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PALEOHISTOLOGIA DE MAMÍFEROS ENCOURAÇADOS (XENARTHRA, CINGULATA): ASPECTOS TAXONÔMICOS, PALEOBIOLÓGICOS E BIOMECÂNICOS

Recife
2020

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Tese apresentada ao Programa de Pós-Graduação em Geociências do Cento de Tecnologia e Geociências da Universidade Federal de Pernambuco como preenchimento parcial dos requisitos para obtenção do grau de Doutora em Geociências.

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Dedico as mulheres cientistas que abriram caminho antes de mim.

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“Não há nenhum erro maior do que o de acreditar que a última palavra dita é sempre a mais correta, que algo escrito mais recentemente constitui um aprimoramento do que foi escrito antes, que toda mudança é progresso.” (SCHOPENHAUER, 1851)

RESUMO

Os osteodermos encontrados em xenartras (tatus, pampatérios, gliptodontes e algumas preguiças) apresentam alto potencial de fossilização e proporcionam inúmeras informações sobre a paleobiologia das espécies fósseis do grupo. A presente tese tem como objetivo descrever a variação microestrutural nos ossos dos cingulatas e mostrar a importância da paleohistologia como ponto de partida para um melhor conhecimento dos táxons extintos. Foi analisado a paleohistologia dos osteodermos de *Pampatherium humboldti*, *Holmesina paulacoutoi*, *Pachyarmatherium brasiliense*, *Glyptodon* sp., *Glyptotherium* sp., *Doedicurus clavicaudatus* e *Hoplophorus euphractus*, além de um úmero do gliptodonte *Hoplophorus euphractus*. A análise demostrou como o padrão da microestrutura óssea dos osteodermos, refletido principalmente no tipo de arranjo das fibras de colágeno e na morfologia das lacunas dos osteócitos, é útil nas inferências sobre a paleobiologia dessas espécies extintas. Os diferentes táxons apresentam grande variação histológica, desde tecidos muito remodelados à tecidos derivados de uma combinação da osteogênese dinâmica e estática. Nas trabéculas dos osteodermos está presente prioritariamente um tecido ósseo lamelar, mais organizado que nas outras regiões do osso, onde a característica mais variável dentre as espécies é o tamanho e distribuição das áreas trabeculares. A microestrutura do úmero de *H. euphractus*, descrita aqui pela primeira vez, mostra um osso derivado de osteogênese dinâmica, onde lacunas de osteócitos alongadas e fibras de colágeno com orientação preferencialmente transversal são visíveis. A análise da microestrutura do osteodermo de *P. brasiliense* mostra uma proximidade da espécie com o padrão esperado para dasipodídeos, indicando uma possível afinidade com este grupo. Além disto, características como aumento da espessura da camada esponjosa na zona central e presença de tecidos ósseos com alto nível de organização das fibras de colágeno pode ser considerados adaptações biomecânicas para os osteodermos dos gliptodontes. Em relação ao desenvolvimento do osteodermo, indícios de ossificação metaplástica e crescimento periosteal são vistos em todos os espécimes. Portanto, as características descritas para ambos tipos de ossos indicam adaptações ao estilo de vida desses animais extintos.

Palavras-chave: Osteodermo. Paleohistologia. Cingulata. Microestrutura óssea.

ABSTRACT

The osteoderms found in xenarthrans (armadillos, pampatheres, glyptodonts and some sloths) are integumentary bones with high fossilization potential that hold information about the paleobiology of the fossil taxa of this group. This work aims to describe the microstructural variation in cingulate bones and to show the importance of paleohistology as a starting point for a better knowledge of extinct taxa. The paleohistology of osteoderms from *Pampatherium humboldti*, *Holmesina paulacoutoi*, *Pachyarmatherium brasiliense*, *Glyptodon* sp., *Glyptotherium* sp., *Doedicurus clavicaudatus* and *Hoplophorus euphractus* were analyzed, in addition to a long bone of *H. euphractus*. The analysis demonstrated how the osteoderm bone microstructure pattern, mainly reflected in the type of collagen fiber arrangement and the morphology of the osteocyte lacunae, is useful in inferring the paleobiology of these extinct animals. In general, the different taxa present a large histological variation, from very remodeled tissues to tissues derived from a combination of dynamic and static osteogenesis. In the trabeculae of the osteoderms, lamellar bone tissue is mostly common, more organized than in other bone regions and the most variable characteristic among species is the size and distribution of the trabecular areas. The humerus histology of *H. euphractus*, described here for the first time, shows a bone derived mainly from dynamic osteogenesis, where elongate osteocytes lacunae and collagen fibers with a preferably transverse orientation are visible. The microstructure analysis of *P. brasiliense* osteoderm shows the proximity of the species to the expected pattern for dasipodidae, indicating a possible affinity with this group. In addition, characteristics such as increased thickness of the spongy layer in the middle zone and presence of bone tissues with a high level of collagen fiber organization can be considered biomechanical adaptations for glyptodonts osteoderms. Regarding osteoderm development, evidence of metaplastic ossification and periosteal growth are seen in all specimens. Therefore, the features seen on both kind of bones indicate lifestyle adaptations of these extinct animals.

