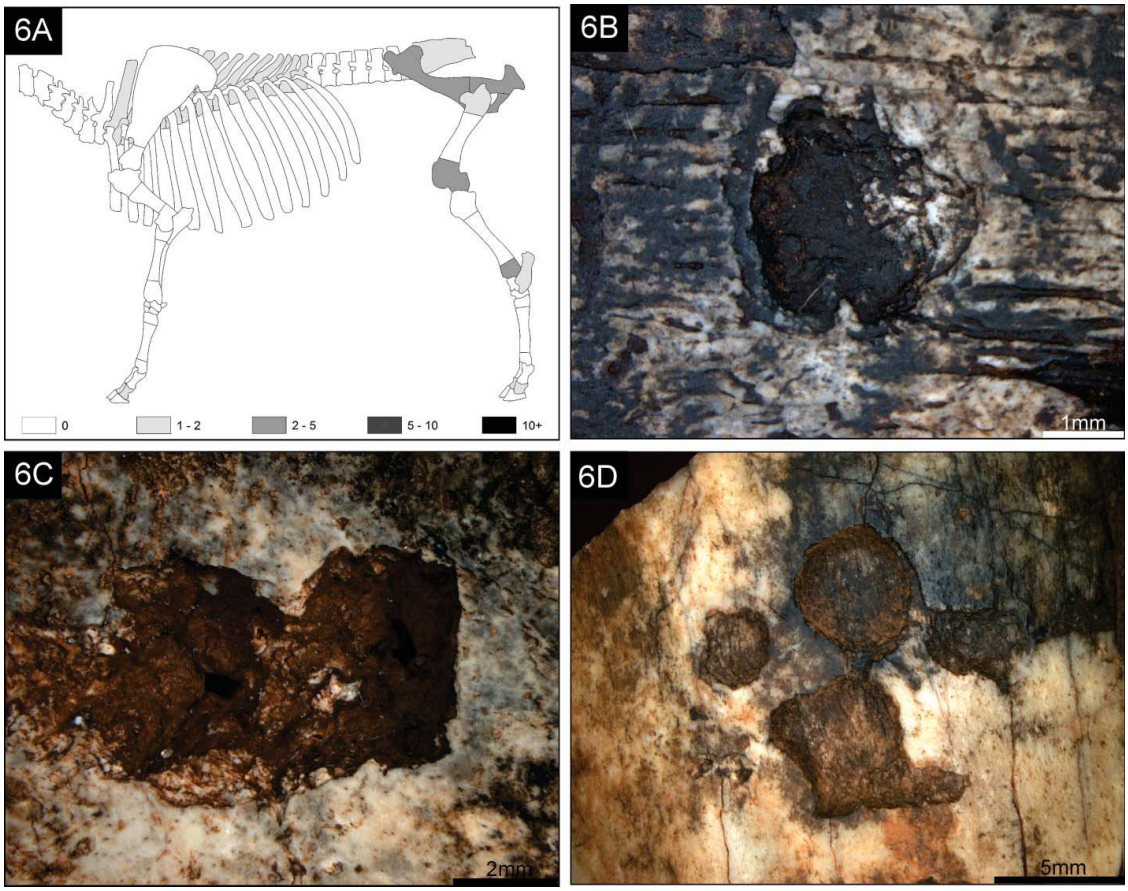

Figure 6. A. Spatial distribution of holes across a generalized quadruped skeleton. B. Individual hole recorded on U.W.27-2870. C. Two clustered holes recorded on U.W.27-1570. D.Four clustered holes on U.W.27-853.

of a long bone. Two instances were recorded in which the entrance of a bore (described later) was located at the bottom of *C. cooperi* (Fig. 4B) suggesting that the creation of bores potentially postdates the creation of the *C. cooperi*. Comparative measurement data for reported *Cubiculum ichnotaxa* is summarized in Table 2.

Other trace morphologies

A single representative occurrence of both an ovoid (type 1) and elongated (type 2), and only two instances of obliquely orientated oval chambers (type 3) were identified in the sample. The plan and cross-section views of each are presented in Fig. 2C. It is the author's opinion that the restricted sample sizes do not warrant the establishment of independent ichnotaxa. All three bones in which the chambers were recorded displayed weathering stage 1, sensu Behrensmeyer (1978).

Type 1: Ovoid chamber (Fig. 4D). A single chamber recorded on a metatarsal shaft fragment belonging to a bovid size class III (U.W.27-6743). The chamber is rounded at one end and constricted at the other, giving it a characteristic egg like (ovoid) shape. The bottom of the chamber does not run parallel to the bone surface but is angled downwards towards the rounded end of the chamber. The walls are angled inwards towards the center of the chamber, and thus the base appears slightly constricted when compared to the entrance. No evidence of fill was identified. The walls as well as the base of the chamber display bioglyphs. The following descriptive statistics were obtained for the length of the bioglyphs: minimum 0.9 mm, maximum 1.3 mm, mean 1.1 mm, and std. deviation 0.18 mm. The chamber itself measures 10 mm in length, with a 5 mm maximum diameter, and ranges from 2–3 mm deep. The unique ovoid shape, angled base and walls, as well as the presence of bioglyphs differentiate this morphology from all other reported *Cubiculum* ichnospecies including: *C. cooperi, C. ornatus*, *C. levis*, and *C. inornatus* (Roberts et al., 2007; Pirrone et al., 2014; Xing et al., 2015).

Type 2: Elongated Chamber (Fig. 4E). A single chamber recorded on a shaft fragment of a radius belonging to an indeterminate mammal taxon (U.W.27-3787). The elongated ellipsoidal shape of the chambers has a length (13 mm) three times that of the associated width (4 mm)

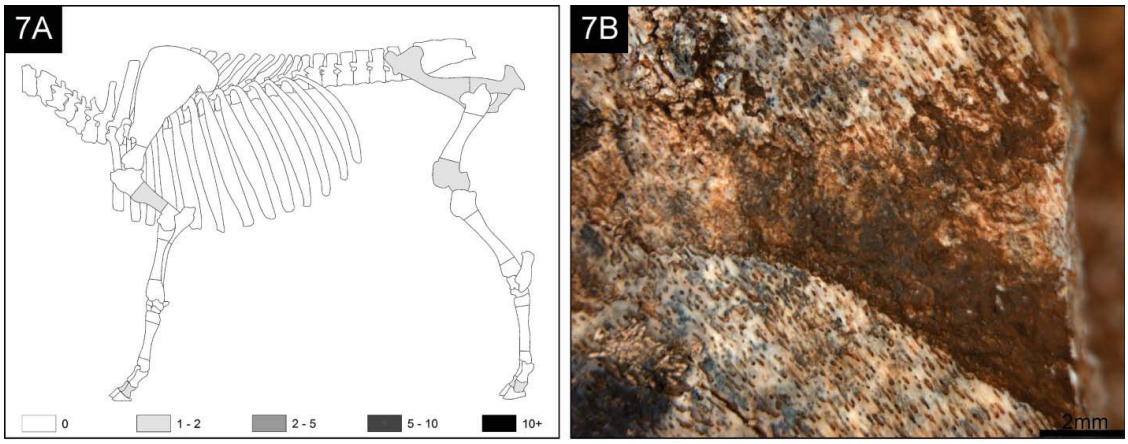

Figure 7. A. Spatial distribution of furrows across a generalized quadruped skeleton. B. Individual furrow recorded on U.W.27-1570 showing a forked branching pattern.

and is 2 mm deep. Slight constriction was noted in the middle of the chamber. The base of the chamber is orientated parallel to the bone surface, whilst the walls are slightly concave. The only morphological characteristic which differentiates this elongated chamber from *Cubiculum ornatus* (Roberts et al., 2007) is the absence of bioglyphs. However, *C. ornatus* is also characterized by dense concentrations of the traces on single skeletal elements and the traces regularly intersect (Roberts et al., 2007). This pattern of occurrence is clearly absent from our current sample. Thus this elongated chamber is distinguishable from *C. ornatus* and bears no similarities to either *C. cooperi*, *C. levis*, or *C. inornatus* (Pirrone et al., 2014; Xing et al., 2015).

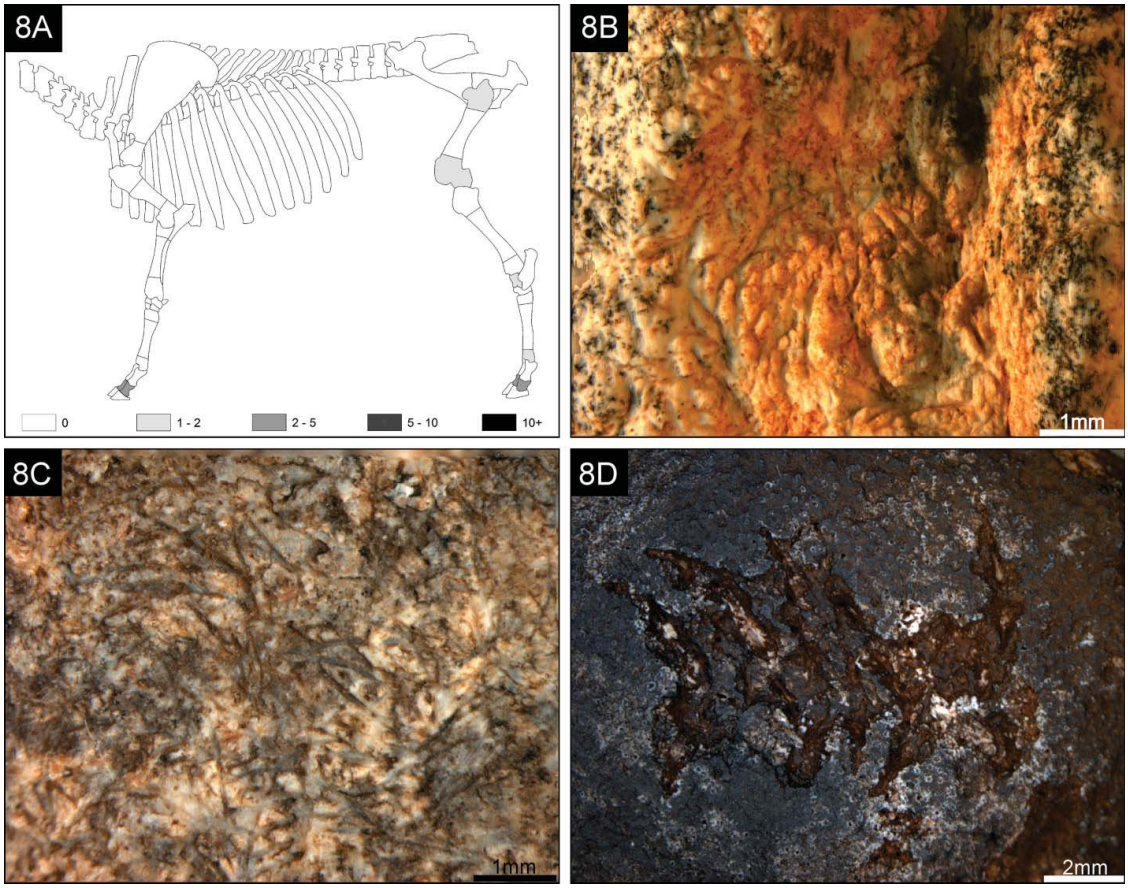

Figure 8. A. Spatial distribution of grooves across a generalized quadruped skeleton. B, C. Clusters of overlapping, intersecting, and randomly orientated grooves recorded on U.W.27-6092 and U.W.27-1628, respectively. D. Concentration of relatively parallel grooves recorded on U.W.27-7894.

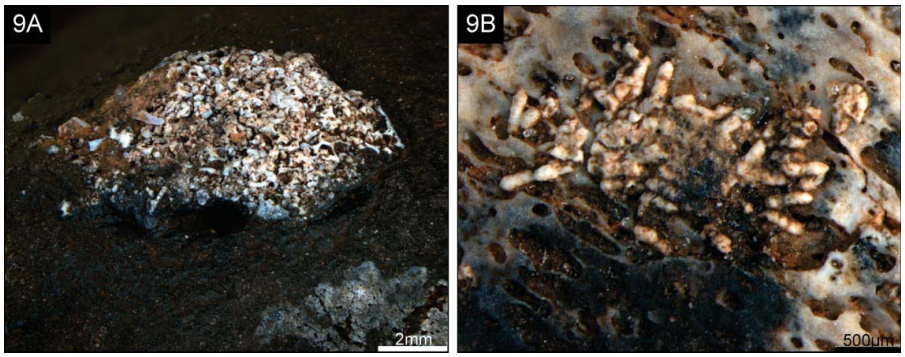

Figure 9. A. Pile of frass associated with a small bore on U.W.27-1327. D. Pile of coprolites preserved close to the furrow (Fig. 7B) on U.W.27-1570.

Type 3: Obliquely orientated oval chamber (Fig. 4F). Two bones recorded a single oval chamber which was obliquely orientated to the bone surface. One was recorded on a shaft fragment of a humerus belonging to a bovid size class II (U.W.27-8566), and the other was recorded on the proximal epiphysis of a femur belong to an indeterminate taxon (U.W.27-1327). In plan view, the chambers display an oval shape (9.5 mm × 4.2 mm) but the cross-sectional view is the most distinctive. The chambers have been constructed at an oblique acute angle relative to the bone surface. Thus, the back wall of the chamber is extremely concave, and from the bottom of the wall the base consistently projects upwards until it comes into contact with the original bone surface (Fig. 2, Type 3). Neither filling nor bioglyphs were identified in the chamber. Type 3 morphology bears no resemblance to any existing *Cubiculum* ichnospecies (Roberts et al., 2007; Pirrone et al., 2014; Xing et al., 2015).

Bores (Fig. 5). A total of 67 bores were recorded across 40 different bone fragments, and their spatial distribution across a generalized quadruped skeleton is displayed in Fig. 5A. In plan view, the entrance of the bore is circular, whilst in cross-section, the bore travels perpendicularly (except in one instance) to the bone surface to a variable depth and has straight walls and a rounded base. In six instances, the bore completely passed through the bone. The vast majority do not display branching (n = 59), but eight bores recorded four different branching styles (Fig. 2). Style 1 is not representative of branching, but rather a directionality change and is characterized by a sharp 90° turn in the bore which then runs parallel to the bone surface and terminates. Due to the fragmentation of the bone specimens on which Style 2 is recorded, it is unclear whether the primary branch either changes direction or records false branching as a result of accidental intersection (D'Alessandro and Bromley, 1987; Pirrone et al., 2014). Similarly, Style 4 could either be interpreted as secondary successive branching sensu D'Alessandro and Bromley (1987) or once again as false branching due to accidental intersection (D'Alessandro and Bromley, 1987; Pirrone et al., 2014). Despite the differences in gross morphology between styles 2, 3, and 4, they all share a common characteristic; all three have a secondary branch with a diameter two thirds that of its associated primary branch. The size difference may be indicative of secondary successive branching (sensu D'Alessandro and Bromley, 1987), which means the two branches were produced by

Table 2. Measurement data from traces identified at Cooper's D as well as from other data gleaned from the literature.

		Maximum Diameter (mm)				Minimum Diameter (mm)				Citation
		Minimum	Maximum	Mean	Std. Deviation	Minimum	Maximum	Mean	Std. Deviation	
						Chambers				
Ichnotaxa	C. cooperii	5.90	14.20	9.90	3.00	3.80	7.20	5.60	1.10	—
	C. ornatus	5.20	41.70	15.70	5.60	1.30	9.00	4.60	1.50	Roberts et al. 2007
	C. levis	4.00	9.00	—	—	3.00	5.00	—	—	Pirrone et al. 2014
						Tubes				
Site	Cooper's D	0.80	9.20	5.30	1.80	0.80	7.90	4.40	1.40	—
	Swartkrans	2.00	5.00	—	—	—	—	—	—	Newman, 1993
	Makapansgat	4.00	5.00	—	—	—	—	—	—	Kitching, 1980
Experimental Data	Termites	3.41	4.20	—	—	2.64	2.83	—	—	Backwell et al. 2012
	Deremstids	0.21	0.68	0.44	0.13	0.14	0.63	0.33	0.16	Parkinson, 2012
						Holes				
Site	Cooper's D	1.40	8.60	4.90	2.10	1.00	7.20	3.60	1.50	—
Experimental Data	Termites	1.97	3.63	—	—	1.54	3.09	—	—	Backwell et al. 2012

different individuals during different boring intervals. The majority of bores occurred as an individual trace (Figs. 5B, 5C) with only a single instance of two bores being recorded as clustered (Fig. 5D). However, as many as two (n = 10), three (n = 1), four (n = 2), five (n = 1), or six (n = 1) bores were recorded on individual bones as "associated" but were not considered clustered. Four bores were embedded in trabecular bone with no point of entry on the cortical surface, which either suggest modification post-fragmentation, or is a product of preservation. Descriptive statistics for width of bore entrances are summarized in Table 2. Descriptive statistics for depth were calculated in two ways: including all specimens (minimum 4.5 mm; maximum 110 mm; mean 14 mm; std. deviation 17.5 mm) and excluding a single specimen (minimum 4.5 mm; maximum 28 mm; mean 11 mm; std. deviation 5 mm). The reason for the exclusion of the single specimen is that the depth of the bore was recorded at over 110 mm, which is anomalous. Bores were found in association with the majority of trace morphologies and ichnotaxa described in this study. Bores do not record evidence of filling or bioglyphs.

Holes (Fig. 6). A total of 21 holes were recorded across nine different bone fragments, and their spatial distribution across a generalized quadruped skeleton is presented in Fig. 6A. In plan view, holes display a circular morphology, whilst in cross-section the profile is bowl shaped (Fig. 2). Descriptive statistics for the width of hole entrances is summarized in Table 2, whilst the following was obtained for depth: minimum 1 mm; maximum 5 mm; mean 2.8 mm; std. deviation 1.1 mm. Measurement data suggests that holes likely represent incipient bores; however, based on the pattern of occurrence this is clearly not always the case. All bores (except in one instance) were recorded as individual traces, whereas holes were recorded as either single (Fig. 6B), or in clusters of two, three, or four (Figs. 6C, 6D), which supports the establishment of an independent category. Holes were found in association with bores in all except two instances but were only found in single instances each with furrows, *Munitusichnus pascens* and *Cubiculum cooperi*. Holes were not found in association with grooves. The majority of specimens on which holes occurred displayed weathering stage 0 or 1 (*sensu* Behrensmeyer, 1978).

Furrows (Fig. 7). Five furrows were identified on four separate bone fragments, and their spatial distribution across a generalized quadruped skeleton is presented in Fig. 7A. Furrows are meandering trails, with no discernible walls, roughly excavated into cortical bone, and in cross-section have shallow rounded profile (Fig. 2F). In two of the four recorded instances, bioglyphs were discernible, and branching was only identified in a single instance (Fig. 7B). The bioglyphs are comparable to Type 1 grooves described below. Furrows measured 3–4 mm wide, 12–47 mm in length and roughly 0.6 mm deep. They were found in association with all other traces described in this study.

Grooves (Fig. 8). Grooves were recorded on nine bone fragments, and their spatial distribution across a generalized quadruped skeleton is presented in Fig. 8A. Grooves range from straight to slightly curved, which have a U-shaped profile (Fig. 2). Type 1 grooves are found in dense clusters, having no consistent orientation to one another, but consistently intersect and overlap (Figs. 8B, 8C). The length of grooves associated to two of these clusters were measured, and the following descriptive statistics were obtained: cluster one (Fig. 8B) (minimum 42 μm; maximum 518 μm; mean 200 μm; std. deviation 109 μm) and cluster two (Fig. 8C) (minimum 195 μm; maximum 913 μm; mean 532 μm; std. deviation 176 μm). Despite the consistent pattern of occurrence and general morphology, the length of striations between the different specimens is highly variable. However, experimentation has shown that groove length is highly variable even when produced by a single insect species (Backwell et al., 2012; Parkinson, 2013); thus variability in the sample does not necessarily suggest multiple agents. However, Type 2 grooves occur in small groups of relatively parallel striae that occasionally intersect (Fig. 8D). The available sample was substantially smaller for Type 2, but the following descriptive statistics were obtained for their length (minimum 2.5 mm; maximum 6.6 mm; mean 3.9 mm; std. deviation 1.3 mm). Type 2 can clearly be differentiated from Type 1 based on length, pattern of occurrence, and orientation of the grooves. Grooves were not found in association with *Munitusichnus pascens* or holes. In one instance, grooves were recorded on a specimen displaying either weathering stages 0, 1, 2, or 3 (sensu Behrensmeyer, 1978).