Keywords: Osteoderm. Paleohistology. Cingulata. Bone microstructure.

SUMÁRIO

1 INTRODUÇÃO

Apesar da monofilia da Ordem Cingulata nunca ter sido profundamente questionada, a relação entre os três principais grupos (tatus, pampatérios e gliptodontes) tem sido difícil de resolver detalhadamente (DELSUC et al., 2016). A Ordem é tradicionalmente dividida em duas superfamílias: Dasypodoidea (Dasypodidae) e Glyptodontoidea (Pampatheriidae e Glyptodontidae) (MCKENNA; BELL, 1997). A presença de uma carapaça constituída de inúmeros ossos dérmicos (osteodermos) dispostos em arranjos regulares representa um dos recursos de diagnóstico mais útil do grupo (KRMPOTIC et al., 2009), sendo o seu arranjo e morfologia empregados como uma fonte de informação taxonômica (HOFFSTETTER, 1958 ; VICKARYOUS; HALL, 2006; KRMPOTIC et al., 2009). Osteodermos são ossos tegumentares com alto potencial de fossilização (HILL, 2006), constituindo os elementos fósseis mais frequentes e abundantes no registro dos Cingulata (CARLINI; ZURITA, 2010).).

Os gliptodontes apresentam uma história biogeográfica e evolutiva bastante complexa e dinâmica (CUADRELLI et al., 2019), distribuindo-se do Eoceno final, Idade Mustersense (ZURITA et al., 2016), até o Holoceno Inicial, Idade Lujanense, Estágio Platense (CIONE; TONNI; SOIBELZON, 2003). Diferem dos restantes cingulados por apresentarem uma dentição fortemente trilobada, coluna vertebral fundida e uma carapaça dorsal essencialmente imóvel que cobre o topo e as laterais do tronco (HOFFSTETTER, 1958).

Já os pampaterídeos são considerados parentes próximos dos gliptodontes, e são registrados do Mioceno ao Pleistoceno (SCILLATO-YANÉ et al., 2005); apresentam inúmeras características similares aos dasipodídeos, como as bandas móveis na carapaça, e características dos gliptodontes, como núcleos de osteodentina (GAUDIN; WIBLE, 2006). Gaudin e Wible (2006) demonstraram que gliptodontes e pampatérios são táxons irmãos, formando um grupo monofilético baseado em dezesseis sinapomorfias não ambíguas. Mesmo que essa hipótese seja amplamente aceita, a taxonomia e as afinidades entre os táxons individuais de pampatérios ainda são discutidas (WOLF; KALTHOFF; SANDERS, 2012; LIMA, 2013).

A hipótese de que os gliptodontes teriam divergido dos outros cingulados em um ponto muito cedo em sua história filogenética é tradicionalmente aceita (HOFFSTETTER, 1958). No entanto, Gaudin e Wible (2006), baseando-se em caracteres crânio-dentais, sugeriram que os gliptodontes estariam dentro do grupo dos tatus, indicando uma relação próxima com a subfamília Euphractinae. Recentemente, Delsuc et al. (2016) apresentaram uma reconstrução filogenética baseada em DNA mitocondrial (GIBB et al., 2015) que também estabelece os gliptodontes dentro do grupo dos tatus, porém representando uma subfamília (Glyptodontinae) dentro da Família Chlamyphoridae. Ao mesmo tempo em um estudo independente, Mitchell et al. (2016) também demostraram a mesma proximidade entre os gigantes gliptodontes e os pequenos tatus da Família Chlamyphoridae, integrando aos seus dados moleculares trabalhos anteriores de caracteres morfológicos do crânio (BILLET et al. 2011) e DNA mitocondrial de xenartras (GIBB et al., 2015).