Frass (Fig. 9A). Two structures (BP/1/727 and BP/1/728) were identified in association with chambers on U.W.27-8566 and U.W.27-1327, and both structures have been interpreted as frass. The frass has a granular texture. In one instance forming a pile at the point of contact between the wall of the chamber and the bone surface (Fig. 4F) and projects above the bone surface and extends around roughly 30% the chambers perimeter in plan view. The other pile of frass is positioned next two a small bore entrance and also projects well above the bone surface. In plan view, this pile of frass has an oval morphology (Fig. 9A). The last pile of frass (BP/1/729) was identified next to the entrance of a larger bore on specimen U.W.27-1570. This pile is much smaller and does not project as far above the bone surface as in the previous specimens but is also

granular and encompasses 30% of the perimeter of the bore entrance.

Coprolites (Fig. 9B). Another interesting trace was identified in association with a pile of frass, and in close proximity to a furrow (Figs. 7B, 9B). This trace was a pile (3×1.5 mm in size) of elongated tube-like structures, which appear rounded in cross-section, segmented, with a rounded and a pointed end (Fig. 9B). This is interpreted as a pile of coprolites (BP/1/730). Individual coprolites are discernible along the edges of the pile but in the middle appear slightly flattened, and individual coprolites are less discernible. The following descriptive statistics were obtained for the length (minimum 308 μm; maximum 363 μm; mean 335 μm; std. deviation 17 μm) and width (minimum 84 μm; maximum 125 μm; mean 103 μm; std. deviation 10 μm) of the coprolites.

Discussion

Ethology and taphonomy

It is hypothesized that the agent responsible for the creation of *Munitusichnus pascens* would use the main boring as a protected dwelling space (domichnia) during the late stages of larval development. It would emerge from its dwelling to feed. Feeding would occur in a circular motion relative to the main boring and thus culminate in the crescent shaped excavation (fodinichnia). Ultimately the boring could have functioned as a pupal chamber (pupichnia), as similar circular morphologies are regularly interpreted as such (Kitching, 1980; Rogers, 1992; Martin and West, 1995; Huchet et al., 2013). To the author's knowledge, this behavior has not been observed among extant insects, but the above interpretation is believed to be the most parsimonious explanation of the morphology. The absence of isolated crescent shaped borings, as well as obliquely orientated borings, further supports the interpretation that the two components do not represent individual structures, but rather two components of the same trace. Therefore, *Munitusichnus pascens* is not interpreted as being reflective of a single behavior, but rather records a portion of the lifecycle of an unknown invertebrate but most likely a beetle. Spatial distribution of *M. pascens* across a generalized quadruped skeleton (Fig. 3A) suggests that this trace was produced by a terrestrial invertebrate with mouth parts capable of modifying spongy bone, but perhaps incapable of modifying denser cortical bone. The taphonomic signature associated with *M. pascens* is different from that of *Cubiculum cooperi* as none of the bones on which *M. pascens* was identified displayed signs of weathering. This suggests that the bones were rapidly incorporated into the cave system. The lack of reports of any similar traces (locally or internationally), the limited number of this trace at Cooper's D and its distribution across multiple individuals at the site suggest that this trace is not habitually produced. Thus it could be argued that all instances of *M. pascens* are potentially contemporaneous. Spatially, *M. pascens* was recovered in both the eastern ($n = 3$) and western ($n = 1$) sections of the deposit. It is not possible for faunal remains from the eastern section to be transported to the western section, or vice versa, due to the dolomites separating the two deposits (de Ruiter et al., 2009). Thus two possible scenarios exist. The modification occurred outside of the cave at a location from which materials could be transported to both the eastern and western entrances of the cave. Alternatively, modification could have occurred in an upper cave chamber which was directly connected to the vertical fissures previously reported (de Ruiter et al., 2009) and was able to contribute depositional material to both the eastern and western section of the current deposits. Finding additional evidence of this trace at other sites in the region such as Sterkfontein, Swartkrans, or Kromdraai may provide additional insight into the exact taphonomic circumstances under which *M. pascens* was produced. A similar taphonomic process is proposed for furrows due to similarity in the taphonomic signature and spatial distribution of this trace morphology. Furrows were also recorded on remains from both the eastern and western sections of the site, and the bone on which they were recorded showed no evidence (except in a single instance) of weathering. Interestingly, the hominin remains recovered from Cooper's D have an almost identical spatial distribution to that of *M. pascens* see (de Ruiter et al., 2009, fig. 2b).

The morphology of *Cubiculum cooperi* supports its incorporation into the *Cubiculum* ichnogenus (Roberts et al., 2007; Pirrone et al., 2014). *Cubiculum cooperi* has been interpreted as a pupal chamber (Laudet and Antoine, 2004; Roberts et al., 2007; Kirkland and Bader, 2010; Huchet et al., 2013; Pirrone et al., 2014; Xing et al., 2015). Its distribution on skeletal remains suggests that the causal agent has a limited ability to modify cortical bone, focusing on less dense skeletal elements. The taphonomic signature of *C. cooperi* is distinctive from that of all others traces described in this study. Half of the bones on which *C. cooperi* are identified display weathering stages 1, 2, or 3 (*sensu* Behrensmeyer, 1978). The variability in depth of the trace may be accounted for by the variable presence of soft/desiccated tissue during the construction of the trace (Martin and West, 1995; Hasiotis et al., 1999; Bader et al., 2009; Kirkland and Bader, 2010; Huchet et al., 2013). Lastly, partially preserved *C. cooperi* could be attributed to transportation of the bones either from surface into the cave and later within the

cave. It is thus hypothesised that *C. cooperi* was produced whilst various decomposing carcasses were sub-aerially exposed and that the responsible agent is likely associated with the final stages of decomposition (Kulshrestha and Satpathy, 2001; Byrd and Castner, 2009; Amendt et al., 2010). However, inferring an exact causal agent for the production of *C. cooperi* would merely be speculative due to a lack of actualistic or experimental data.

Bores record the greatest diversity of associated taphonomic variables, thus suggesting they were likely produced at different stages in the taphonomic sequence. The following observations and associated deductions are being proposed: at least two bores were identified at the base of a *Cubiculum cooperi* chamber indicate they were likely produced after *C. cooperi* and thus perhaps after the incorporation of the remains into the cave. Four bores were partially preserved indicating post-production damage (possibly linked to transportation). A further four bores were found embedded in trabecular bone with no direct link to the cortical surface which suggests that the bores were likely created post-fragmentation. A single bore in association with frass and coprolites (Fig. 9B) suggests this bore was created at the point of deposition, as subsequent transportation may have destroyed the frass. Lastly, six bores completely pass through the bone which has previously been argued as evidence of post-burial modification (Paik, 2000; Novas et al., 2008; Kirkland and Bader, 2010). It is thus hypothesized that the associated causal agent had repeated access to the skeletal remains inside of the cave at different stages of the taphonomic process.

Unfortunately, not all trace morphologies are as well represented and thus as informative; limited occurrence or morphological simplicity of grooves, holes, ovoid or elongated chambers provide little evidence to elucidate their individual taphonomic histories. The limited occurrence of these chambers suggests that their creation is perhaps incidental when compared to the frequency of other trace morphologies recorded at the site (e.g., *Cubiculum cooperi* and bores). However, the occurrence of preserved frass in association with an obliquely oriented oval chamber may have taphonomic implications. The majority of pupal chambers in bone reported in the literature are constructed perpendicular to the bone surface, whilst variability in the depth of the chambers is motivated by the presence of soft and/or desiccated tissue during the construction (Kitching, 1980; Martin and West, 1995; Hasiotis et al., 1999; Laudet and Antoine, 2004; Bader et al., 2009; Kirkland and Bader, 2010; Huchet et al., 2013). However, no one has considered the possibility of the presence of sediment. In that, if an insect tunnels into sediment at a perpendicular angle relative to the surface and comes into contact with a buried bone which is lying at an oblique angle, it would result in a chamber which has an oblique angel relatively to the bone surface, but in its primary context it is constructed perpendicular to the soil horizon. Under normal conditions, when insects burrow through soil, they tend to avoid hard substances. However, if the motivation for burrowing is in search of food, then bone is just as likely to be modified after burial as it would be if the traces were produced on the surface. In fact, the most regularly proposed reason as to why insects gnaw on bone is for nutritional purposes (Watson and Abbey, 1986; Freymann et al., 2007; Backwell et al., 2012). In the current study, I believe that some bones where modified post burial and that this hypothesis is supported by the preserved frass. Frass is inherently fragile, and it is unlikely that frass would have remained in its original position if the bone had undergone transportation following the creation of the frass. Furthermore, if frass is produced post-burial, it is likely to remain undisturbed and ultimately preserve. A similar taphonomic scenario could thus be inferred for specimen U.W.27-1570 which also recorded frass and a pile of coprolites. However, the frass on U.W.27-1570 appears slightly compressed at its center and does not project above the bone surface as much as the frass associated with specimen U.W.27-8566. It is thus hypothesised that the frass and coprolites on U.W.27-1570 were produced at the point of final deposition but prior to the burial of the bone. Thus the reduction of the frass pile and compression of the coprolites could be attributed to sedimentary processes during burial.

Comparisons and causal agent determination

The morphological disparity of the three chambers described in this study suggests that all three chambers were produced by different invertebrate agents, but no attempt is made to identify any specific agent based on the current evidence. However, all three chamber types are interpreted as insect pupation chambers, or pupichnia. Bores are most frequently interpreted as pupal chambers (Kitching, 1980; Martin and West, 1995; Huchet et al., 2013), but modern experimental and observational data tends to be more supportive of feeding behavior (Hall and Russell, 1933; Russell, 1947; Watson and Abbey, 1986; Backwell et al., 2012; Parkinson, 2012; Holden et al., 2013; Zanetti et al., 2014; Zanetti et al., 2015). In reality, borings could represent either or both behaviors, but inferring a specific behavior from morphologically simple traces would be speculative and is thus avoided. However, based on the size variation of the borings, it is possible that they were produced either by different taxa, or the same taxa but at different larval

Table 3. Measurement data of grooves identified at Cooper's D as well as from other data gleaned from the literature.

	Length (μm)				
	Minimum	Maximum	Mean	Std. Deviation	Citation
		Sites			
Cooper's D (Random Orientation)	42	913	304	204	—
Cooper's D (Parallel Orientation)	421	1608	800	280	—
Laetoli	200	3000	—	—	Kaiser, 2000
		Causal Agents			
Termites (Star-shaped marks)	171	832	518	137	Backwell et al. 2012
Termite (sub-parallel)	244	889	599	97	Backwell et al. 2012
Cockroaches	179	726	468	63	Parkinson, 2012
Dermestids	123	837	388	137	Parkinson, 2012

development stages. Holes, furrows, and grooves are interpreted as feeding traces. The identification of insect coprolites/frass is not uncommon as it is regularly identified in fossilised wood or amber (Light, 1930; Rogers, 1938; Lance, 1946; Ash, 2000; Nuorteva and Kinnunen, 2008; Schmidt et al., 2010; Colin et al., 2011); for a summary of reports see (McMillan, 2003). However, this is the first instance in which such microscopic coprolites have been identified and preserved on the surface of a fossil bone, and the preservation of frass/coprolites would suggest rapid mineralization.

Unfortunately comparison with other geographically and temporally relevant sites and traces is limited due to the paucity of available data. Similarly, there is a lack of comparable experimental data to aid in the determination of specific causal agents at Cooper's D. However, the diversity of trace morphologies at Cooper's D is unique when compared to all other reported trace morphologies from the African continent. Most reports either focus solely on the occurrence of borings (Kitching, 1980; Newman, 1993) or describe a collection of traces associated to star-shaped marks (Kaiser, 2000; Backwell et al., 2012). Comparative length data for grooves is available for termite damage from Laetoli (Kaiser, 2000) and from experimental data for termites (Backwell et al., 2012), dermestids and cockroaches (Parkinson, 2013). Experimentation has shown that termite damage is characterized by the occurrence of star-shaped marks (Kaiser, 2000; Backwell et al., 2012). Unfortunately, star-shaped marks were not identified on Cooper's D faunal materials, which eliminates termites as a potential causal agent. However, the length of grooves from Cooper's D is by comparison closer to grooves produced by dermestids than those produced by cockroaches (Table 2). Whilst holes produced by dermestids fall within the lower range of holes recorded at Cooper's D and bores made by dermestids fall outside of the reported range from Cooper's D (Table 3). Therefore, based on modern experimental data, both cockroaches and dermestids can be eliminated as causal agents for the modifications at Cooper's D (Roberts et al., 2003; Parkinson, 2012). The only conclusion that can be drawn based on existing data is that bores at Cooper's D, Makapansgat, and Swartkrans are broadly similar in size (Table 3). Bores from Makapansgat were attributed to dermestids, but they are not comparable to modifications produced under experimental conditions (Kitching, 1980; Parkinson, 2013) or from observational reports (Hall and Russell, 1933; Borell, 1938; Russell, 1947; Voorhies, 1948; Hooper, 1950; Hefti et al., 1980; Timm, 1982). Bores from Swartkrans were attributed to either beetles or termites (Newman, 1993), but a lack of star-shaped marks reported from Swartkrans likely eliminates termites (Backwell et al., 2012), and inferring a particular beetle based on current evidence would simply be misleading. Future studies should focus on comprehensively describing traces of terrestrial invertebrate damage to bone from as many regional sites as possible to facilitate systematic regional comparisons. Furthermore, additional experimental work should be undertaken to gather data to aid in the identification of potential causal agents in the fossil record.

Conclusion

A total of nine morphologically distinctive traces were identified at Cooper's D including *Munitusichnus pascens, Cubiculum cooperi*, ovoid, elongated and oval chambers, bores, holes, furrows, and grooves. *Munitusichnus pascens* and *C. cooperi* are the first ichnotaxa formally diagnosed in bone from the Pleistocene of Africa, whilst the identification of frass and coprolites preserved on bone is a world first. Experimental data eliminates the role of dermestids, cockroaches and termites as potential causal agents. However, based on distribution patterns of the trace and morphological disparity of chamber morphologies, it is likely that more than one agent is responsible for the traces at Cooper's D. Despite not being able to identify a specific causal agent, an examination of the associated taphonomic variables and general trace morphologies has been used to hypothesize that traces were produced at different stages of the taphonomic process including whilst bones were sub-aerially exposed, at the

point of final deposition in the chambers as well as after burial of some of the remains. Future research should focus on comprehensive descriptions of modifications from other geographically and temporally relevant sites such as Swartkrans, Sterkfontein, Makapansgat, Malapa Cave, and Dinaledi chamber. Lastly, experimentation may be a critical resource to enable the accurate identification of causal agents in the fossil record.

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CHAPTER 5 (PAPER 4)

OSTEOPATHOLOGY AND INSECT TRACES IN THE *AUSTRALOPITHECUS AFRICANUS* SKELETON StW 431

AUTHORS:
Edward J. Odes[1]
Alexander H. Parkinson[2]
Patrick S. Randolph-Quinney[1,2,3]
Bernhard Zipfel[2]
Kudakwashe Jakata[2]
Heather Bonney[4]
Lee R. Berger[2]

AFFILIATIONS:
[1]School of Anatomical Sciences, University of the Witwatersrand, Johannesburg, South Africa
[2]Evolutionary Studies Institute, School of Geosciences, University of the Witwatersrand, Johannesburg, South Africa
[3]School of Forensic and Applied Sciences, University of Central Lancashire, Preston, Lancashire, United Kingdom
[4]Department of Earth Sciences, Natural History Museum, London, United Kingdom

CORRESPONDENCE TO:
Edward J. Odes

EMAIL:
eddieodes@gmail.com

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Osteopathology and insect traces in the *Australopithecus africanus* skeleton StW 431

We present the first application of high-resolution micro computed tomography in an analysis of both the internal and external morphology of the lumbar region of StW 431 – a hominin skeleton recovered from Member 4 infill of the Sterkfontein Caves (South Africa) in 1987. The lumbar vertebrae of the individual present a number of proliferative and erosive bony processes, which were investigated in this study. Investigations suggest a complex history of taphonomic alteration to pre-existing spinal degenerative joint disease (SDJD) as well as post-mortem modification by an unknown insect. This study is in agreement with previous pathological diagnoses of SDJD which affected StW 431 and is the first time insect traces on this hominin are described. The results of this analysis attest to the complex series of post-mortem processes affecting the Sterkfontein site and its fossil assemblages.

Significance:

- First application of high-resolution micro computed tomography of the lumbar region of StW 431, a partial skeleton of *Australopithecus africanus,* attests to pre-existing degenerative joint disease and identifies post-mortem modification by an unknown insect.
- The co-occurrence of degenerative pathology and insect modification may not be unique to StW 431. A combination of traditional morphoscopic analysis and non-invasive high-resolution tomography is recommended.

Introduction

The StW 431 hominin skeleton was discovered by excavation teams from the University of the Witwatersrand during February and March 1987,[1] at the karstic cave site of Sterkfontein, Cradle of Humankind, South Africa. This site has yielded the largest sample of the taxon *Australopithecus africanus,* fossil members of the genus *Homo* and archaeological evidence of Oldowan and more recent lithic technologies.[2-7]

The StW 431 specimen comprises a partial skeleton of *Australopithecus africanus,* consisting of 48 fragments reconstructed into 18 partial elements.[1] The skeletal remains (Figure 1) consist of portions of the right scapula and clavicle, right humerus, radius and ulna, a right rib, five thoracic vertebrae, five lumbar vertebrae, the first three sacral segments and os coxae, and part of the right acetabulum. The individual is skeletally adult (based on sacral vertebral fusion) and has been previously assigned as male on the basis of a number of morphological characteristics. These characteristics include overall robusticity and muscular markers, the proportions between the body of the first sacral segment and the sacral base, and a relatively large estimated body mass (41.1–42.5 kg) based on reduced major axis regression consistent with the male range of body size for *A. africanus.*[1] The postcranial remains were associated to a single individual, based partly on position, refit, similar colour and physical condition, state of preservation and morphology.[1]

Stratigraphic understanding of the site at the time of excavation attributed the remains to Bed B of Member 4 within the Sterkfontein Formation.[1] While the exact chronological age of this stratigraphic bed is unknown, age estimates range between 1.5 Ma and 2.8 Ma for the member as a whole.[2-7] It is widely acknowledged that accurate dating of South African cave sites has been historically problematical.[8] Age ranges of between 2.4 mya and 2.8 mya of Sterkfontein Member 4 have been established by faunal analysis and archaeology[9-12], where absolute dating methods have proved problematic[13]. Electron spin resonance methods have also been used to date South African Plio-Pleistocene sites, and dates between 1.6 mya and 2.87 mya for Member 4 Sterkfontein have been suggested, with an average electron spin resonance estimated age of 2.1 ± 0.5 Ma.[14] Using U-Pb dating methods, a new absolute age range of between 2.65 ± 0.30 Ma and 2.01 ± 0.05 Ma has been assigned to fossiliferous deposits of Sterkfontein Member 4.[15] Electron spin resonance, isotopic and palaeomagnetic studies carried out on speleothem and siltstone material from Member 4 Sterkfontein Cave suggests the date of the deposits and *A. africanus* fossils at between 2.58 Ma and 2.16 Ma. Thus largely based on arguments of parsimony, provenance and morphology, most researchers studying the skeleton have accepted a taxonomic assignment to *A. africanus.*[16,17]

StW 431 is additionally important as the skeleton has been cited with regard to ongoing debate concerning the presence of skeletal pathology in the specimen; researchers have previously identified two conditions – brucellosis and spondylosis deformans – affecting this specimen.[18] Staps[18] diagnosed spondylosis deformans with osteophytic formation, and osteoarthritis of the facet joints from L4 to S1. In contradistinction, D'Anastasio and colleagues[19] diagnosed a case of possible brucellosis. In this paper, we attempt to resolve this debate and clarify the nature of ante- versus post-mortem processes affecting the specimen.

Scale = 50 mm

Figure 1: StW 431 – a partial skeleton of *Australopithecus africanus* discovered at Sterkfontein Caves in 1987. Stw 431 represented only the third partial skeleton attributed at the time to *A. africanus*, and represents the only probable male skeleton attributed to this taxon to date.

Materials and methods

The fourth and fifth lumbar vertebrae of StW 431 were studied macro- and microscopically to record surface morphology. Internal bone structure was imaged using micro computed tomography (micro-CT). Comparative skeletal material – including healthy modern human and pathological vertebrae from the Bone Teaching Collection and Raymond A. Dart Collection of Human Skeletons housed in the School of Anatomical Sciences at the University of the Witwatersrand – was also imaged. Comparative material with known and purported brucellar pathology from radiographical and palaeopathological literature was also studied (see Supplementary table 1 for a list of all comparative materials used in this study).

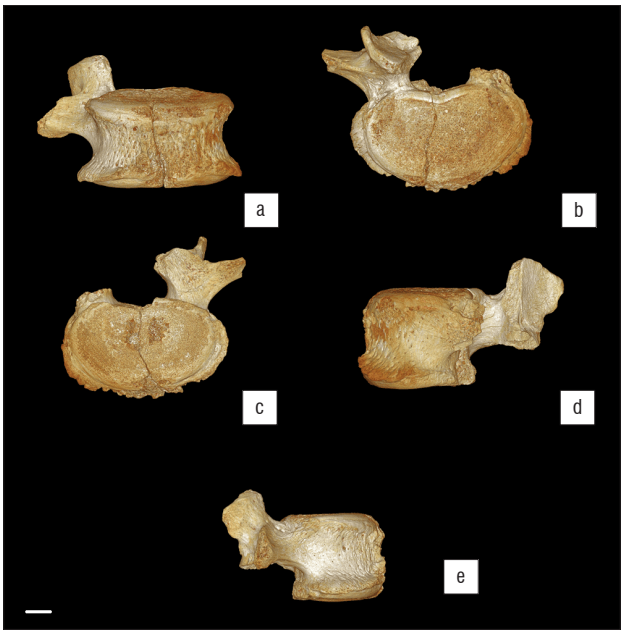
Gross surface morphology of the vertebral specimens was studied microscopically at magnifications of 7–25 times under reflected light using an Olympus SZX 16 multifocus microscope fitted with a digital camera. Micrographic imaging of the anterior and lateral bodies, antero-superior margins and endplates of the two lumbar vertebrae was carried out by applying Analysis 5.0, which includes a Z-stacking function. This function operates as an automated smoothing process whereby multiple sub-images are transformed into a single high-quality, high-resolution image with greater depth of field than a single micrograph.

In order to investigate internal (as well as external) morphology, imaging of the StW 431 vertebral specimens was carried out using micro-CT undertaken with a Nikon Metrology XTH 225/320 LC dual source industrial CT system housed in the Evolutionary Studies Institute of the University of the Witwatersrand. Both specimens were scanned using a potential difference of 85 kV and a current of 75 μA at a resolution of 33 μm; a TIFF format image stack was generated in VG Studio Max following volume reconstruction. Further reconstruction was undertaken using Avizo Amira 5.4 to generate both two-dimensional orthoslice and three-dimensional surface rendered views based on the volume data; multiplanar mode was used to allow the recovery of homologous orthoslices (superior, inferior, coronal and sagittal) through both specimens for comparative purposes.

Results

Macroscopic analysis

The fourth lumbar vertebra (L4) is largely complete (Figure 2) and presents as the bulk of the vertebral centrum, the right pedicle, with right superior articular and transverse processes. Some degree of post-mortem damage has occurred, resulting in the loss of the left pedicle, lamina and superior, inferior and transverse processes. The remaining superior and transverse processes display some degree of post-mortem damage, with apices of both processes truncated, leading to exposure of internal trabeculae. The vertebral body further displays a fracture which runs slightly antero-laterally from the margin of the vertebral foramen to the anterior border of the body, just to the right of the midline. This fracture has led to the loss of a wedge of cortical bone at the antero-inferior margin of the centrum, with loss of cortex and exposure of underlying trabecular bone either side of the plane of the fracture. This erosion is contiguous with a zone of cortical erosion affecting the anterior surface of the body, with greatest removal of cortex on the left side of the body. Additionally, there is a small area of post-mortem erosion at the antero-superior rim of the centrum, on either side of the fracture, which has shaved off part of the labrum of the body over approximately 5–7 mm of the margin.



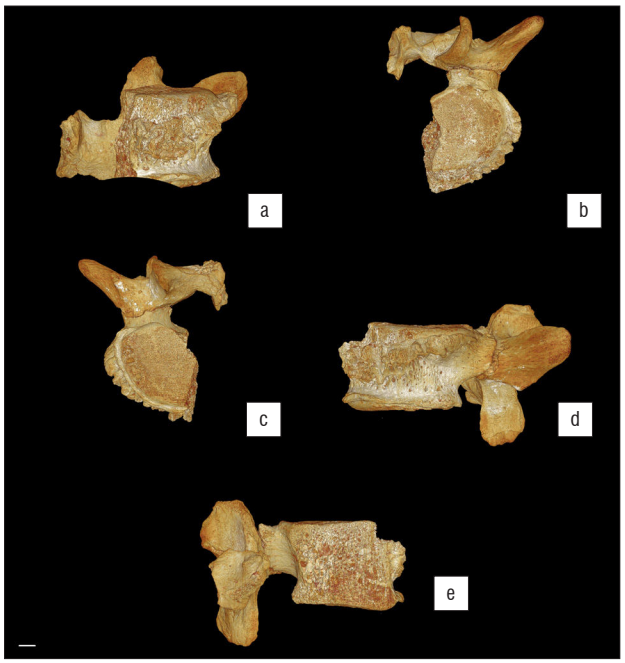
Scale = 10 mm

Figure 2: Three-dimensional volume rendered micro-CT views of the L4 vertebra of StW 431: (a) anterior, (b) superior, (c) inferior, (d) left lateral and (e) right lateral.

Slight osteophytic lipping occurs around the antero-superior region of L4, which creates a rolled appearance to the margin of the labrum, which is further disturbed by the erosion of this surface (Figure 2a). Extensive osteophytosis is expressed around the circumference of the inferior border of the vertebral body, present as a crenulated skirt of bone running from the anterior roots of the pedicles (the pedicle on the left, remaining as a short stump of the original process) around the margin of the body (Figure 2b,c). This skirt projects anteriorly a maximum of 5 mm beyond the inferior margin, and is most pronounced at the lateral

borders of the body. The superior endplate exhibits a generalised pattern of mild porosity. The surface of the inferior endplate exhibits more extensive erosion, with two major areas of cortical loss on either side of the midline of the vertebra – these are approximately 4 mm by 5 mm in diameter, the left of which is transected by the fracture which runs through the body.

The fifth lumbar vertebra (Figure 3) is less complete than L4 (Figure 2), displaying extensive post-mortem fracturing and damage. L5 presents as the left half of the vertebral body, with the pedicle, superior and inferior articular processes, and transverse process largely intact. The left lamina is present, together with a small portion of right lamina still attached, thus preserving a significant portion of the spinous process. In keeping with L4, L5 exhibits extensive osteophytic deposition, but both superior and inferior endplate margins, and the anterior surface of the body in the midline, which forms a series of cranio-caudally orientated buttresses, are more pronounced on the lateral side of the body. The osteophytic lipping extends from the left pedicle antero-laterally and continues anteriorly until it reaches the most anterior point of the vertebral body before it is interrupted by the fracture, which slices through the approximate midpoint of the vertebral body. The anterior projection of the superior osteophytic formation is interrupted by a large zone of removal of osseous tissue, which presents as a scooped, hollowed-out region that has removed both the osteophytic rim and a portion of the anterior body (Figure 3): this zone of removal has previously been described elsewhere as a region of osteolysis, possibly related to the presence of infectious disease.[19]

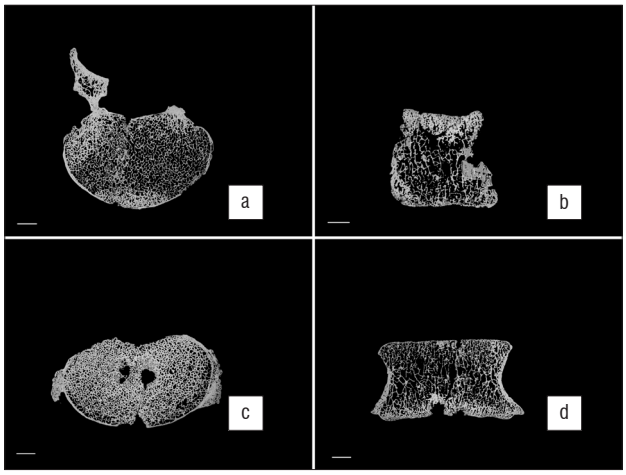


Scale = 10 mm

Figure 3: Three-dimensional volume rendered micro-CT views of the L5 vertebra of StW 431: (a) anterior, (b) superior, (c) inferior, (d) left lateral and (e) right lateral. Note the osteophytic formation on the superior and inferior endplate margins, as well as the anterior surface of the body in midline.

Microscopic analysis

Micro-tomographic imaging of the internal morphology of L4 indicates areas of both bone deposition and removal. The superior body displays very slight osteophytic lipping around the antero-superior margin, which overall presents a slightly rolled appearance in cross section. This is clearly seen in Figure 4a,b, with a slight degree of remodelling and an increase in trabecular thickness and concomitant reduction in trabecular spacing in the antero-superior margin of the centrum. The greatest expression of osteophytosis occurs in the inferior body, where the original cortical bone making up the surface of the body can be seen (Figure 4c). Secondly extensive areas of new bone formation of varying densities (from open woven to sclerotic) can be seen in transverse and coronal sections (Figure 4c,d). In addition to the marginal osteophytes, the two zones of endplate erosion are clearly evidenced. These appear as punched out cavities within the inferior body, where struts of individual trabeculae are truncated (and subsequently infilled with breccia in areas) with no evidence of sclerosis or reactive bone formation around the cavity margins (Figure 4c).

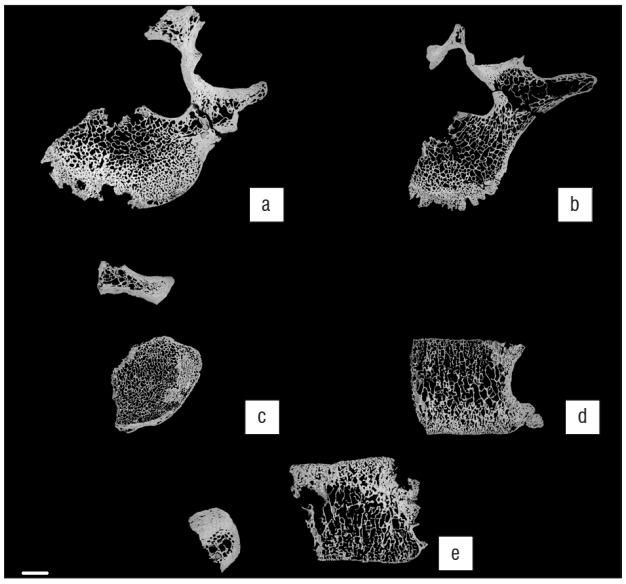


Scale = 10 mm

Figure 4: Micro-CT orthoslice views of the L4 vertebra of StW 431: (a) transverse superior, (b) sagittal midline, (c) transverse inferior and (d) anterior. Note the bilateral osteophytic formation and two zones of erosion evident on the inferior endplate surface of the L4 in (c). There is no evidence of sclerosis or new bone formation around the margins of the cavities (c). Also note areas of new bone formation ranging from open woven (c) to sclerotic (d).

In keeping with gross morphological assessment, micro-tomographic imaging of the internal morphology of L5 indicates extensive areas of both bone deposition and removal, with pronounced osteophytosis affecting the superior, anterior and inferior margins of the vertebral body. Figure 5a shows a transverse cross section through the superior region of the endplate (just inferior to the labrum), which demonstrates the extensive projection of osteophytes at the antero-superior margin. Unlike those affecting L4, these osteophytes express as direct remodelling of the cortical bone, appearing as both a thickened sclerotic margin and as crenulated buttresses of porous bone devoid of internal trabeculae; such a pattern is further reflected in the mid-transverse region of the body (Figure 5b). The inferior endplate, on the other hand, only presents osteophytosis as a thin ordered sclerotic rim extending around the inferior circumference, without buttressing (Figure 5c).

The pattern of erosion affecting L4 was identified by us (AHP) as being of potential post-mortem origin, and specifically surface modification caused by insects. In order to clarify this modification, potential insect damage was further imaged at magnifications between 7x and 115x using an Olympus SZX 16 multifocus microscope fitted with a digital camera. Terminologies used to describe the morphology of traces observed is based on a recent summary of general morphologies of bioerosional traces in bone.[20] Length and width measurements were taken using Stream Essentials© image processing software linked to the Olympus SZX multifocus microscope. Depth measurements were obtained using digital callipers. Two comparative collections of insect damage to bone were utilised in this study: these experimental collections comprise bones exposed to the following agents under control conditions; a southern African termite (*Trinervitermes trinervoides*)[21] and a dermestid beetle (*Dermestes maculatus*)[22]. Furthermore, insect damage was compared to available data gleaned from the literature.[21-24]



Scale = 10 mm

Figure 5: Micro-CT orthoslice views of the L5 vertebra of StW 431: (a,b) transverse to the midline (close to surface), (c) transverse to inferior, (d) coronal midline, (e) sagittal superior to bottom. Note new bone formation on the anterior wall (e) and major osteophytic formation at the antero-superior margin indicating remodelling of the cortex, revealing a sclerotic margin, and as crenulated buttresses of porous bone devoid of internal trabeculae (a,b). There is no evidence of sclerosis or reactive bone formation around the cavity margins (a). The inferior endplate (c) exhibits an osteophytic formation as a thin ordered sclerotic rim around inferior circumference with no presence of buttressing. Notice the channel interpreted as invertebrate damage in (e).

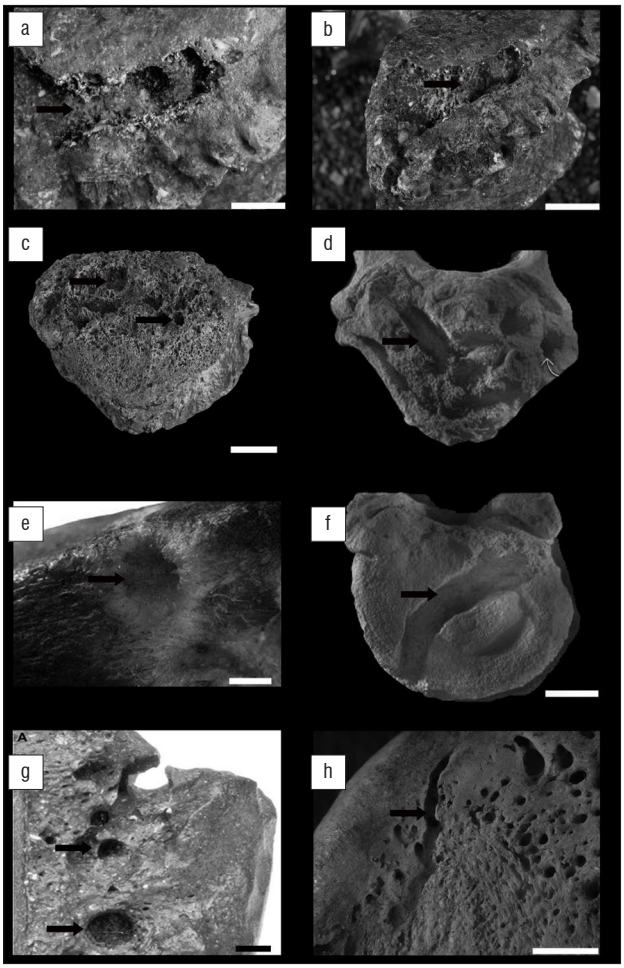
Insect traces

A channel-like structure is present on the antero-superior margin of the L5 vertebral body (Figure 6a,b). The channel comprises a series of conjoined excavations and cavities and extends from approximately the midpoint of the antero-superior rim of the body (where the vertebra has been fractured post-mortem) until the mid-lateral antero-superior margin of the upper endplate. Externally, there is no evidence of sclerosis or reactive bone formation around the cavity margins. The channel narrows from 8 mm to 3 mm in diameter and penetrates to a maximum depth of 4 mm. Embedded in this channel are distinctive circular holes which are 3–4 mm in diameter and 3–4 mm deep (Figure 5e demonstrates this pattern in cross section). No discernible mandible marks were found in association with the insect traces. The micro-CT images demonstrate an absence of bone remodelling related to either the channel or holes.

Discussion

Pathology

Gross observation of surface morphology and micro-tomographic imaging of the internal cortical and trabecular morphology of the StW 431 hominin vertebrae have indicated the presence of osseous proliferation (osteophytosis) consistent with degenerative spinal joint disease. Degenerative joint disease (DJD) is one of the most common pathological ailments observed in archaeo-skeletal assemblages, and is seen in many cases as a natural internal response of the body to 'wear and tear'.[25-29] Skeletal involvement in DJD usually consists of the covarying processes of bone formation and bone destruction, including: (1) degeneration of articular cartilage with exposure of the bone surface, leading to progressive erosive porosity in the subchondral bone; (2) bone remodelling which produces stabilising focal nodules (osteophytes) of new bone formation at joint margins or ligament/tendon insertions (entheses); (3) subchondral cysts or lytic cavities in cartilage-depleted areas; and (4) eburnation produced by direct bone-on-bone abrasion, often leaving polished wear facets on the affected areas.[30-32]



Scale = 10 mm

Figure 6: Insect traces excavated into the L5 vertebrae on StW 431 are indicated by arrows (a and b); (c) circular boring on a cercopithecus vertebra from Cooper's D; (d, f) furrows attributed to *Dermestes maculatus* from the Jurassic period[34]; (e) hole produced by the termite *Trinervitermes trinervoides*[21]; (g) holes produced by dermestids under experimental conditions[39]; and (h) furrow produced by the termite *T. trinervoides*[21].

DJD is a chronic disease process affecting joints, particularly large weight-bearing joints and is common in older individuals, but can occur in younger individuals either through a genetic mechanism or, more commonly, because of previous joint trauma.[30-32] Affected individuals may express reduced mobility, reduced flexibility and chronic pain. In modern clinical practice, DJD is equally prevalent in men and women in their mid-forties to mid-fifties, although the disease has been shown to affect much younger age groups in forensic, historical and archaeological populations in individuals with physically stressful lifestyles or occupations.[33]

The presence of osteophytosis and other associated degenerative traits elsewhere in the lumbar and sacral region of StW 431 has been proposed by Staps[18] as symptomatic of spondylosis deformans in this specimen, and our results are broadly consistent with this diagnosis, although the degenerative changes observed could also be attributed to normal age-related degenerative joint changes. However, the causal mechanism behind the destructive erosion seen on L4 and L5 has yet to be unequivocally addressed from a pathological perspective and here we attribute it to post-mortem alteration by insects.

Pirrone et al.[20] proposed that traces in bone produced by insects can be classified into one of eight general morphological categories. Subsequently, Parkinson[23] synonymised grooves and striae, as well as furrows and channels, thus reducing the number of general morphological categories to only six. These categories now are: pits, holes, chambers, tubes, furrows and grooves. The insect traces identified during the course

of this study can be classified into two of these morphological categories, namely furrows and holes. Holes (Figure 6c,e,g) are considered vertical excavations in bone which display a circular morphology in plan view and a bowl-shaped morphology in cross section. Holes are primarily found embedded in the outer cortical surface or as shallow excavations which penetrate the cortical surface and terminate in the trabecular bone.[20,23] Furrows (Figure 6d,f,h) are horizontal excavations which present as a meandering trail across the surface of a bone. Furrows are distinguishable from chambers as they lack the characteristic ellipsoidal morphology in plan view. Furrows are constructed to variable depths, and thus either record the presence or absence of vertical walls, and in cross section either display a bowl-shaped or shallow, rounded profile (see Parkinson[23(Fig.2)] and Pirrone et al.[20(Fig.3)] for a summary of plan and cross-sectional views of these common morphologies). Thus, the primary basis for identifying the insect traces on StW 431 is morphological similarity with traces widely attributed to insects in the literature (Figure 6).