Estudos relacionados à histologia de osteodermos têm fornecido informações valiosas em diversos aspectos biológicos, incluindo evidências úteis em análises sistemáticas (SCHEYER; SANDER, 2004; SCHEYER et al., 2007). Hill (2005) ressalta o valor sistemático das características histológicas dos osteodermos para reconstruções filogenéticas, mas, infelizmente, trabalhos mais antigos não abordam esse tópico com muitos detalhes (WOLF; KALTHOFF; SANDERS, 2011). A paleohistologia poderia finalmente ajudar a definir as relações filogenéticas de espécies problemáticas que apresentam relações filogenéticas controversas. No entanto, o conhecimento das características histológicas de osteodermos de cingulados é até agora limitado a poucos espécimes de um número muito pequeno de táxons superiores (WOLF; KALTHOFF; SANDERS, 2011; ARAÚJO; PORPINO, 2018).

Os estudos como os de Hill (2006), Wolf (2007), Krmpotic et al. (2009) Wolf, Kalthoff e Sanders (2011) e Da Costa Pereira et al. (2014), Asakura et al. (2017), Araújo & Porpino (2018) demonstram a grande variação interespecífica e intraespecífica da microestrutura dos osteodermos, tanto de cingulados como de milodontídeo (HILL, 2006). As ossificações dérmicas fornecem informações sobre estruturas de tecido mole que não foram preservados em fósseis; tais estudos também demonstram a importância deste tipo de análise para inferir diversos aspectos paleobiológicos (WOLF, 2008; WOLF; KALTHOFF; SANDERS, 2011; STRAEHL et al., 2013).

1.1 OBJETIVO GERAL E ESPECÍFICOS

Este estudo pretendeu expandir o conhecimento acerca da histologia de osteodermos de mamíferos encouraçados, preenchendo esta lacuna no tocante das espécies de gliptodontes e pampatérios sul-americanos. Os resultados obtidos ampliam o conhecimento sobre os padrões histológicos dos osteodermos dos mamíferos cingulados, incluindo aspectos da sistemática e a paleobiologia do grupo. Para tanto foram estabelecidos como objetivos específicos:

a) Descrever a histologia dos osteodermos dos cingulatas *Pampatherium humboldti*, *Holmesina paulacoutoi*, *Pachyarmatherium brasiliense*, *Glyptodon* sp., *Glyptotherium* sp., *Doedicurus clavicaudatus* e *Hoplophorus euphractus*;
b) Discutir as possibilidades de inferências sistemáticas baseado na paleohistologia de

osteodermos;

c) Inferir os mecanismos ontogenéticos presentes durante o desenvolvimento dos osteodermos;
d) Investigar evidências para adaptações biomecânicas nos osteodermos.

2 MATERIAIS E MÉTODOS

Os materiais e métodos utilizados nesta pesquisa estão descritos abaixo.

2.1 MATERIAL

O material utilizado consistiu em fósseis depositados na Coleção de Macrofósseis do Laboratório de Paleontologia do Departamento de Geologia, Centro de Tecnologia e Geociências da Universidade Federal de Pernambuco (DGEO-CTG-UFPE) e de fósseis doados por Coleções Científicas de outras Universidades (Ver Apêndice I).

2.2 METODOLOGIA

A metodologia usada em estudos paleohistológicos envolve processos destrutivos dos espécimes. Assim, todo material foi previamente medido e fotografado, tendo sido registrados seus dados relacionados à forma original e a morfologia externa. Os espécimes menos comuns foram também moldados e replicados.

A metodologia adotada para a confecção das lâminas delgadas seguiu as propostas de Chinsamy e Raath (1992) e Lamm (2013). Os cortes histológicos foram realizados no Laboratório de Preparação de Amostras e Laminação (DGEO/UFPE) e no Laboratório de Preparação do Instituto de Geociências da Universidade de São Paulo (IGc/USP).

O material foi impregnado com resina poliéster para garantir resistência durante o seccionamento na máquina de corte petrográfico com lâmina diamantada. A superfície cortada foi então polida para ser colada na lâmina, onde a superfície oposta foi desbastada até a que o material se tornar translúcido. Cada osteodermo originou duas lâminas, sentido transversal e longitudinal.

As lâminas foram analisadas em um microscópio óptico Zeiss, modelo Axioskop 40 e Axio Vision Imager A2 com câmera AxioCam MRc acoplada, utilizando o programa Axio Vision Release 4.8 para execução de fotomicrografias, ambos pertencentes ao Laboratório de Paleontologia – PALEOLAB/DGEO/CTG/UFPE.

As feições macroscópicas foram registradas com câmera Nikon Coolpix P520 e estereomicroscópio Zeiss Discovery.V8 com câmara AxioCam MRc acoplada, utilizando o programa Axio Vision Release 4.8. As imagens, quando necessário, foram editadas nos programas ImageJ e Adobe Photoshop CC 2017.