The holes identified on StW 431 have a diameter range of 3–4 mm which are within the range of holes recently reported from Cooper's D (1–8.6 mm)[23] (Figure 6c) and those produced by the southern African termite *T. trinervoides* under experimental conditions (1.54–3.63 mm)[21] (Figure 6e). However, it is necessary to compare size data to data across all morphological categories because of the transitional nature of these traces. In that the morphological categories proposed by Pirrone et al.[20] and Parkinson[23] are by no means independent of one another – it is widely accepted that they represent transitional morphotypes which relate to the orientation of the excavation relative to the bone surface.[34-37] For example, if an insect excavates perpendicular to the bone surface and thus penetrates vertically in the bone, the trace would transition through a number of morphologies: initially it would be a pit, transition into a hole, and culminate in a tube. Alternatively, if the excavation is orientated parallel to the bone surface, the initial excavations would potentially take the form of a chamber and culminate in a meandering furrow. The transitional nature of these morphologies is an important consideration when one compares measurement data available in the literature. Thus the holes identified on StW 431 also fall within the range of tubes reported from Swartkrans (2–5 mm)[38], as well as those produced by *T. trinervoides* (3.41–4.2 mm)[21]. The holes on StW 431 fall outside of the range of tubes produced by *D. maculatus* (0.21–0.68 mm) and that of pits (1.5–2.5 mm) produced by dermestids[39] under experimental conditions (Figure 6g). Actualistic experimental results of bones exposed to *D. maculatus* suggests that dermestids rarely produce tubes or holes in bones, even after extended periods of exposure.[22] Lastly, the StW 431 holes also fall outside of the range of holes or tubes reported from Makapansgat which were attributed to dermestids by Kitching[40].

Morphological and metrological variables are key in the identification of insect traces on bone but they have little application in identifying a specific causal agent responsible for their creation.[23,35-37] Traces produced by insects are morphologically consistent despite geographical and/or temporal distribution; for example, furrows as described in this study from the Plio-Pleistocene are consistent with traces recently reported from the Jurassic of China.[36] Shallow circular holes have been reported throughout the Mesozoic and well into the Late Cenozoic; the most commonly inferred causal agent of traces during the Mesozoic are dermestids[34,41-44], but this shifts during the Cenozoic to termites being the most commonly inferred agent[21,24,45-47]. This temporal and geographical consistency of trace gross morphology relates to the similarity in the associated behaviour of insects whilst producing traces in bone. This unfortunate reality suggests that gross morphological categories should not be attributed to a specific agent, or should be attributed only with a high degree of caution. The trace that best illustrates this is a star-shaped pit mark.[24,45] Star-shaped pit marks have been widely reported from the Cenozoic of Africa and have been attributed to termites. Backwell and colleagues[21] sought to test this hypothesis and found that *T. trinervoides* do in fact produce star-shaped pit marks comparable to those reported in the literature.[21] Subsequent to this research, Parkinson[22] experimentally tested the impact of *D. maculatus* on bone under controlled conditions. The results of this later study suggest that dermestids also produce traces comparable to star-shaped pit marks attributed to termites. Thus inferring termites as a causal agent of star-shaped pit marks has lost a degree of credibility because various agents can produce similar morphological traces as a result of commonality in the behaviour attributed to the trace production; this then becomes an issue of equifinality which cannot be addressed directly in the palaeorecord.[22,48,49]

Despite the limitations of comparing gross morphological variables across both geologically and temporally disparate traces, one solution may be to describe traces more comprehensively within an ichnotaxonomic framework.[20,23,35] Such a description would require establishing to what degree the traces identified on bones are distinguishable from other trace taxa described in the literature, and whether the trace is substantially different to motivate the establishment of a new ichnotaxa. For example, on a gross morphological level many traces are described as chambers, but various authors have gone one step further and formally diagnosed ichnotaxa belonging to the ichnogenus *Cubiculum*. *Cubiculum ornatus* was described by Robert and colleagues[44] to include traces in bone which display a chamber-like morphology but which are characterised by the additional presence of gnawing marks/grooves. Subsequently, *Cubiculum levis* was described from Argentina as a chamber which is characterised by a bowl-shaped morphology in cross section.[35] However, in the past 18 months *Cubiculum* has expanded to include a further two taxa: *C. inornatus,* which is similar in morphology and size to *C. ornatus* but lacks the characteristic gnawing marks/grooves[36], and, most recently, *C. cooperi*, which has been described from Cooper's D in the Cradle of Humankind[23]. *C. cooperi* is distinguishable from all other *Cubiculum* taxa because of the absence of gnawing marks/grooves, as well as a uniquely consistent length to width ratio of 2:1.

This brief history of *Cubiculum* illustrates that at a gross morphological level, traces are broadly similar, but in fact the minor morphological variables between traces can be used as a basis for comparison and differentiation. The establishment of ichnotaxa in bone produced by insects also links morphological characteristics to the behavioural tendency of the trace makers. For example, *Cubiculum* ichnospecies are believed to represent the behaviour of producing pupation chambers in bone.[23,35-37,44] Interestingly, the majority of these authors who have described ichnotaxa in bone have avoided attributing a specific causal agent to the traces for various reasons.[23,35-37] These authors recognise that the trace is a reflection of behaviour, and that behaviour could easily be mimicked by numerous agents belonging to a diversity of insect groups. Simply put, more experimental research is required to begin to better understand and document the minor morphological variables between traces in bone produced by a substantially wider diversity of potential agents. Expanding research to address concerns of equifinality would be a fundamental step towards establishing practical criteria to enable causal agent determination/differentiation in the palaeorecord. In the case of the traces identified on StW 431, it is clear that these traces can be attributed to an insect based on gross morphological similarity to traces reported in the literature. High-resolution micro-CT provided clear evidence that the traces were produced post-mortem because there was no bone remodelling. However, because of the limited sample size and morphological and metrological similarity with traces produced by both termites and dermestids, we avoid attributing a specific agent. Lastly, the limited number of traces and lack of distinctive morphology do not warrant the diagnosis of an independent ichnotaxa, nor do the traces on StW 431 bear similarity to the existing ichnotaxa diagnosed in the region during the Plio-Pleistocene, namely *Munitusichnus pascens* and *C. cooperi.*[23] Lastly, drawing behavioural conclusions from a limited sample of traces which are not particularly well preserved would be futile in our opinion.

Conclusion

Our results have demonstrated that – with the exception of the presence of osteopathological lesions in the form of osteophytic lipping, demonstrating minor degenerative joint disease – the pre-mortem bone remodelling described by previous researchers[14] as evidence for infectious disease is shown here to have been caused by post-mortem processes. Our results suggest that the areas described to be as a result of infection are in fact post-mortem modifications made by insects or regions exhibiting degenerative joint disease. We draw these conclusions

based upon new imaging modalities applied to the fossil specimens. This is contrast to the methods used by D'Anastasio and colleagues[19] which comprised observation of surface morphology using light and scanning electron microscopy. In relation to the present study, in addition to macroscopic and microscopic methods, micro-CT was used in order to achieve a much higher resolution than in previous studies with full three-dimensional capacities to model and render previously identified lesions. No previous studies have used micro-CT to evaluate the surface bone of StW 431. Until now, insect traces have not been reported on StW 431.

Macroscopic and microscopic examinations are important prerequisites to determine the difference between post-mortem taphonomic and pre-mortem pathological processes as these two processes can look extremely similar, and are difficult to distinguish. This distinction can mean the difference between an interpretation of ante-mortem pathology or post-mortem pseudopathology. The modifications observed on StW 431 cannot be considered unique, as a similar pattern of bone modification is recorded on a fossil cercopithecoid vertebra from nearby Cooper's D (Figure 6c). Skeletal material at both sites (Cooper's D and Sterkfontein) experienced similar taphonomic sequences of events. Quadrupedal and bipedal primates apparently suffered from similar joint disease conditions[50], and after they died their bones appear to have been modified by an unknown insect.

This study is in agreement with previous pathological diagnoses of spinal DJD which affected StW 431, whilst shedding additional light on the complex series of post-mortem processes affecting the Sterkfontein site and its fossil assemblages. The co-occurrence of degenerative pathological processes *and* subsequent insect modifications of the same regions – the former being an ante-mortem in-vivo response and the latter a post-mortem or post-fossilisation modification – presents an interesting taphonomic case that may not be specific to StW 431, with a cercopithecoid vertebra from Cooper's D showing a similar taphonomic sequence of events. We suggest that it is only with a combination of traditional morphoscopic analyses of external gross morphology, coupled with non-invasive high-resolution tomographic methods (i.e. micro-CT, nano-CT or synchrotron tomography), that diagnoses can be rationalised. This comparative approach has been successfully demonstrated elsewhere in the South African fossil record, with the identification of the earliest evidence for neoplastic disease in the hominin record,[51,52] which would have been impossible using conventional diagnostic methods. Such caution is further supported by Mays[53] who suggests that, in future, secure diagnosis of infective disease processes in bone should primarily be undertaken using biomolecular means. Ancient bacterial DNA may, of course, not survive in such deep time scales, lending greater emphasis to the use of comparative non-invasive methods.[51,52]

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Authors' contributions

E.J.O., L.R.B. and P.S.R-Q. wrote the original draft of the manuscript, incorporating additional information and data on Stw 431 from A.H.P., B.Z. and L.R.B., with additional palaeopathological commentary from H.B. K.J. undertook the micro-CT scanning of the hominin specimen and primary reconstruction. E.J.O. and P.S.R-Q. undertook secondary reconstruction from orthoslice and three-dimensional volume data; all authors contributed equally to analysis and editing.

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CHAPTER 6 (PAPER 5)

POSSIBLE BITE-INDUCED ABSCESS AND OSTEOMYELITIS IN LUFENGOSAURUS (DINOSAURIA: SAUROPODOMORPH) FROM LOWER JURASSIC OF THE YIMEN BASIN, CHINA.

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Possible bite-induced abscess and osteomyelitis in *Lufengosaurus* (Dinosauria: sauropodomorph) from the Lower Jurassic of the Yimen Basin, China

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Lida Xing[1,2], Bruce M. Rothschild[3,4], Patrick S. Randolph-Quinney[5,6,7], Yi Wang[8], Alexander H. Parkinson[7,9] & Hao Ran[10,11]

We report an osseous abnormality on a specimen of the sauropod dinosaur *Lufengosaurus huenei* from the Fengjiahe Formation in Yuxi Basin, China. A gross pathological defect occurs on the right third rib, which was subjected to micro-computed tomographic imaging as an aid in diagnosis. The analysis of pathological characteristics and the shape of the abnormality is incompatible with impact or healed trauma, such as a common rib fracture, and instead suggests focal penetration of the rib, possibly due to a failed predator attack. The identification of characteristics based on gross morphology and internal micro-morphology presented by the specimen, suggests an abscess with osteomyelitis as the most parsimonious explanation. Osteomyelitis is a severe infection originating in the bone marrow, usually resulting from the introduction of pyogenic (pus-producing) bacteria into the bone. Micro-tomographic imaging of the lesion suggests a degree of healing and bone remodelling following post-traumatic wound infection with evidence of sclerotic bone formation at the site of pathological focus, indicating that *L. huenei* survived the initial trauma. However, as osteomyelitis can express through widespread systemic effects, including a lowering of immune response and overall condition, this disease may have been a contributing factor to the eventual death of the individual.

We report evidence for pathological lesions in a partial skeleton of *Lufengosaurus huenei* from the Fengjiahe Formation in Yuxi Basin, Yunnan Province, China, which may be attributed to a failed predator attack on this sauropod. The analysis of osseous abnormalities can provide critical insight into palaeo-immunology, behavioural and life history information for dinosaurs, as well as environmental insights into ancient ecosystems[1–3]. A modern understanding of forensic trauma analysis and pathology, as well as pathologies in modern birds, reptiles and mammals provides a platform for identification of similar palaeopathologies in the fossil record. The fundamental principle underlying the inference of palaeopathological conditions from modern comparative analysis is that of uniformitarianism[4], and this approach has yielded significant data regarding the expression and response to disease and trauma in the dinosauria. In particular, the fossil record of China has yielded a range of

[1]State Key Laboratory of Biogeology and Environmental Geology, China University of Geosciences, Beijing, 100083, China. [2]School of the Earth Sciences and Resources, China University of Geosciences, Beijing, 100083, China. [3]Division of Vertebrate Paleontology, Carnegie Museum of Natural History, 4400 Forbes Avenue, Pittsburgh, PA, 15271, USA. [4]West Virginia University School of Medicine, Morgantown, WV, 26506, USA. [5]School of Forensic and Applied Sciences, University of Central Lancashire, Preston, PR1 2HE, UK. [6]Evolutionary Studies Institute, University of the Witwatersrand, Wits, 2050, Johannesburg, South Africa. [7]School of Anatomical Sciences, University of the Witwatersrand Medical School, Johannesburg, South Africa. [8]Yuxi Museum, Yunnan, 653100, China. [9]School of Geosciences, University of the Witwatersrand, Wits, 2050, Johannesburg, South Africa. [10]State Key Laboratory of Genetic Resources and Evolution, Kunming Institute of Zoology, Chinese Academy of Sciences, Kunming, 650223, China. [11]Key Laboratory of Ecology of Rare and Endangered Species and Environmental Protection, Ministry of Education, Guilin, 541004, China. Correspondence and requests for materials should be addressed to P.S.R.-Q. (email: prandolph-quinney@uclan.ac.uk) or H.R. (email: ranhao.cn@gmail.com)

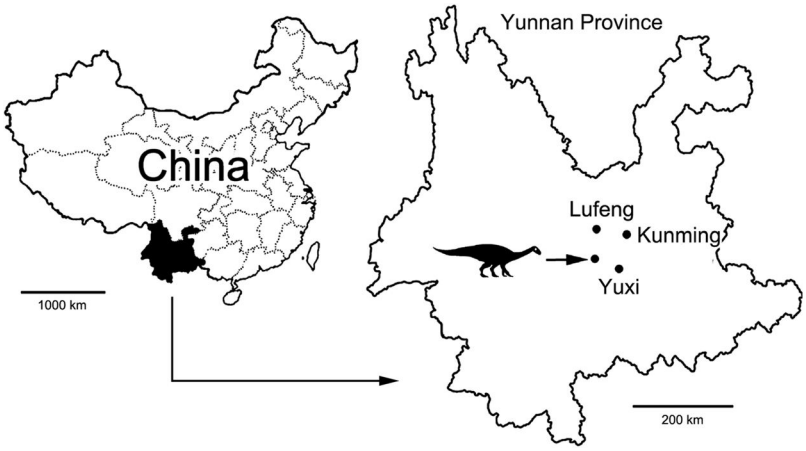

Figure 1. Map showing the position of the *Lufengosaurus huenei* dinosaur fossil locality in Yunnan Province, China. Line drawing modified from Xing and colleagues[1].

reports of palaeo-bone pathologies including healed bite marks in *Sinraptor*[5], bacterial infection in the fibula of the basal ceratopsian *Psittacosaurus*[6], a healed fracture in the theropod *Yangchuanosaurus*[7], osteoarthritis in the theropods *Caudipteryx*, *Confuciusornis* and *Microraptor*[8], tooth loss and alveolar remodeling in *Sinosaurus triassicus*[9], and vertebral fusions in sauropodomorph dinosaurs[1]. The specimen reported here adds evidence of both bone-pathology, as well as inferred behaviour, to this growing hypodigm of skeletal pathologies.

Geological setting

The Fengjiahe Formation is the oldest Lower Jurassic red bed unit in the region and varies in thickness throughout[10]. The formation is stratigraphically positioned between the Upper Middle Jurassic Zhanghe Formation, and the Lower Triassic Shezi Formation. The Fengjiahe Formation is 1500 m thick in some regions, and its upper contact with the Shezi formation is conformable[10]. The formation is characterised by purple-red mudstone and argillaceous siltstone interbedded with gray-green and yellow-green quartz sandstone and feldspathic quartz sandstone. The formation is also associated with a rich track-bearing horizon.

The Fengjiahe Formation has yielded a diversity of vertebrate fossils and is renowned for the *Lufengosaurus* fauna, consisting mostly of the basal sauropodomorphs, *Lufengosaurus* and *Yunnanosaurus*[11]. The fauna recovered from this formation is comparable with fauna from the Lufeng Formation[12]. The Lufeng Formation is the most famous and prolific unit of the Mesozoic strata of Yunnan Province in China. This formation contains a rich vertebrate fauna, including dinosaurs (sauropodomorph, theropod and ornithischian), early mammals and non-mammalian eucynodonts, basal crocodylomorphs, and sphenodontians[13–17].

Materials and Methods

The specimen analysed during the course of this study was recovered from the Yuxi Basin, Yunnan Province, China (Fig. 1) and is housed at Yuxi Museum, Yunnan, under accession number YX V0003. The specimen was referred to *Lufengosaurus huenei* based on the manus being longer than the ulna and the pubis-illium length ratio of 1.1[18–21]. YX V003 comprises a partial articulated skeleton which includes 9 cervical vertebrae, 10 dorsal vertebrae, 3 sacral vertebrae, 7 caudal vertebrae, scapulas, humeri, hip bones, and limb bones. The specimen was recovered from the No.3 bonebed, Jiaojiedian Village, Shijie Township, Yimen County, Yunnan Provence, China. The entire specimen was inspected morphoscopically for any evidence of paleopathological lesions.

Only a single skeletal element showed clear evidence of skeletal pathology. The osseous abnormality is located on the third right rib of YX V003 (Fig. 2); no other osseous lesions or pathological traces were identified elsewhere on the partial skeleton. The rib is complete and has a chord length between the dorsal and ventral ends of 394 mm, with an overall circumferential (curve) length of 452 mm (Fig. 3).

In order to investigate the pathology affecting the specimen, the fossil was subjected to high-resolution imaging using micro-computed tomography (micro-CT). This non-invasive imaging technique has been demonstrated to provide a significant diagnostic advantage over conventional whole-bone morphoscopic or microscopic methods of imaging in cases of fossilised pathology[22,23]. Micro-CT imaging of the YX V003 rib was carried out using a Nikon Metrology XTH 225/320 LC dual source industrial micro-computed tomographic scanner, housed in the China University of Geosciences, Beijing (CUGB), China. Due to the size of the specimen in relation to the detector size and gantry size of the scanner, the lesion was imaged in two focussed portions split into a dorsal half (volume identifier [VI] XLD02) and a ventral half (VI XLD03) with a small zone of overlap between the two volumes of interest (VOI). Both VOI were scanned using a potential difference of 135 kV and a current of 67 µA, at an effective resolution of 37 µm; a TIFF-format image stack was generated in VG Studio Max following volume reconstruction. Further reconstruction by PSR-Q was undertaken using Avizo Amira 5.4 to generate both 2D orthoslice and 3D surface rendered views based on the volume data; multi-planar mode was used in Amira to allow the recovery of homologous orthoslices through the specimen for comparative purposes. Differential diagnosis based on established pathological and palaeopathological criteria was subsequently undertaken[24–27].

Figure 2. Museum reconstruction of the *Lufengosaurus huenei* YX V0003 sauropod. Arrow shows the anatomical location of the affected rib. Photograph by L.X.

Figure 3. External morphology of the YX V0003 third rib. (**A**) lateral view; (**B**) medial view; (**C**) anterior (cranial) view; (**D**) posterior (caudal) view; (**E**) dorsal view. Scale bar is 10 cm. Photographs by L.X.

Description

The defect presents as a well-defined aperture which affects the lateral, medial and anterior surfaces of the rib (see Fig. 3A–C), and is located some 108 mm ventrally in the coronal plane (146 mm circumferentially) from the costovertebral junction. Viewed from the lateral and medial aspects, the defect presents as a semi-circular (concave) removal of bone, the loss of which extends almost half-way through the antero-posterior thickness of the shaft of the rib, to an approximate depth of 15.4 mm on the lateral surface. The defect is teardrop-shaped when viewed from the anterior aspect (Fig. 3C). The defect has a total external length of 54 mm, with the widest point some 20 mm from the blunted (dorsal end), reducing in width inferiorly to form a sharp tail at the ventral end of the aperture. The margin of the defect is largely intact and undamaged, although a comminuted block of cortical bone (15 mm long and 8.4 mm wide) has been lost on the opposing (medial/pleural) side of the rib (Fig. 3C,D), the margins of which clearly follow lines of post-fossilisation cracking – the loss of this area of bone is thus unrelated

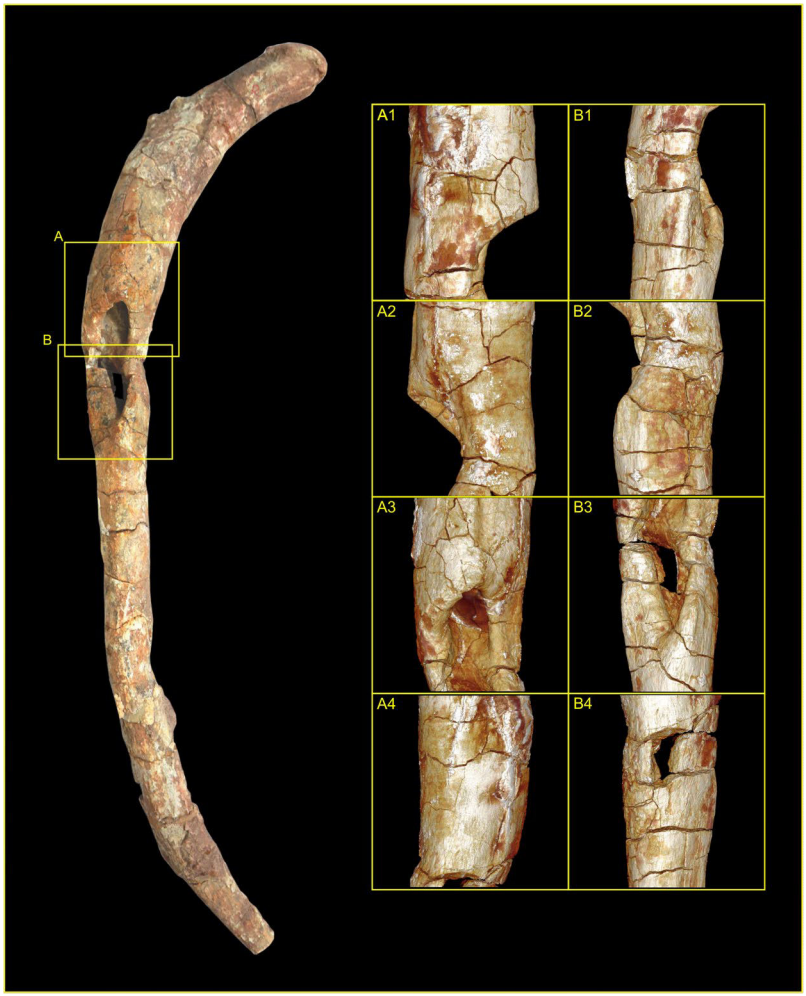

Figure 4. External morphology of the pathological *Lufengosaurus huenei* sauropod rib reconstructed from surface render of micro-computed tomographic (MCT) image volume. Schematic overlay of the photograph highlights the regions of interest (ROI) based on the respective MCT volumes ROIA and ROIB. Each ROI shows **(1)** lateral view; **(2)** medial view; **(3)** anterior (cranial) view; **(4)** posterior (caudal) view. Note, the section lines labelled A to F indicate the anatomical locations of MCT orthoslices presented in Fig. 5. Photograph by Lida Xing. Volume renders produced by P.S.R.-Q.

to the formation of the pathological defect. None of the fractures observed across the wider specimen are consistent with peri-mortem trauma or *in-vivo* failure, and instead present as transverse or right-angled breaks, showing block-comminution between major fracture blocks, and are thus 'dry bone' fractures and post-mortem/fossilisation in nature[28,29]. Many of these fracture boundaries have subsequently been infilled with calcite, particularly those following longitudinal split lines along the grain of the rib shaft.

Externally, the margin of the defect is rounded, with clear softening and remodelling of the bone around the aperture (Fig. 4) – this creates a ridge which extends some 3 mm above the original bone surface. Where remaining cortical surface of the fossil survives intact, the surface is observed as irregular and slightly puffy, suggesting remnants of systematic sub-periosteal reaction or cortical remodelling, indicative of reactive new bone formation. No evidence of bone tumors, exostoses or spurs was observed.

Internally, the defect comprises two distinct zones, separated by a convex bulge or island of bone (see Fig. 4A3,B3) coincidental with an external change of curvature of the rib shaft; there is clear evidence of remodelling and reactive bone formation surrounding the defect which extends into the medulla of the shaft. In cross-section, the defect and surrounding cortical bone present clearly defined zones of active surface and internal remodelling, with both exosteal and endosteal surfaces displaying areas of radiolucency indicative of reactive bone formation (Fig. 5A–F), which is present both as sequestra of necrotic bone and as involcrum formation. This contrasts with the presence of normal un-modified trabecular bone within the structure of the residual (normal) body of the shaft. Well-defined sclerotic zones are noted in cross-section, occurring extensively around the expanded margins of the defect and within the medula (Fig. 5A,D,E), and along the body of the shaft (where they are observed underlying zones of remodelling), indicating a quiescent condition with healing of the lesion.

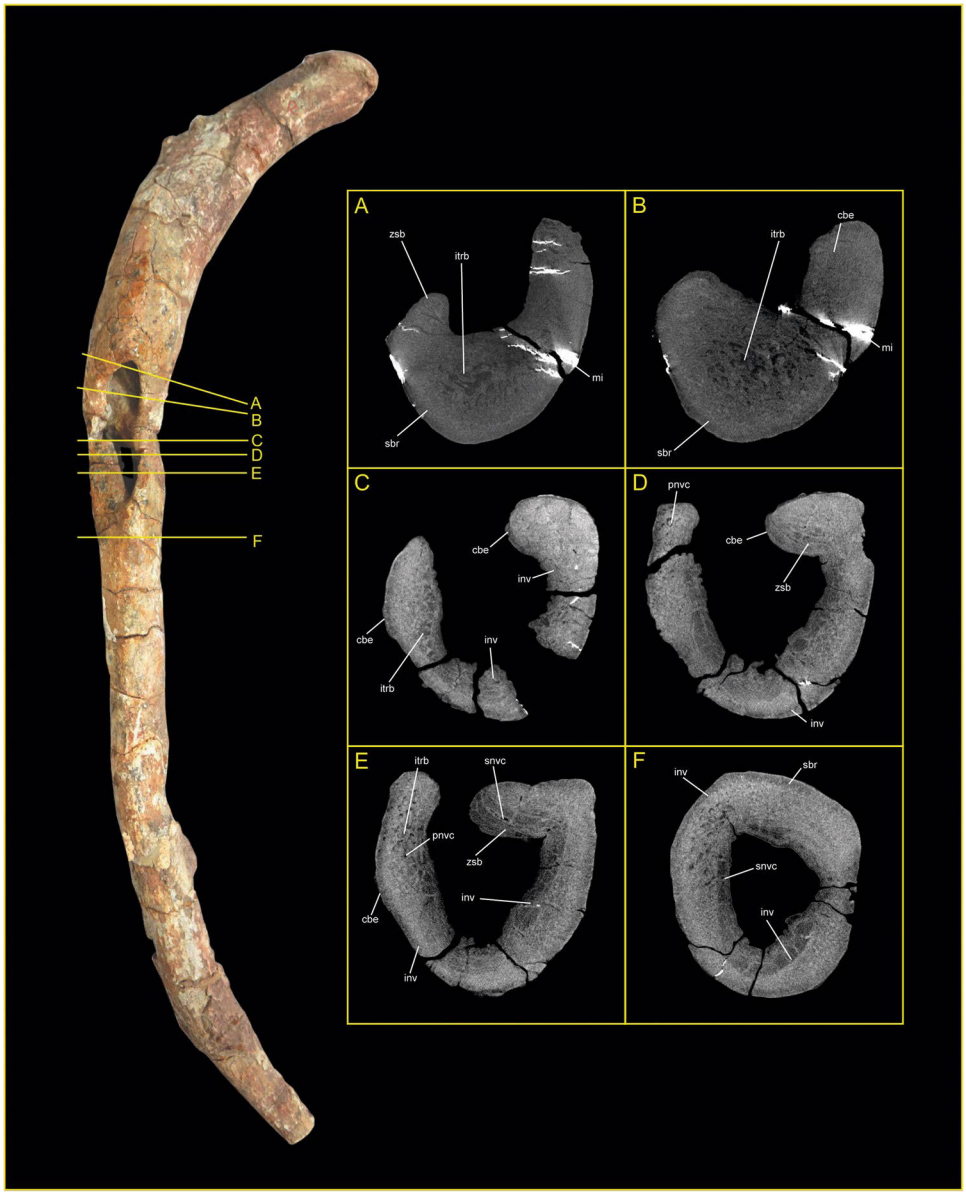

Figure 5. Transverse orthoslices produced from micro-computed tomography of the YX V0003 rib. Each slice represents an aligned transverse x-ray slice taken perpendicular to the cortical surface of the bone at the locations marked A to F in Fig. 4. Abbreviations: cbe = cortical bone expansion; inv = involcrum; itrb = internal trabeculae; pnvc = primary neurovascular canal; sbr = sequestered bone region; snvc = secondary neurovascular canal; zsb = zone of sclerotized bone. Photograph by Lida Xing. Orthoslices produced by P.S.R.-Q.

Differential diagnosis

Diagnosis was undertaken using both palaeopathological and clinical diagnostic criteria[24–27] with clinical character recognition adapted for use in cases of micro-tomographic volume imaging[22,23,30]. The accumulated evidence for both osteolytic and osteosclerotic processes indicates that the disease process was both chronic (long-term) and clinically active at the time of death of the animal. The damage was associated with reactive new bone formation, and thus can be attributed to a phenomenon which occurred during the animal's life[2,26,31]. Clear evidence of bone remodelling suggests that the dinosaur lived past the initial event that caused the damage to the osseous tissue. The limited distribution of the pathology rules out systemic diseases[1,26]. The position of the pathology (unassociated with a joint) also rules out consideration of gout, arthritis, or pseudarthrosis[25,26]. No other bioerosional bone damage was identified on the specimen, with any fractures or breaks post-mortem/fossilisation in nature.

A lytic lesion produced as a result of a bone infection is the most likely explanation due to the associated cortical bone loss and subsequent remodelling of the rib[2,31,32], with osteomyelitis being suggested as the most parsimonious explanation. Osteomyelitis is a severe infection originating in the bone marrow, usually resulting from the introduction of pyogenic (pus-producing) bacteria into the bone; most commonly today this is staphylococcus,

although streptococcus, salmonella, enterococcus and other organisms have been implicated as pathogenic vectors[33]. These pathogens can enter either via the bloodstream, or through wounds and open fractures which will introduce bacteria into the periosteum, cortex, marrow, and cancellous tissue. A distinctive feature of osteomyelitis in the skeleton is extensive new bone formation. This may totally encase the cortex of infected bone (sequestrum) as a shell of new bone, which is termed an involucrum. Sequestrated bone varies in size ranging from one or more small areas within an element to the entire diaphysis. Involucrum is the product of periosteal reaction to tissue necrosis, and although cortical bone may be dead as a consequence of infection, the periosteum may be viable and forms new bone to ensure continued normal function. Involucrum tends to be formed quickly and typically has an irregular surface. Present on the surface of the involucrum are often found large, circular openings or cloacae, which are channels of drainage for pus produced by the bacteria[33]. Such general features are evidenced in the YX V0003 specimen, supporting a diagnosis of osteomyelitis.

Discussion

Osteomyelitis is a bacterial infection frequently distinguished by progressive inflammatory destruction of bone, in which the primary skeletal focus is in the bone marrow, and which thus occurs endosteally within the medulla. This is in contrast with osteitis, which primarily occurs within compact bone, and periostitis, which affects the outer layer of bone and its associated periosteal sheath. It is common for all three areas of bone to be involved in an infectious disorder, and determining the primary skeletal site of pathology may be difficult. In the case of YX V0003 the defect is localised and connects through to the medulla, providing both a focal site and a clear diagnosis. There are two primary categories of acute osteomyelitis: hematogenous osteomyelitis, caused by the seeding of the bacteria from a remote source, and direct or contiguous inoculation osteomyelitis, caused by direct contact of the tissue and bacteria during trauma[34]. In the case of YX V003, the loss of bone marrow with exposure of the medulla supports the hypothesis that direct osteomyelitis is the most likely process to have caused this bone pathology[2,31]. Reactive new bone in the form of periosteal reaction[35,36] is a significant characteristic of bird or reptile osteomyelitis[27]. Other studies have also suggested that in modern mammals bone deformation can also be produced by osteomyelitis[37]. Long-term clinical symptoms include abrupt onset of high fever, local oedema, erythema, non-healing ulcers, sinus tract drainage, sepsis and tissue necrosis[33,38,39].

Osteomyelitis has previously been observed in non-avian dinosaurs[2,8,26,40] and attributed to, fractures and/or other injuries[2]. Reports of bone marrow infection (e.g., osteomyelitis) is rare in dinosaurs, especially Sauropodomorpha dinosaurs[2,32]. Our identification of osteomyelitis in a sauropodomorph is second only to that of García and colleagues[2] who reported the pathology in a *Baurutitan britoi* from Argentina. The pathology reported here from *Lufengosaurus huenei* is the first recognition of an abscess in sauropods, and the third example of surviving osseous abnormalities or pathologies from the Yunnan area[1,9]. This discovery enriches the record of infections in dinosaurs, and extends our understanding of the frequency of paleopathology in Sauropodomorpha dinosaurs.

Given the physical location of the lesion within the thorax, and the relatively exposed nature of the costal margin to external insult, we suggest that the infection may have been initiated by a physical injury, such as a puncture or bite[26,33]. This suggests that the observed pathological processes arose as the result of a subsequently transferred (acquired) infective agent[32,41]. If so, the infective process would have been present for an extended period. If the infection extended in different directions with a similar speed[42,43], the shape of the bone puncture would reflect the shape of the wound, in this instance a wide blunt opening with a sharpened end. The morphology of the damage suggests that the damage was produced as a result of a tooth or claw incision[44]. Whilst the large defect may be representative of a remodelled cloaca following localised necrosis (rather than a bite or puncture) we suggest that the size of the defect would preclude this. Such a large cloaca would likely produce a much greater degree of involucrum formation than is expressed here, and we consider it more likely that the primary area of bone loss represents a bite or tooth puncture as a consequence; the general tear-drop shape and overall pattern of the defect is in keeping with the morphology of bite-induced trauma and predatory behaviours recorded elsewhere in the dinosaur fossil record[5,45–48].

No theropod skeletons have yet been recovered from the Fengjiahe Formation. However, the faunally-similar Lufeng formation has yielded four theropod genera *Sinosaurus*[9,49,50], *Eshanosaurus*[51], *Panguraptor*[52]. *Eshanosaurus* belongs to therizinosaur, which is considered to be herbivore[51,53]. *Panguraptor* are small coelophysids and may have not have the ability to attack *Lufengosaurus*. However, *Sinosaurus* is a famous and larger bodied predator, and would be able to attack *Lufengosaurus*[9,50], and thus is a possible suspect for producing the injuries which resulted in the abscess and osteomyelitis. Whilst the exact identity of the proposed attacker is not critical, it is clear is that the attack was unsuccessful, and that the associated symptoms of the infection likely influenced the future life-history of the *Lufengosaurus*. Osteomyelitis is known to produce the fever, fatigue, nausea, loss of range of motion, tenderness, and discomfort, although necrotizing tracts of pasteurella bacteria extending to the brain have been reported in clinical cases in reptiles[54], which are associated with elevated morbidity. Any or all of these symptoms would have negatively impacted the survival potential of the injured *Lufengosaurus* in the dynamic and complex ecosystems during the Jurassic.

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Author Contributions

L.X., Y.W., H.R. and B.M.R. undertook the primary analysis and wrote the original draft of the manuscript. P.S.R.-Q. undertook the micro-tomographic reconstruction and subsequent analysis in conjunction with A.H.P., and edited the original manuscript. A.H.P. produced the composite plates from photographic and orthoslice and three-dimensional volume data; all authors contributed equally to analysis and editing.

Additional Information

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CHAPTER 7 (PAPER 6)

MODERN BONE MODIFICATIONS BY *DERMESTES MACULATUS* AND CRITERIA FOR THE RECOGNITION OF DERMESTID TRACES IN THE FOSSIL RECORD

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Modern bone modification by *dermestes maculatus* and criteria for the recognition of dermestid traces in the fossil record

Alexander Parkinson [a,b]

[a]Evolutionary Studies Institute, University of the Witwatersrand, Johannesburg, South Africa; [b]the Centre for Exploration of the Deep Human Journey, University of the Witwatersrand, Johannesburg, South Africa

ABSTRACT

Various insect taxa are known to modify bone with their mandibles, including members of the orders Dermestidae, Tenebrionidae, Calliphoridae, Tineidae and Termitidae. Dermestidae are one of the most frequently inferred causal agent of bone surface modifications but little neoichnological/actualistic data exists to support such inferences. The primary aims of this investigation were to determine the nature of bone damage caused by the cosmopolitan species *Dermestes maculatus* and develop an interpretative framework to aid in the identification on modern and fossil and differentiation of associated bone. Four experiments were conducted utilising 79 bones exposed to *D. maculatus* for a period of 4 months. Five primary modification types were established for *D. maculatus* including furrows, destruction of bone, bore-holes, striae, and three classes of pits. This study has vindicated *D. maculatus* as a causal agent of reported instances of bone surface modifications in existing archaeological and palaeontological literature, and more focussed studies may find greater application within the forensic sciences.

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INTRODUCTION

A large body of literature exists attributing bone modifications to terrestrial invertebrates, particularly insects, including members of the orders Dermestidae (Gabel, 1955; Hefti, 1980; Kitching 1980; Coe, 1980; Rogers 1992; Martin and West 1995; Hasiotis et al. 1999; Paik 2000; Chin and Bishop 2004; Hasiotis 2004; Laudet and Antoine 2004; Bader 2005; Robinson 2005; Roberts et al. 2007; West and Hasiotis 2007; Fernández-jalvo and Monfort 2008; Britt et al. 2008; Thompson et al. 2018), Tenebrionidae (Zacher 1929; Mcfarlane 1971), Calliphoridae (Kitching 1980; Huchet and Greenberg 2010), Tineidae (Zacher 1929; Gentry 1987; Hill 1987) and Termitidae (Derry 1911; Thorne and Kimsey 1983; Hill 1987; Watson and Abbey 1986; Wylie et al. 1987; Gautier 1993; Kaiser 2000; Kaiser and Katterwe 2001; Dangerfield et al. 2005; Fejfar and Kaiser 2005; Guapindaia 2008; Huchet et al. 2009; Parkinson 2010; Parkinson et al. 2010a, b; Backwell et al. 2012). The most comprehensive descriptions of bone surface modifications are found within the archaeological and palaeontological literature. The unfortunate reality is that due to preservation conditions, little direct evidence exists in which bone modifications are found in direct associated with the causal agent(s) responsible for their creation (except Huchet et al. 2009). This has resulted in modifications being described and their makers either being attributed to 'insects' in general or justifications being provided for a particular insect.