O úmero foi analisado quantitativamente através do programa BONE PROFILER Versão 4.5.8 (GIRONDOT; LAURIN, 2003). Para usar o programa, a imagem da lâmina foi convertida em um desenho esquemático binário utilizando os programas ImageJ e Adobe Photoshop CC 2017, onde a cor preta representa o osso e a cor branca representa as áreas vasculares (canais vasculares, ósteons e espaços de reabsorção). Os seguintes parâmetros foram analisados usando o programa: compactação global (*Cg*); a distância relativa do centro da seção até o ponto abrupto de mudança no nível de compactação (*P*); largura da zona de transição entre o tecido compacto e esponjoso.

2.3 TERMINOLOGIA BÁSICA

A terminologia usada segue o conceito convencional visto na literatura de referência na histologia óssea (principalmente FRANCILLON-VIEILLOT et al., 1990; HILL, 2006; HUTTENLOCKER, WOODWARD & HALL, 2013, STEIN; PRONDVAI, 2014, PRONDVAI et al., 2014).

Em relação à disposição das camadas histológicas, optou-se por utilizar o termo “superficial” para a face do corte voltada para o exterior e “profundo”, para a face voltada para o interior do animal, seguindo a proposta de Hill (2006). Para elementos referentes à morfologia macroscópica foram empregados os termos “externo” e “interno”.

A classificação em osso compacto e esponjoso foi considerado como uma feição macroscópica relacionada apenas ao nível de porosidade do osso, seguindo a proposta de Francillon-Vieillot et al. (1990).

3 RESULTADOS E DISCUSSÃO

Os resultados obtidos na tese e suas respectivas discussões são apresentados nos artigos científicos que seguem:

3.1 ARTIGO 1 - A HISTOLOGIA COMO UMA FERRAMENTA NO ESTUDO DOS FÓSSEIS: CONSIDERAÇÕES SOBRE A PALEOHISTOLOGIA EM MAMÍFEROS COM CARAPAÇA ÓSSEA

3.2 ARTIGO 2 - AN INSIGHT INTO A LARGE CINGULATE PALEOBIOLOGY: THE CASE OF *HOPLOPHORUS EUPHRACTUS* LUND, 1839 (CINGULATA, GLYPTODONTOIDEA)

3.3 ARTIGO 3 - ELUCIDATING CINGULATE'S OSTEODERMS HISTOLOGY – IMPLICATIONS FOR SYSTEMATICS, OSTEODERM GROWTH, AND BIOMECHANICAL ADAPTATION

3.1 A HISTOLOGIA COMO UMA FERRAMENTA NO ESTUDO DOS FÓSSEIS: CONSIDERAÇÕES SOBRE A PALEOHISTOLOGIA EM MAMÍFEROS COM CARAPAÇA ÓSSEA

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RESUMO

Mesmo após milhões de anos de soterramento, muitos vertebrados ainda apresentam a estrutura histológica do tecido ósseo preservada. Aspectos ontogenéticos, reconhecimento de feições fossildiagenéticas, aprimoramento de estudos sistemáticos e anatômicos são os principais pontos abordados pelos estudos paleohistológicos. Dentre os vertebrados, os grupos mais estudados por esta ótica foram os dinossauros, pterossauros, ictiossauros, crocodilomorfos, testudines, cinodontes e peixes, sendo ainda discretos os estudos realizados com mamíferos, especialmente os mamíferos com carapaça óssea. Este trabalho pretende reunir o conhecimento disponível acerca da histologia de osteo- dermos de mamíferos cingulados, de forma a apresentar um panorama dos trabalhos atuais e um ponto de partida para futuros trabalhos.

Palavras chave: Microestrutura óssea, osteodermos, Cingulata, Xenarthra, tecido ósseo.

ABSTRACT

Even after millions of years of burial, many vertebrates still display the histological structure of preserved bone tissue. Ontogenetic aspects, recognition of fossildiagenetic features, improvement of systematic and anatomical studies are the main points addressed by paleohistological studies. Among the vertebrates, the most studied groups were the dinosaurs, pterosaurs, ichthyosaurs, crocodilomorphs, testudines, cynodonts and fish, being also discreet the studies carried out with mammals, especially the cingu- lates. This study aims to review the work on osteoderms histology of cingulate mam- mals, presenting a panorama of current works and a starting point for future works.

Keywords: Bone microstructure, osteoderms, Cingulata, Xenarthra, bone tissue.