The role of dermestid beetles in animal decomposition is well documented; they consume soft sub-dermal tissue and skin, hence their name *Dermestes* derived from the Greek to 'consume skin' (Smith 1986; Byrd and Castner 2009). Observational reports of dermestids modifying bone relate to the use of dermestids for the cleaning of skeletal materials, but unfortunately these reports lack any substantial qualitative or quantitative descriptions of the bone modifications. Dermestids are reported to either do no damage (Hall & Russel, 1933; Borell, 1938; Hooper 1950; Osuji, 1975) or little damage to delicate bones (Howell 1932; Hefti et al. 1980; Timm 1982; Weichbrod 1987). Hefti (1980) suggests that lack of food availability may result in damage, whilst other authors suggest specific areas which are infrequently damaged such as; tympanic bullae (Howell 1932), iliac crest of the pelvis, vertebrae (Hefti et al. 1980), humerus and the acetabulum (Schroeder et al. 2002). However, a recent study suggested that under natural conditions dermestids do not modify bones, but these experimental trails are potentially limiting in their scope due to only bird bones being used (Adams, 2018).

Direct evidence of dermestid modification to bone has been obtained from various neoichnological studies. Roberts and Rogers (2003) suggested that a wide variety of modification types were produced by dermestids but that the identified suite of modifications differed markedly from those attributed to dermestid beetles in the palaeontological literature (unfortunately, full results of this study were not comprehensively published but only briefly presented at a conference). Whilst investigating various skeletal preparation techniques it was established that *Dermestes sp*. can destroy bone, make grooves, holes and chew-marks (Fernández-jalvo and Monfort 2008). Zanetti et al. (2014) conducted forensically focused research which reported the presence of mandibular striae on bones but these were not described in detail. Holden et al. (2013) made a concerted neoichnological effort by establishing that dermestids produce pits, bore holes and scallops (also referred to as 'quarries' in the text). Unfortunately, the established criteria do not display sufficiently distinctive morphological characteristics that would aid in the identification and differentiation of dermestids as a causal agent in the fossil record. This limitation may have been as a result of the bone specimens used, as my personal experience has shown that the use of cancellous bone (i.e., ribs and chicken bones) primarily results in modifications without distinctive morphological characteristics, and thus limited in their practical application for interpreting the fossil record. All the above-mentioned researchers have made substantial contributions to the study of insect bone interactions. Zanetti et al. (2019) recent research suggests that

CONTACT Alexander Parkinson palaeoparky@gmail.com

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dermestids do in fact pupate in bone, but the study lacks direct evidence, and the inference of surface modifications relating to pupation is done with a high degree of caution. The study also states that the described traces could equally be a result of feeding, and not pupation.

The modifications described and attributed to dermestidae (Figure 1) are from chronologically and geographically disparate specimens, ranging from the Upper Jurassic Morrison Formation of Wyoming (Britt et al. 2008a) to Holocene aged settlement in Palestine (Huchet et al. 2013). Britt *et al.* (2008) provided a very strong motivation for their identification of *D. maculatus* as the exact causal agent based on their tooth width, distance between the teeth and the occurrence of striae being paired. This was supported by the fact that extant dermestids have a primary mandibular cusp in addition to a secondary marginal cusp. It was thus suggested that during the creation of striae, both the primary and secondary cusps came into contact with the bone surface and thus create a pair of parallel striations with every bite. Whilst Huchet et al. (2013) examine modifications and interprets them to be dermestid pupation chambers due to a correlation in widths with modern pupal chambers constructed by dermestids in Styrofoam, wood and paper (Huchet et al. 2013). In fact, boreholes in bone are often interpreted as pupal chambers and attributed to dermestids (Figure 1). This deduction is surprising when you consider that several researchers having directly observed the ways in which dermestids modify bone (Howell 1932; Hefti et al. 1980; Timm 1982; Weichbrod 1987; Schroeder et al. 2002; Roberts and Rogers 2003; Fernández-jalvo and Monfort 2008; Holden et al. 2013; Zanetti et al. 2014) but none have reported dermestids producing pupation chambers in bone.

Neoichnological experimentation has been shown to be a useful method in testing the ways in which insects modify bone. Investigations of this nature conducted on termites in Southern Africa has highlighted several additional benefits to undertaking such studies, in that; micro-environmental conditions as well as the season of deposition of the modified remains may be inferred by correctly identifying the modifying agent (Backwell et al. 2012). It was also suggested that termites as agents of bone modification could potentially bias taxonomic and skeletal element representation, minimum number of individuals as well as age profiles inferred from faunal remains that have been subjected to termite damage (Backwell et al. 2012). These potentialities identified by Backwell et al. (2012) highlights the need for such research and the contributions that such efforts can make in the reconstruction and interpretation of taphonomic biases and conditions.

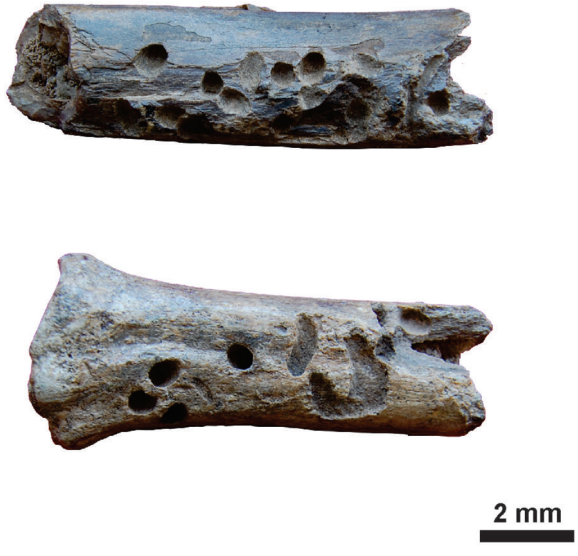

Figure 1. Fossil bovid bones displaying insect modifications interpreted as dermestids pupation chambers by Kitching (1980) . Two bovid long bone fragments showing multiple circular bore holes, and elongated surface furrows excavated into the bone. Modifications previously attributed to dermestid beetles.

The goals of this study are to determine how dermestids modify bone and describe these modifications in detail. To quantify the impact of the absence of sand and the impact of reduced feeding conditions, on the type, frequency and distribution of modifications produced. Lastly, this study aims to establish practical modification criteria to aid in the identification of fossil dermestid traces on bone.

MATERIALS AND METHODS

Seventy-nine bones were used in this study (Table 1 and Table 2). The bones were divided between four experiments. Bones were specifically selected to be as comparable as possible in terms of size, taxon, and skeletal element. The primary selection criteria for these bones were condition (fresh, dry, weathered, fossilised) and bone density (thin cortical, thick cortical, cancellous, and compact bone). Some bones (e.g., complete long bones) display a variety of different bones densities and as such were classified as 'variable'. Unidentified dry or fossil, large-sized bovid specimens are simply referred to as 'bovid', indicating animals in the range 23–84 kg (after Brain 1974). All dry and weathered bone specimens were selected from field collections, fresh specimens were sourced from a local butchery or taken from a *Damaliscus pygargus phillipsi* (Blesbok) skeleton donated to the project, and fossil specimens were sourced from unprovenanced and unidentifiable remains from the early hominin-bearing cave site of Coopers D, in the Cradle of Humankind World Heritage Site, South Africa. The surfaces of all bones underwent comprehensive pre-experimental screening. Any pre-existing surface modifications (Figure 2.C) that may potentially be misinterpreted as insect modification were identified using stereo microscopes at magnifications between 7 and 115x and noted on the images. The Dermestid species used was sent to the South African National Biodiversity Institute (SANBI) for formal identification. The results confirmed *D. maculatus* was used during all experimental trials.

The frequency and distribution of modifications produced by *Dermestes maculatus* during the experiments were recorded; frequency was expressed either as a percentage of the total available surface area of the bone specimen effected or as an *n*- value which represents physical occurrences, whilst distribution was described in terms of the area (periosteal, medulla and edge) on which the modifications occurred. Specimens were labelled with unique numbers using a permanent marker, imaged in ventral and dorsal view on a flatbed scanner, and the resulting images printed in colour.

Experiment Protocol

Bones were placed in 600 × 300 mm fish tanks housed within a dedicated insectary maintained at a temperature of 28°C at 40% humidity, with 12 hours of light and 12 hours of darkness. Wooden framework lids were treated with creosote to prevent dermestid damage and the top of the frames were covered with a double layer of 2 mm mosquito wire mesh to prevent escape. The bottom of each tank was covered with 50 mm of fine to medium grained, sterile river sand, unless otherwise specified.

Four experiments (1–4) were conducted; Experiments 2 & 3 were duplicated under the same conditions of 50 mm of sterilised sand at the base of the tank and receiving 100 g of

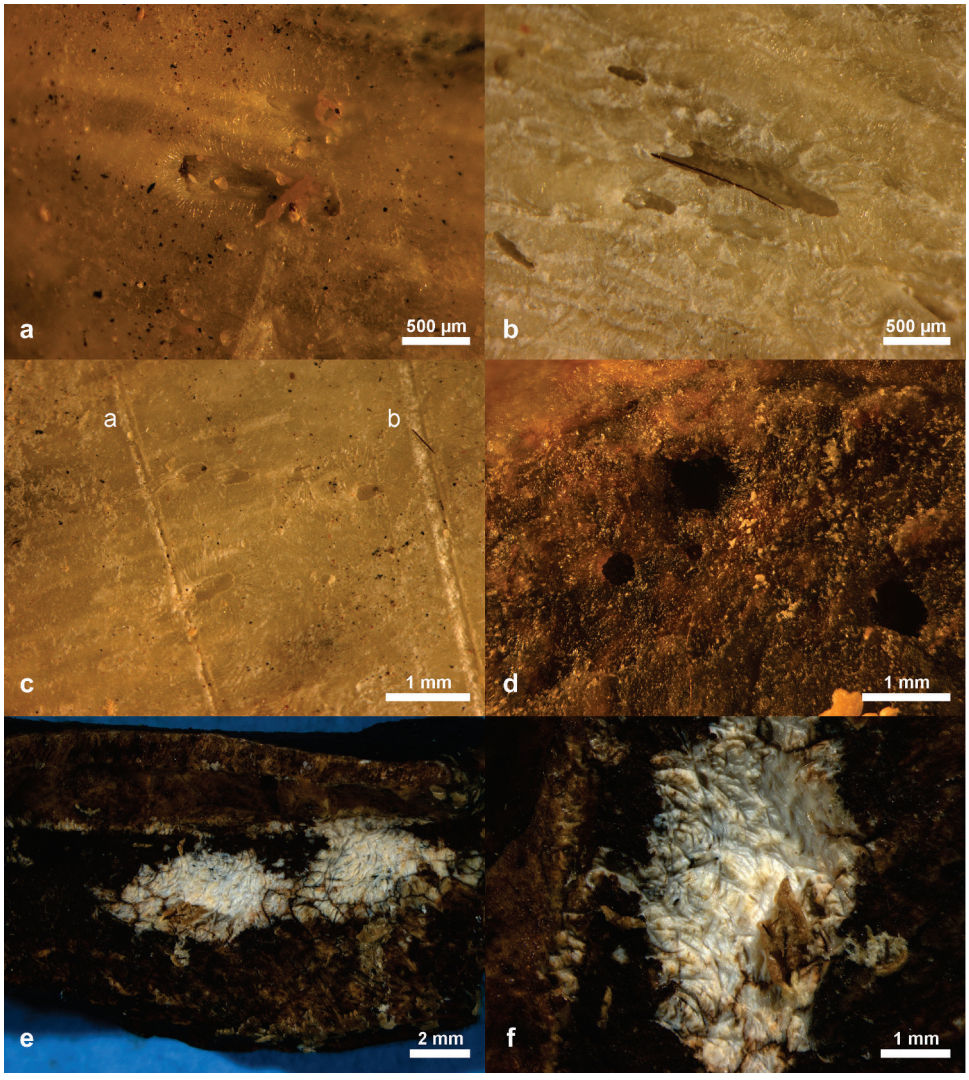

Figure 2. (A to D) Class 1 surface pits produced by *D. maculatus* after a period of four months. (A, B, C) dry *O. aries* scapula (specimen #22). (D) fresh proximal epiphysis of a *D. pygargus phillipsi* femur (specimen #20). The 'a' and 'b' indicate existing surface damage created prior to exposure to *Dermestes maculatus*. (E, F) Class 2 surface pits produced on the enamel of a fossil tooth (specimen #12) after four months of exposure. Pit classes 1 and 2 are UID 1 rated modifications and as such should be used as the primary criteria for identification and differentiation of dermestids as a causal agent in the fossil record. The image presents several examples of Class 1 and Class 2 surface pits. These modification types are considered UID1 rated which suggests the modifications are useful in identification and differentiation of Dermestid modifications in the fossil record. Images show complex arrangement of gnawing marks/striae centred around a boring, exposed haversian canal or depression (Class 1). Else dense clustering's of gnawing marks (striae) on a previously fossilised bone fragment (Class 2).

canned meat twice a week. Experiment 1 (substrate experiment) aimed to test whether the absence of a sandy substrate would impact the type, frequency, or distribution of modifications. Whilst, Experiment 4 (food experiment) aimed to test the impact of reduced food availability on the type, frequency and distribution of modifications. These two variables were selected based on observations made by Roberts and Rogers (2003) which suggested that both variables impact on modification frequency. However, the impact was never quantified and neither the occurrence of modification types nor distribution thereof were discussed in relation to the variables. The substrate (Experiment 1) and food experiments (Experiment 4) did not run concurrently, thus the same tanks were re-used. Bones from the substrate experiment (Experiment 1) were randomly distributed across the base of the first tank and this layout was duplicated in subsequent runs. After placing the bones in the tank 100 larvae (instars 4 or 5) and 35 adult beetles were introduced. The tanks each received 100 g of canned meat twice a week for the duration of the experiments, unless otherwise specified. The amount of food provided coincided with the quantities being provided to the primary colonies from which the larvae and adult beetles were taken. Specimens were left undisturbed for 4 months, roughly three reproductive cycles (Howell 1932; Russell 1947; Weichbrod 1987), after which bones were removed, labelled, and bagged for analysis.

Standard Experiments – 50 mm sand, 100 g food bi-weekly

Experiments 2 and 3 were conducted under this condition and were allocated specimen numbers (Experiment 2: #22–36, #38–41; Experiment 3: #85–94, #96–104).

Substrate (sand) Experiment – No sand, 100 g food bi-weekly

This experiment aimed to test the influence of the absence of substrate on the type, frequency, and distribution of bone modifications. Experiment 1 was conducted under this condition and was allocated

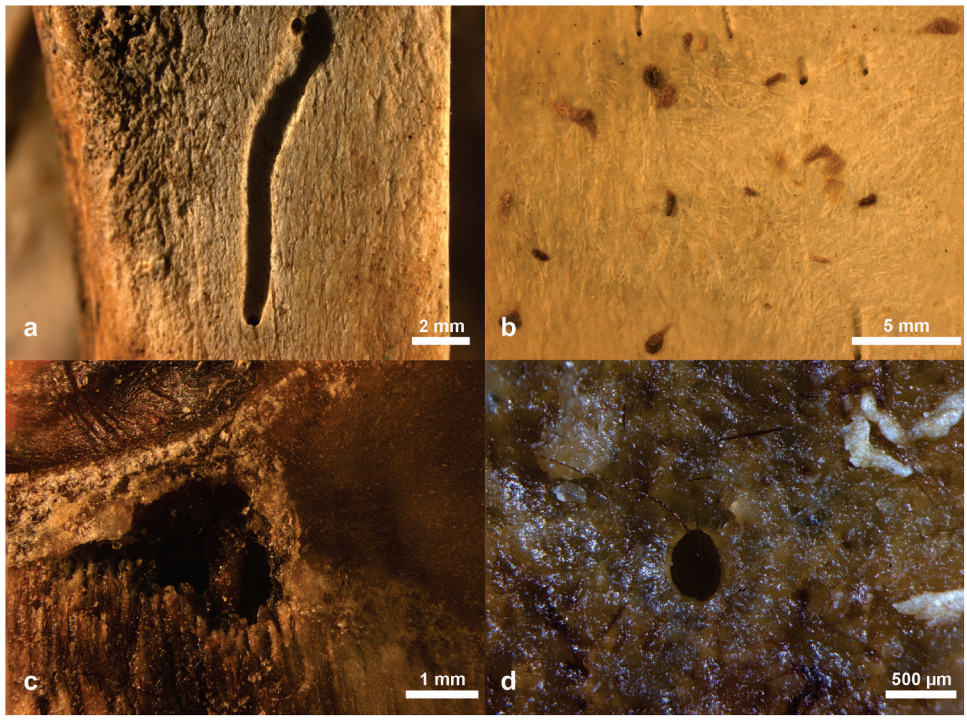

Figure 3. (A, B) Furrows made by *D. maculatus* after four months of exposure. (A) A single furrow made on a dry distal epiphysis of an indeterminate bovid humerus (specimen #30). (B) Five smaller furrows evident on a dry *O. aries* scapula (specimen #85). (C, D) Boreholes produced on a fresh *G. domesticus* femur (specimen #38) and a previously dry *O. aries* scapula (#1) respectively. Furrows and boreholes are UID 2 rated modifications which suggests these modification types are not sufficiently distinctive to enable identification of dermestids as a causal agent when found in isolation in the fossil record, but that they may be attributed to dermestids when found to co-occur with UID 1 rated modification types. The image presents several examples of surface furrows and bore holes. Modification types considered UID2 rated which suggests the modifications could be attributed to Dermestid if identified in association with UID1 modifications. Thus, only being potentially useful in the identification and differentiation of Dermestid modifications in the fossil record. The micrographs show macro and microscopic furrows which are excavated parallel to the bone surface. Additionally, boreholes excavated perpendicular to the bone surface and associated to gnawing marks.

specimen numbers (#1–20). The bone specimens were scattered over bare glass at the base of the tank and the associated layout was duplicated for all other experiments conducted.

Food experiment – 50 mm sand, 50 g food bi-weekly

This experiment aimed to test the influence of reduced food availability on the type, frequency, and distribution of bone modifications. Experiment 4 was conducted under this condition and was allocated specimen numbers (#95, 106–123). This tank received only 50 g of canned meat twice a week.

Morphological descriptive terminology

The modification-type morphologies described in this study fall within accepted general morphologies described for bioerosion trace fossils in bone (Pironne et al. 2014). However, the modification-type terminology adopted in this study are not directly related to definitions summarised by Pironne et al. (2014), but rather reflect a more simplistic morphological categorisation. The following list will indicate the term used in this text followed by synonymised terms summaries by Pironne et al. (2014) which are shown in brackets. Furrows (furrows and channels), pits classes 1–3 (pits, holes, and chambers), boreholes (tubes), striae (grooves and striae). The associated definitions of terms used in this study are presented in Table 3, archetypal images of each modification type are presented in Fig. 2–5, and descriptive statistics for sizes of the various modification types are listed in Table 4.

Analytical Protocol

After an exposure period of 4-month bones were removed and inspected for macroscopic modifications. Thereafter, they were inspected using an Olympus SZX 16 Multifocus microscope at magnifications between 7 and 115x, fitted with a digital camera. Modifications were categorised and recorded in a Table. Modifications were measured in microns using image processing software linked to the Olympus SZX Multifocus microscope. Measurements taken varied depended on the modification type being measured. In the majority of instances two measurements were taken, unless otherwise specified; Length was considered the larger of the two measurements whilst breadth was taken as the smaller of the two. Descriptive statistics were obtained using IBM SPSS Statistics version 20. The resulting experimental samples are curated at the Evolutionary Studies Institute, University of Witwatersrand, South Africa.

RESULTS

At the conclusion of all experiments 36 (46%) of 79 bones exhibited dermestid damage. Results show that *Dermestes maculatus* modify bone in several different ways. They can destroy bone (destruction) or create furrows, boreholes, pits (classes 1, 2 and 3) and striae. Furrows display a U-shaped profile excavated across the surface of a bone, regularly displaying small boreholes at either one or both ends of the furrow. Boreholes are semi-circular features without a discernible bottom. Class 1 surface pits are highly variable in shape from semi-circular to elliptical shaped shallow depressions with U-shaped profiles displaying striae which radiate around the

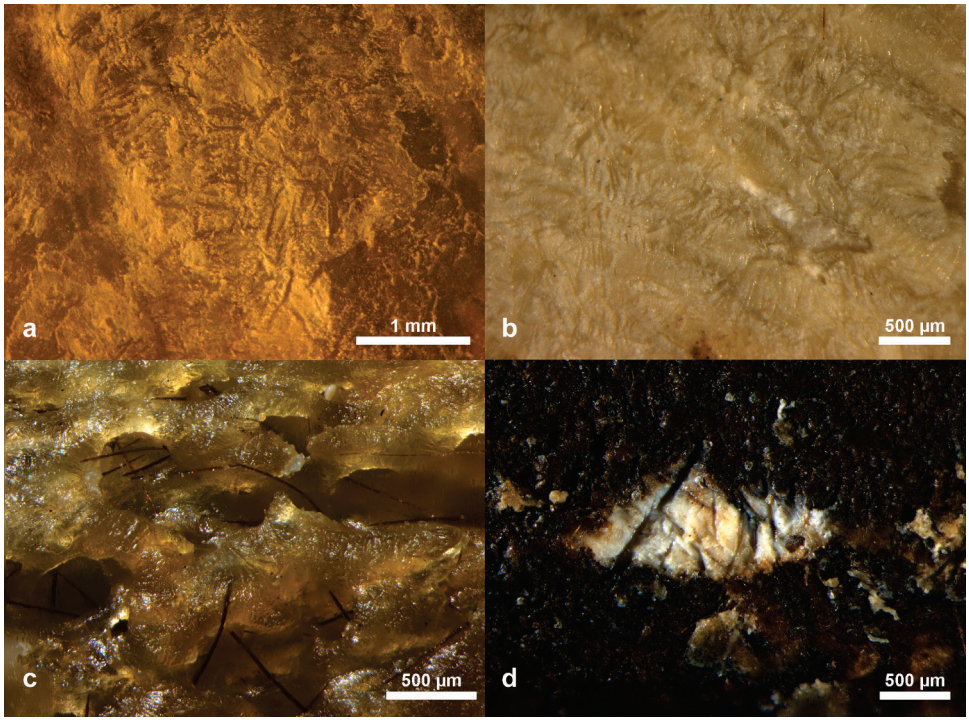

Figure 4. (A to D) Striae produced by *D. maculatus* after four months of exposure. (A, B) Clusters of striae evident on dry *O. aries* scapula (specimen #1 and #22). (C) Striae on the inner trabecular bone of a dry *O. aries* rib (specimen #6), note the presence of larval spicules. (D) Isolated and intersecting striae recorded on a fossilised piece of tooth enamel (specimen #12). Striae are UID 2 rated modifications which suggests they are not sufficiently distinctive to enable identification of dermestids as a causal agent when found in isolation in the fossil record, but that they may be attributed to dermestids when found to co-occur with UID 1 rated modification types. The image presents examples of striae/gnawing marks. Striae are considered UID2 rated. Striae present as either straight or slightly convex lines excavated into the bone surface. The cross-sectional profile appears U-shaped under high magnification. The striae occur as small patches of clearly distinguishable single 'bites' in a small area, else as dense clusters of multiple 'bites' covering a much wider area. One image shows how microscopic striae can even be identified on trabecular bone.

outer circumference. Class 2 surface pits are large semi-circular shallow depressions with randomly orientated striae over the entire feature, individual striations are lenticular in shape, which intersect and crosscut one another and are found in large clusters. Class 3 surface pits are irregular-shaped depressions with vertical walls and complex profiles not associated with striae. The lack of associated striae is due to the modification occurring only on cancellous bone, thus small pieces of bone and trabeculae are being removed without leave any discernible mandibular traces. Lastly, clusters of randomly orientated lenticular-shaped striae which cover a large surface area. Striations have an u-shaped profile and range from 123 to 837 µm in length with an average width of 20 µm.

Experimental results shown in (Tables 5 – 8) have been grouped according to the level of visibility of each of the modification types. Macroscopically visible modifications (furrows) are considered modifications that can be seen with the naked eye, intermediate modifications (destruction and bore holes) can be seen at relatively low magnifications ranging from 7–20x, whilst microscopic modifications (pits and striae) can only be seen at magnifications higher than 20x. However, Class 2 surface pits are macroscopically visible but have been grouped with the rest of the pit classes (1 and 3) for ease of presentation. Only 12 specimens (4 fresh, 6 dry, 2 weathered) displayed macroscopically visible modifications (Table 4), 31 specimens (4 fresh, 20 dry, 6 weathered, 1 fossil) displayed intermediately visible modifications (Table 5) and 32 specimens (4 fresh, 20 dry, 6 weathered, 2 fossil) shows microscopically visible modifications (Table 6).

Several bones remained unmodified at the end of the experiments; under the standard condition of 50 mm sand and 100 g food twice a week; Experiment 2 showed that 11 (2 fresh, 4 dry, 2 weathered, 3 fossil) out of 20 specimens were not modified representing 55% of the sample. Whilst experiment 3 showed that 11 (3 fresh, 3 dry, 2 weathered, 3 fossil) out of 19 specimens were not modified representing 58% of the sample. In the absence of substrate seven (1 fresh, 4 dry, 1 weathered, 1 fossil) out of 21 specimens remained unmodified representing 33% of the sample. Whilst, under reduced feeding 14 (4 fresh, 5 dry, 2 weathered, 3 fossil) out of 19 specimens displayed no modifications representing 74% of the sample. Figure 6 summaries the type and percentage of modifications observed on specimens across the four experiments.

Standard Condition

Experiment 2

Furrows (n = 7) were recorded on 15% of specimens (1 fresh, 2 dry). Pits (n = 71) were evident on only 30% of specimens (1 fresh, 4 dry, 1 weathered). Destruction was recorded on 40% of specimens (2 fresh, 6 dry, 1 weathered), whilst striae were recorded on 35% of specimens (1 fresh, 6 dry). Lastly, boreholes (n = 15) were recorded on 30% of specimens (2 fresh, 4 dry).

Experiment 3

Furrows (n = 21) were recorded on 11% of specimens (1 fresh, 1 dry in both instances). Pits (n = 22) were recorded on 16% of specimens (1 fresh, 1 dry, 1 weathered). Boreholes (n = 10) were recorded on 21% of specimens (1 fresh, 3 dry), whilst destruction and striae were recorded on 32% of specimens (Destruction - 1 fresh, 3 dry, 2 weathered; striae – 4 dry, 2 weathered).

Substrate Experiment

All modification types except boreholes are more frequent in the absence of sand when compared to the results from experiments 2 & 3. Furrows (n = 12) were recorded on 24% of specimens (3 fresh, 2 dry). Pits

Table 1. Specimens used in experiment A that were exposed to *D. maculatus* for a period of 4 months. Dataset D2 was in a tank with 50 mm of sterilised sediment at the base, whilst for D1 no sediment was present.

Trial	Condition	Taxon	Element	Conservation	Bone Type	Spec No.
D1	Dry	*O. aries*	Scapula	Complete	Variable	1
D1	Dry	*O. aries*	Radius	Complete	Variable	2a
D1	Dry	*O. aries*	Ulna	Complete	Variable	2b
D1	Dry	Indet. bovid	Tibia	Shaft fragment	Thick cortical	3*
D1	Dry	*O. aries*	Humerus	Shaft fragment	Thin cortical	4*
D1	Dry	*O. aries*	Tarsus	Complete	Compact	5
D1	Dry	*O. aries*	Rib	Complete	Spongy	6
D1	Dry	Indet. bovid	Humerus	Distal epiphysis	Variable	9
D1	Dry	*O. aries*	M^2	Complete	Tooth	13*
D1	Dry	*Antidae sp.*	tibiotarsus	Complete	Variable	15*
D1	Dry	*Phocid sp.*	Femur	Complete	Variable	16
D1	Fossil	Indet.	Tibia	Shaft fragment	Thick cortical	10
D1	Fossil	Indet. bovid	Indet.	Shaft fragment	Thin cortical	11*
D1	Fossil	Indet. bovid	Indet.	Enamel fragment	Tooth	12
D1	Fresh	*G. domesticus*	Femur	Complete	Variable	17
D1	Fresh	*D. pygargus*	Rib	Fragment	Spongy	18
D1	Fresh	*D. pygargus*	Tibia	Shaft fragment	Thick cortical	19*
D1	Fresh	*D. pygargus*	Femur	Proximal epiphysis	Variable	20
D1	Weathered	Indet. bovid	Vertebrae	Complete	Spongy	7
D1	Weathered	Indet. bovid	Phalange	Complete	Compact	8
D1	Weathered	*B. domesticus*	P^4	Complete	Tooth	14*
D2	Dry	*O. aries*	Scapula	Complete	Variable	22
D2	Dry	*O. aries*	Radius	Complete	Variable	23a
D2	Dry	*O. aries*	Ulna	Complete	Variable	23b
D2	Dry	Indet. bovid	Metapodial	Shaft fragment	Thick cortical	24*
D2	Dry	*O. aries*	humerus	Shaft fragment	Thin cortical	25*
D2	Dry	*O. aries*	Tarsus	Complete	Compact	26
D2	Dry	*O. aries*	Rib	Complete	Spongy	27
D2	Dry	Indet. bovid	Humerus	Distal epiphysis	Variable	30
D2	Dry	*O. aries*	M^2	Near complete	Tooth	35*
D2	Dry	*Antidae sp.*	Tibiotarsus	Complete	Variable	36*
D2	Fossil	Indet.	Indet.	Shaft fragment	Thick cortical	31*
D2	Fossil	Indet.	Indet.	Shaft fragment	Thin cortical	32*
D2	Fossil	Indet. bovid	Indet.	Enamel fragment	Tooth	33*
D2	Fresh	*G. domesticus*	Femur	Complete	Variable	38
D2	Fresh	*D. pygargus*	Rib	Fragment	Spongy	39
D2	Fresh	*D. pygargus*	Tibia	Shaft fragment	Thick cortical	40*
D2	Fresh	*D. pygargus*	Femur	Proximal epiphysis	Variable	41*
D2	Weathered	Indet. bovid	Vertebrae	Complete	Spongy	28
D2	Weathered	Indet. bovid	Phalange	Complete	Compact	29*
D2	Weathered	*B. domesticus*	P^4	Near complete	Tooth	34*

Table 2. :Specimens used in experiment B that were exposed to *D. maculatus* for a period of 4 months with 50 mm of sterilised sediment at the base of both tanks. The tank containing dataset D3 received 100 g of canned meat biweekly, whilst the tank containing dataset D4 received only 50 g biweekly.

Trial	Condition	Taxon	Element	Conservation	Bone Type	Spec No.
D3	Dry	*O. aries*	Femur	Distal epiphysis	Variable	86
D3	Dry	Indet. bovid	Metacarpal	Shaft fragment	Thick cortical	87
D3	Dry	Indet. bovid	Metapodial	Shaft fragment	Thin cortical	88*
D3	Dry	*O. aries*	Tarsus	Complete	Compact	89
D3	Dry	Indet. bovid	Rib	Fragment	Spongy	90
D3	Dry	*O. aries*	P_3	Complete	Tooth	98*
D3	Dry	*Aves sp.*	Humerus	Complete	Variable	99*
D3	Dry	*Phocid sp.*	Femur	Complete	Variable	100
D3	Fossil	Indet. bovid	Tibia	Shaft fragment	Thick cortical	93*
D3	Fossil	Indet.	Metapodial	Shaft fragment	Thin cortical	94*
D3	Fossil	Indet. bovid	Tooth	Root fragment	Tooth	96*
D3	Fresh	*G. domesticus*	Femur	Complete	Variable	101
D3	Fresh	*D. pygargus*	Rib	Shaft fragment	Spongy	102*
D3	Fresh	*D. pygargus*	Tibia	Shaft fragment	Thick cortical	103*
D3	Fresh	*D. pygargus*	femur	Distal epiphysis	Variable	104*
D3	Weathered	Indet. bovid	Rib	Fragment	Spongy	91*
D3	Weathered	Indet. bovid	Phalanx	Complete	Compact	92
D3	Weathered	*B. domesticus*	P_3	Near complete	Tooth	97*
D3	Weathered	*O. aries*	Scapula	Complete	Variable	85
D4	Dry	*O. aries*	Scapula	Near complete	Variable	106
D4	Dry	Indet. bovid	Metacarpal	Shaft fragment	Thick cortical	108*
D4	Dry	*O. aries*	Metapodial	Shaft fragment	Thin cortical	109*
D4	Dry	*O. aries*	Tarsus	Complete	Compact	110
D4	Dry	Indet.	Rib	Fragment	Spongy	111
D4	Dry	*O. aries*	P_4	Complete	Tooth	117*
D4	Dry	*Phocid sp.*	Femur	Complete	Variable	118*
D4	Dry	*Aves sp.*	Humerus	Complete	Variable	119*
D4	Fossil	Indet. bovid	Tibia	Shaft fragment	Thick cortical	114*
D4	Fossil	Indet.	Indet.	Shaft fragment	Thin cortical	115*
D4	Fossil	Indet. bovid	Tooth	Enamel fragment	Tooth	95*
D4	Fresh	*G. domesticus*	Femur	Complete	variable	120*
D4	Fresh	*D. pygargus*	Rib	Shaft fragment	Spongy	121*
D4	Fresh	*D. pygargus*	Tibia	Shaft fragment	Thick cortical	122*
D4	Fresh	*D. pygargus*	femur	Distal epiphysis	Variable	123*
D4	Weathered	*O. aries*	Femur	Distal epiphysis	Variable	107
D4	Weathered	Indet. bovid	Phalange	Complete	Compact	112
D4	Weathered	Indet.	Indet.	Shaft Fragment	Spongy	113*
D4	Weathered	*B. domesticus*	P_3	Complete	Tooth	116*

(n = 103) were evident on 38% of specimens (2 fresh, 5 dry, 1 fossil). Destruction and striae were recorded on 48% of specimens (destruction - 1 fresh, 7 dry, 1 weathered, 1 fossil; striae - 1 fresh, 6 dry, 1 weathered, 2 fossil) and lastly boreholes (n = 3) where only recorded on 10% of specimens (2 dry). The increase in modification frequency in the absence of sand likely relates to time expenditure; in that, when sand was available both beetles and larvae were frequently cited burrowing into the sand, whilst in its absence *Dermestes maculatus* were potentially more focused on feeding and exploring the bone as a medium for pupation. Interestingly, even despite the absence of sand as a pupation medium, *D. maculatus* were not found to bore pupation chambers into bone as suggested in the palaeontological literature (Figure 1.), rather they simply pupated underneath specimens or in the open.

Food Experiment

Modification frequency was lower under reduced feeding conditions when compared to the results of experiments 2 and 3. Furrows (n = 7) were recorded on 11% of specimens (1 dry, 1 weathered). Pits (n = 6) were recorded on 11% of specimens (2 dry). Boreholes (n = 1) were recorded on only 5% of specimens (1 dry), whilst destruction and striae were recorded on 26% of specimens (3 dry, 2 weathered for both types). These observations suggested that reduced food availability has a negative effect on the frequency of modification which is in contradiction to existing literature (Hefti et al. 1980). It is hypothesised that the reduction of modification frequency under reduced feeding conditions may be representative of *Dermestes maculatus* not being able to acquire sufficient energy from food to expend the energy to modify bone. However, this requires further testing.

Modification distribution with respect to density and level of conservation

Across all experimental trials; furrows were recorded on cancellous bone and bones of varying density. Destruction and surface pits were also primarily restricted to cancellous bone but were also recorded on compact bone and teeth. Boreholes were recorded on cancellous, compact, and variable bone specimens. Striae occurred across all bone densities except for thin cortical bone. Primarily, teeth, thin and thick cortical specimens appear less favourable to *Dermestes maculatus* for modification. This is supported by majority of modifications being recorded on less dense area of bone, on cancellous bone or close to the epiphysis

Table 3. :Types of bone surface modifications produced by *Dermestes maculatus* after 4 months exposure.

Modification Type	Descriptions and level of observation	Fig. No
Destruction	Obliteration of bone. Completely destroying cancellous bone or articular facets resulting in exposure of the underlying trabecular bone. Intermediately visible at 7–20x magnification.	5.A, B
Furrows	Shallow depressions with a U-shaped profile excavated across the surface of a bone. Bore holes often occur at either one or both ends of the furrow. Primarily occurring as a single furrow, however, occasionally as a complex of interconnected furrows. Macroscopically visible.	3.A, B
Bore holes	Deep semi-circular holes which may or may not have a discernible bottom. Penetrating through cortical or cancellous bone either in to the medullary cavity or underlying trabecular bone. Intermediately visible at 7–20x magnification.	3.C, D
Pit	Class 1: Highly variable in shape, however, most often semi-circular to elliptical shallow depressions with a U-shaped profile that have striations radiating from around the outer circumference of the depression. Visible at magnifications higher than 20x.	2.A – D
	Class 2: Semi-circular shallow depressions with randomly orientated, often intersecting, striations occurring over the entire feature. Macroscopically visible.	2.E, F
	Class 3: Irregular-shaped depressions with complex profiles not associated with striations. Visible at magnifications higher than 20x.	5.C, D
Striae	Clusters of mandible gnawing marks which cover a large surface area. Visible at magnifications higher than 20x.	4.A – D

Table 4. Descriptive statistics of modification sizes produced by *D. maculatus* after 4 months exposure.

		Length				Breath/Width			
Type	*n*	Min.	Max.	Mean	Std. Dev.	Min.	Max.	Mean	Std. Dev.
Boreholes	9	215	689	448	135	149	639	337	165
Pit Class 1	18	110	1381	482	367	63	550	235	154
Pit Class 2	2	5188	5334	5261	103	2872	3077	2974	145
Furrows	26	984	9946	4286	2817	82	1644	448	327

diaphysis junction of long bones. In terms of bone condition, destruction, pits, and striae were found to occur across all conservation types (fresh, dry, weathered, fossil), furrows occurred on specimens of all types except fossil, whilst boreholes were limited to either fresh or dry specimens. Apart from a few isolated instances only fossil specimens appears less favourable to *D. maculatus*.

Table 5. :Results of macroscopically visible modifications.

		Furrows				
		Location		Ornt	Vis	No
#	**Trial**	**P**	**M**			
1	D1	Y	X	Vn	Clr	3
9		2	1	-	Ft, Clr	3
17		Y	X	Ds	Clr	3
18		Y	-	-	Ft	1
20		Y	-	Px	Ft, Clr	2
22	D2	Y	X	Vn	Mod	3
30		Y	-	-	Mod, Clr	3
38		Y	X	Px	Clr	1
85	D3	Y	X	Dr	Mod	18
86		Y	-	Px	Clr	3
106	D4	Y	X	Vn	Mod	5
107		Y	Y	Px	Clr	2

DISCUSSION

Identification and differentiation criteria

This study highlights the disparity between neoichnologically established bone surface modification criteria and the practical application of such criteria in the identification and differentiation of causal agents within the palaeontological taphonomic record. Dermestids have been shown to modify bone in several different ways, but the majority of modifications criteria do not display sufficient qualitative characteristics to enable the identification of dermestids as a causal agent and eliminate the potential influence of equifinality and/or behavioural convergence. Equifinality suggests that in any open system a given end state can be achieved by many potential means. Termites have been shown to produce a very similar morphological range of bone surface modifications to those of dermestids established in this study (Backwell et al. 2012). The apparent morphological overlap between these two causal agents is likely driven by the similarities in feeding behaviours of the two agents. It is thus likely that many other agents, both known and unknown, share similar behavioural tendencies whilst feeding on bones and would thus produce similar traces. Thus, behavioural convergence is another potential driver which could explain the disparity between modern dermestid traces and those identified in the fossil record. It is possible that one or several unknown invertebrate agents developed similar feeding habits to that of modern dermestids, and in doing so left behind traces in bone. Thus, when attempting to identify dermestids, or infer any specific causal agent, in the fossil record it is advisable to do so with caution. Irrespective of the potential limitations of

Table 6. :Results of modifications visible at 7–20x magnification.

		Destruction						Boreholes				
		Location			Ornt	Vis	Cat	Location		Ornt	Vis	No
#	**Trial**	**P**	**M**	**E**			**% Cover**	**P**	**M**			
1	D1	Y	X	-	-	Ft	<5%	Y	X	Vn	Clr	1
2a		Y	X	-	Px	Ft	<10%	-	-	-	-	-
2b		Y	X	-	Px	Ft	<10%	-	-	-	-	-
5		Y	X	-	-	Mod	<5%	-	-	-	-	-
6		Y	X	-	-	Mod	<5%	-	-	-	-	-
7		Y	X	-	-	Ft	<5%	-	-	-	-	-
9		Y	Y	-	-	Clr	<40%	Y	-	Px	Clr	2
12		-	Y	Y	-	Clr	<10%	-	-	-	-	-
16		Y	X	-	Ds, Px	Mod	<5%	-	-	-	-	-
17		Y	X	-	Ds, Px	Mod	<40%	-	-	-	-	-
22	D2	Y	X	Y	-	Ft	<40%	Y	X	Vn	Ft	6
23a		Y	X	Y	Ds, Px	Ft	<10%	Y	X	Ds	Clr	5
23b		Y	X	Y	Ds, Px	Ft	<10%	-	-	-	-	-
26		Y	X	-	-	Clr	<10%	Y	X	-	Ft	1
27		Y	X	-	-	Ft	<15%	Y	X	-	Ft	1
28		Y	X	-	-	Ft	<5%	-	-	-	-	-
30		Y	Y	-	-	Ft	<5%	-	-	-	-	-
38		Y	X	-	Ds, Px	Clr	<40%	Y	X	Ds	Clr	1
39		-	-	-	-	-	-	Y	X	-	Ft	1
85	D3	Y	X	-	Vn, Dr	Ft	<10%	-	-	-	-	-
86		Y	Y	-	-	Ft	< 10%	Y	-	Px	Mod	2
89		-	-	-	-	-	-	Y	X	-	Ft	3
90		Y	X	Y		Clr	<5%	Y	X	-	Clr	3
92		Y	X	-	-	Ft	<10%	-	-	-	-	-
100		Y	X	-	-	Ft	<5%	-	-	-	-	-
101		Y	X	-	Px, Ds	Mod	<15%	Y	X	Ds	Mod	2
106	D4	Y	X	-	Vn, Dr	Ft	<5%	-	-	-	-	-
107		Y	Y	-	Px	Ft	<5%	-	-	-	-	-
110		Y	X	-	-	Ft	<5%	Y	X	-	Ft	1
111		Y	N	-	Ds	Clr	<10%	-	-	-	-	-
112		Y	X	-	-	Ft	<10%	-	-	-	-	-

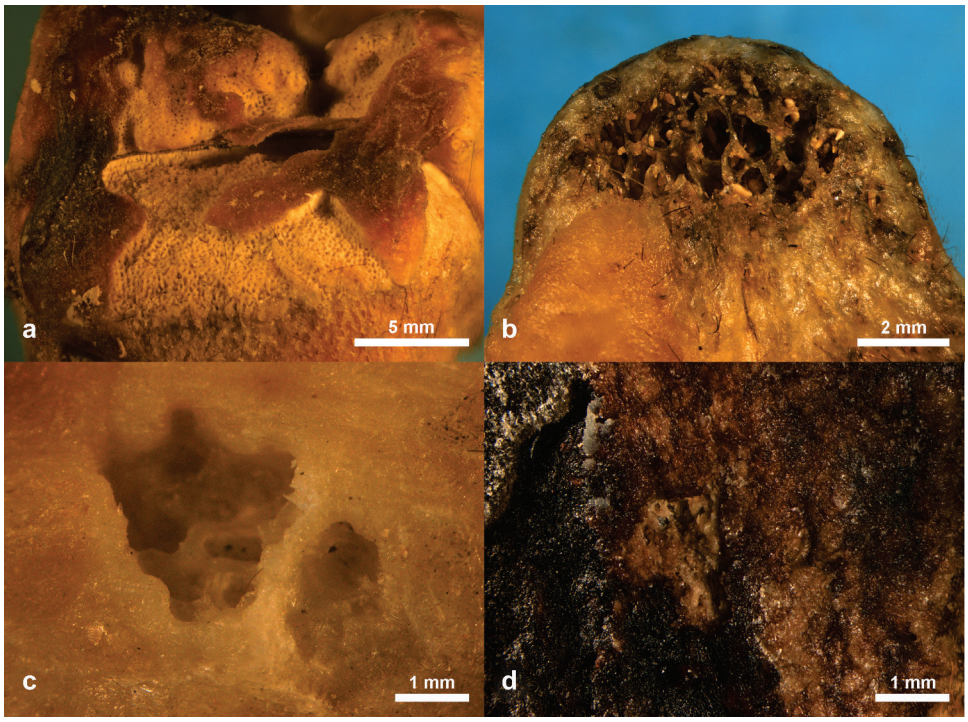

Figure 5. (A and B) Destruction of bone evident after four months exposure to *D. maculatus*. (A) Destruction evident on a fresh *G. domesticus* femur (specimen #17). (B) Destruction evident on a dry *O. aries* tarsal (specimen #5). (C, D) represent pit class 3 modifications evident on a dry *O. aries* Ulna (specimen #23b) and a fresh *G. domesticus* femur (specimen #38) retrospectively. Destruction and class 3 surface pits are UID 3 rated modification types, which suggests that neither criteria should be used as a basis for identification nor differentiation of dermestids as a causal agent if identified in the fossil record due to an inability to eliminate equifinality. The image presents several examples of bone destruction or irregular surface pits. These two modification types are considered UID3 rated which suggests they have little application in the identification and differentiation of Dermestid beetle activity in the fossil record. The micrographs show concentrated areas in which bone has been removed, mostly on softer cancellous bone. Additionally, images of irregular-shaped surface pits which lack any characteristic morphology.

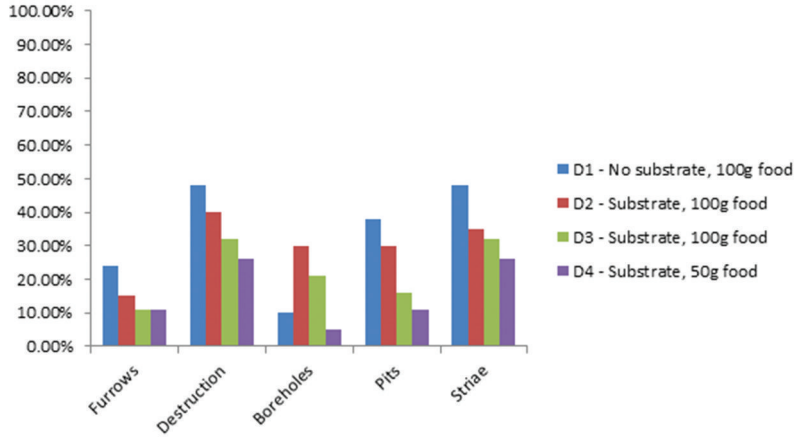

Figure 6. Type and percentage of modifications observed on all specimens used during experiments. A graph which plots the percentage of the various modification types (Furrows, Destruction, Boreholes, Pits, Striae) against the total number of bone specimens used during the experimental trails. Interestingly, in the absence of substrate and with 100 g of food the highest number of modifications were recorded. Whilst in the presence of substrate with the least amount of food provided (50 g) the lowest frequency of modifications where identified.

identifying specific agents, or enabling the differentiation between agents in deep time. To aid in this discussion a classification system is adopted which rates modification types accordingly to its 'usefulness in identification and differentiation', hereafter the UID Index. I define three UID Index classifications: UID 1 = traces sufficiently distinctive to facilitate identification of the trace maker and enable differentiation from other trace types, or traces made by other insects (Figure 2); UID 2 = traces which alone, are not distinctive but when found to co-occur with UID 1 modification types can be referred to a maker (Fig. 3, 4); UID 3 infers a modification type which has little practical application in identification or differentiation of an associated causal agent to the genus level (Figure 5).

Classes 1 and 2 surface pits show sufficiently distinctive morphology to enable the identification of dermestids as a causal agent and are distinctive enough from other reported modifications as to enable differentiation when discovered in the fossil record. Star-shaped marks (Backwell et al. 2012) associated to a termite (*Trinervitermes trinervoides*) from South Africa could be confused with class 1 pits described in this study. However, several characteristics can be used

Table 7. Results of modifications visible at magnifications higher than 20x.

		Pits							Striae					
		Location		Ornt	Vis	Class			Location			Ornt	Vis	Cat
#	**Trial**	**P**	**M**			**1**	**2**	**3**	**P**	**M**	**E**			**% Cover**
1	D1	Y	X	Vn, Dr	Ft	>60	0	0	Y	X	Y	Ds, Px	Ft	<60%
2a		Y	X	Ds, Px	Ft	13	0	0	Y	X	-	Ds, Px	Ft	<20%
2b		Y	X	Px	Ft	16	0	0	Y	X	Y	Px	Ft	<10%
5		-	-	-	-	-	-	-	Y	X	-	-	Ft	<5%
6		Y	X	Px	Ft	6	0	0	Y	X	-	Ds, Px	Ft	<20%
8		-	-	-	-	-	-	-	Y	X	-	Ds	Ft	<5%
9		-	-	-	-	-	-	-	Y	-	-	-	Ft	<10%
10		-	-	-	-	-	-	-	-	Y	-	-	Ft	<5%
12		-	Y	-	Clr	0	2	0	-	Y	Y	-	Clr	<10%
16		-	-	Ds	Clr	0	0	1	-	-	-	-	-	-
17		Y	X	Ds	Ft	0	0	1	-	-	-	-	-	-
20		Y	-	-	Mod	4	0	0	Y	-	-	Px	Ft	<5%
22	D2	Y	X	Vn, Dr	Mod	44	0	0	Y	X	-	Vn, Dr	Ft	<60%
23a		Y	X	Ds	Ft	4	0	0	Y	X	Y	Ds	Ft	<40%
23b		Y	X	Px	Clr	8	0	2	Y	X	Y	Ds, Px	Ft	<40%
26		-	-	-	-	-	-	-	Y	X	-	-	Ft	<5%
27		Y	X	-	Ft	9	0	1	Y	X	-	-	Ft	<5%
28		Y	X	-	Ft	1	0	0	-	-	-	-	-	-
30		-	-	-	-	-	-	-	Y	-	-	Ds	Ft	<10%
38		Y	X	Ds	Mod	0	0	2	Y	X	-	Ds	Ft	<5%
85	D3	y	x	Vn, Dr	Ft	7	1	0	y	x	-	Vn, Dr	Mod	<15%
86		-	-	-	-	-	-	-	y	-	-	-	Ft	<10%
87		-	-	-	-	-	-	-		y	-	-	Ft	<5%
89		y	x	-	Ft	13	-	-	y	x	-	-	Ft	<5%
90		-	-	-	-	-	-	-	y	y	y	-	Ft	<20%
92		-	-	-	-	-	-	-	y	x	-	-	Ft	<5%
101		y	x	Ds	Clr	-	-	1	-	-	-	-	-	-
106	D4	y	x	Ds	Ft	2	-	-	y	x	-	Vn, Dr	Ft	<10%
107		-	-	-	-	-	-	-	y	-	-	Px	Ft	<5%
110		y	x	-	Ft	4	-	-	y	x	-	-	Ft	<5%
111		-	-	-	-	-	-	-	y	x	-	-	Ft	<10%
112		-	-	-	-	-	-	-	y	x	-	-	Ft	<5%

Table 8. Summary of experimental variables and results.

Experiment	1	2	3	4
Substrate	0	1	1	1
Food (grams)	100	100	100	50
Damaged bones %	67	45	42	16

to differentiate the two modifications and thus the associated agents. Star-shaped marks are infrequently produced by termites, they are associated with existing surface features such as foramina (e.g. haversion canals), the have a circular morphology, the density of radiating striae is higher and star-shaped marks range from 1500 to 3000 µm in diameter. Class 1 pits are more readily produced by dermestids, are not associated to any existing surface features, have lower concentrations of radiating striae, they display an elliptical morphology and are smaller with a maximum size of 1381 x 550 µm (refer to Table 4). Unfortunately, due to their size Class 1 surface pits may be limiting by their preservational potential and as such may only prove useful under exceptional conditions. Class 2 surface pits are substantially larger than class 1 pits and are unlikely limited by their size. Due to the uniqueness of class 2 pits it is unlikely that it could be confused with any other modification types associated to known agents. However, pits described by Britt et al. (2008b) from the Upper Jurassic Morrison formation are similar to class 2 pits described in this study. However, the pits described by Britt et al. (2008b) are different in terms of the parallel and opposing nature of the associated striae. Class 2 pits made by dermestids display randomly orientated singular striae and are by no means parallel or opposing. The similarities between class 1 pits and star-shaped marks (Backwell et al. 2012) as well as class 2 pits and pits described by Britt et al. (2008b) likely relate to similarity in the behaviour of the agent during the creation process. However, it is the subtle differences between these modification types which are vitally important and it is thus recommended that classes 1 and 2 surface pits should form the primary basis for the identification *Dermestes maculatus* as a causal agent in the fossil and/or archaeological record.

The most limiting factor relating to the use of furrows, boreholes, and striae as a basis for identification and differentiation are imposed by the principle of equifinality. For example, boreholes lack distinctive qualitative characteristics and thus descriptions are overly dependent on shape and size (length, width, and depth). Thus, the identification of causal agent based on associated measurements is largely superficial as similar sized boreholes could easily be produced by a multitude of different agents which simply have a similar head width and body size. In such an instance the possibility of equifinality cannot be overlooked and thus boreholes should not be used in isolation to determine nor associate a causal agent. Striae and furrows present similar limitations due to a lack of qualitative characteristics. The length of 250 striae was measured and produced a mean of 388 µm, whilst the diameter of 100 striae were measured providing a mean of 19.72 µm (Table 4). Unfortunately, the descriptive statistics for dermestids are within the range of striae measured for termites in South Africa (Backwell et al. 2012). This unfortunately limits the application of size as an indicator of associated agent. In reality striae of comparable sizes could be produced by a diversity of similarly sized causal agents. Additionally, striae are unlikely to be preserved in the fossil record or may be confused with mechanical preparation damage. Following the same argument, similarly sized furrows could be produced by any invertebrate agents sharing a similar head width. Therefore, it is proposed that furrows, boreholes, and striae should not be used as criteria for identification of dermestids as a causal agent of bone modification in the fossil record. However, when such modification types are found in association with UID 1 modifications (Classes 1 and 2 surface pits) they may be attributed to dermestids based on associational inference.

The principle of equifinality limits the usefulness of destruction and Class 3 surface pits as a primary criterion for identification. These modifications do not display sufficient qualitative morphology and/or display highly variable morphology and thus have no practical application in identification of the associated causal agent. Destruction for

example could easily be attributed to differential preservation or an entire host of other taphonomic process which would have the same result. Realistically, the only conditions under which these modifications criteria could be identified would be by a neoichnologist who has recorded the condition of the specimens prior to exposure. Class 3 surface pits show highly variable morphology with no other associated characteristics which may be used to attribute this modification to *Dermestes maculatus*. Thus, when found in isolation in the fossil record equifinality cannot be eliminated and thus such modifications could be reasonably attributed to a diversity of abiotic or biotic agents. As such it is unwise to attribute such modifications to a particular causal agent.

Implications for the interpretation of the fossil record

The current neoichnological data suggests that *Dermestes maculatus* certainly do modify bone but that the modifications are disparate from those attributed to dermestids in the palaeontological literature (Kitching 1980; Martin and West 1995; Hasiotis et al. 1999; Chin and Bishop 2004; Hasiotis 2004; Laudet and Antoine 2004; Bader 2005; West and Hasiotis 2007). Britt et al. (2008b) provided a very strong motivation for their identification of *D. maculatus* as the causal agent based on their tooth width, distance between the teeth and the occurrence of striae being paired. This assumption was supported by the fact that extant dermestids have a primary mandibular cusp in addition to a secondary marginal cusp. It was thus suggested that during the creation of striae, both the primary and secondary cusps came into contact with the bone surface and thus create a pair of parallel striations with every bite. However, the creation of a pair of parallel striations during bone modification was not observed/recorded during this research. Thus, the identification of *D. maculatus* as the causal agent of modifications from the Upper Jurassic Morrison Formation does not find direct support when experimentally tested. However, in a broader context the general modification-type morphologies represented (i.e., furrows, pits and bore holes) as well as their associated size variability would suggest a member of the dermestidae family is the most likely suspect responsible for the Morrison Formation modifications. Traces bearing morphological similarities to those produced by dermestids during this study were recently reported on a early Homo GAR IVE hemimandible and associated faunal remains discovered in Ethiopia, dating to 1.8 million years old (Le Cabec et al. 2021). The identification was based on similarities on a gross morphological level. Unfortunately, at this level trace signatures across both time and space are grossly similar, not due to being made by the same agents, but rather due to the behavioural similarities of the invertebrate agents producing the traces. Furthermore, the traces on the GAR IVE samples including those on the hemi-mandible of the hominin are metrologically disparate to modifications produced by dermestids under experimental conditions. Future studies may wish to ascertain whether a link exists between mandibular width and/or head width of insect agents in respect to the maximum size of the bone modifications they produce. In doing so, size may prove to be a very useful indicator and/or eliminator of potential causal agents when interpreting damage to faunal remains from either an archaeological or palaeontological context.

The most common modification described and attributed to dermestids in palaeontological literature is the occurrence of boreholes, which are suggested to act as pupation chambers (see Figure 1). However, no comparable modifications were identified during the current investigation. The neoichnological data presented in this study suggest that the assumption that dermestids bore into bone to form pupation chambers, does not find credence when tested using extant dermestids under controlled conditions. Despite the limitation of this current study, I believe these finding have significant implications for the fossil record and perhaps warrants a re-interpretation of existing literature. The lack of correlation between extant *Dermestes maculatus* modifications and those described in the palaeontological record, does not categorically prove that dermestids are not responsible for the creation of many palaeontological modifications. The current investigations only examined a single species of the genus *Dermestes*, but the possibility remains that other species of *Dermestes* may produce slightly variable modification types and distributions to those described in this study. As such, the aims of future research should focus on establishing whether different *Dermestes* species produce different modification suites or whether they mimic one another based on similarities in feeding behaviour and/or mandibular morphology. Lastly, the influence of predation pressures by other arthropods, birds or even lizards should be tested to establish whether or not predation affects represented modification types and/or their associated distribution patterns.

Taphonomically, the most frequent modifications are microscopic and thus it is unlikely that dermestids would bias taxonomic and skeletal element representation, minimum number of individuals or age profiles as suggested for termites (Backwell et al. 2012). Nonetheless, the identification of dermestid bone surface modification could significantly contribution to reconstructing taphonomic history and site formation processes. The occurrence of dermestid modifications would likely suggest; the corpses was sub-aerially exposed long enough to have reached the bloated and/or dry stage of decomposition prior to modification and burial. This information in combination with other proxy information may further elucidating the taphonomic and site formation history of any fossil site being investigated.

CONCLUSION

This study has shown that dermestids modify bone in several different ways including destroying bone, creating furrows, boreholes, pits (classes 1, 2 and 3) as well as striae. The principle of equifinality imposes limitations on the application of these criteria for the identification and differentiation of dermestids as a causal agent in the fossil record. Thus, a classification system was used to rate the 'useful for identification and differentiation' (UID) of the neoichnologically established criteria. Thus, it is suggested that Pit Classes 1 and 2 (UID 1) modifications should be used as primary criteria for identification and differentiation. Whilst the use of other modification criteria (UID 2 and UID 3) established during the course of this investigation should be interpreted with a degree of caution. Once a positive identification has been established various inferences can be drawn to better reconstruct the total taphonomic history of a fossil deposit. In my opinion it has long been inferred that dermestids pupate in bone, but to date no substantial body of direct/primary evidence exists to support such inferences. Despite ongoing neoichnological efforts this evidence still eludes us.

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ORCID

Alexander Parkinson http://orcid.org/0000-0001-9067-7266

Highlights

- Dermestids can destroy bone, creating furrows, boreholes, pits and striae.
- *Dermestes maculatus* are unlikely responsible for modifications in palaeontological literature.
- The absence of sediment increases modification frequency
- Whilst, reduced food availability negatively impacts on modification frequency.
- When determining causal agent influence of equifinality needs to be considered.

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