INTRODUÇÃO

A Ordem Cingulata é caracterizada principalmente pelo corpo coberto por uma armadura dérmica (osteoder- mos), que são os elementos ósseos mais frequentes e abundantes no registro fóssil desta ordem (Vizcaíno *et al.*, 2008; Carlini & Zurita, 2010). Os osteodermos constituem um osso tegumentar com alto potencial de fossilização (Ameghino, 1889; Hill, 2006) e apresentam ornamentação que varia muito de um gênero para outro, bem como de uma espécie para outra, razão pela qual essa característica é utilizada com frequência em estudos taxonômicos (Ameghino, 1889; Scillato-Yané, 1976; Paula Couto, 1979; Vickaryous & Hall, 2006; Carlini & Zurita, 2010; Fernicola & Porpino, 2012). Justamente por serem os elementos esqueletais mais abundantes no registro fóssil do grupo, os osteodermos são o principal foco das pesquisas morfológicas e histológicas de cingulados.

A estrutura do tecido ósseo é composta por um tecido conjuntivo mineralizado produzido por deposição de hidroxiapatita, fosfato de cálcio cristalino e, nas partes interiores, por osteócitos e numerosos canais vasculares (Chinsamy *et al.*, 1995; Hall, 2005). Após a morte, os componentes orgânicos, incluindo as células e vasos sanguíneos, se decompõem, ao passo que a porção inorgânica fossiliza, mantendo, assim, a microestrutura óssea e preservando a forma dos componentes decompostos (Ricqlès *et al.*, 1998).

Os estudos sobre xenartros conduzidos por Hill (2006), Wolf (2007), Krmpotic et al. (2009), Wolf *et al.* (2012) e Da Costa Pereira *et al.* (2014), mostram que as características da mi- croestrutura anatômica dos osteodermos são altamente relevantes para os estudos das relações evolutivas, além de fornecerem caracteres diagnósticos em níveis taxonômicos mais baixos (gênero e mais raramente espécie), e informações sobre estruturas de tecido mole que não foram preservados em fósseis.

Atualmente, é notável o significativo aumento da utilização da paleohistologia aplicadas a inúmeros grupos fósseis (Chinsamy *et al.*, 2013; Nakajima *et al.*, 2014; Sayão *et al.*, 2016; Evans *et al.*, 2018). Inferências sobre aspectos ontogenéticos e fisiológicos, reconhecimento de feições fossildiagenéticas, diagnóstico de paleopatologias, aprimoramento de estudos sistemáticos e anatômicos tem sido os principais pontos abordados por esses estudos (Chinsamy *et al.*, 2009; Ricqlès, 2011; Houssaye, 2014).

Este trabalho objetiva sintetizar as informações disponíveis sobre a paleohistologia de cingulados fósseis, apresentando um panorama da atual situação das pesquisas envolvendo essa técnica.

BREVE HISTÓRICO

O processo de fossilização envolvendo ossos revela muito mais do que a forma externa dos tecidos esqueletais, preservando também a sua estrutura microscópica, e o seu estudo é chamado de paleohistologia (Padian, 2013). O termo “paleohistologia”, criado por Goodrich em 1913, refere-se ao estudo dos diferentes níveis de organização dos tecidos mineralizados, como osso, cartilagem calcificada, dentina, esmalte, tendões ossificados, etc. através da análise de sessões delgadas de fósseis (Lamm, 2007; Houssaye, 2014).

A origem da paleohistologia de vertebrados é incerta, sendo obvio que o domínio da técnica de cortes em materiais duros e o desenvolvimento dos equipamentos de microscopia foram um pré-requisito importante para o início desta área de estudo (Ricqlès *et al.*, 2004).

A análise óssea em nível celular data da invenção do microscópio, pois foram iniciados pelos próprios inventores do equipamento, Leeuwenhoeck & Havers (Ricqlès *et al.*, 2004; Hoole, 2010; Sanchez *et al.*, 2012). Em 1673, Van Leeuwenhoek foi um dos primeiros cientistas a analisar cortes de material de animais e plantas usando um microscópio óptico (Sanchez *et al.*, 2012). Na mesma época, Robert Hook observou uma grande diversidade de organismos, sendo inclusive o primeiro pesquisador a analisar fósseis ao microscópio, estudando madeiras fossilizadas e conchas de moluscos (Davidson, 2010).

O considerado pai da petrografia microscópica, Henry Clifton Sorby realizou seu primeiro corte petrográfico em 1849 (Lamm, 2013). A pesquisa de Sorby data quase 200 anos depois dos primeiros trabalhos de Leeuwenhoeck e de Hooke relacionados à histologia de ossos de animais (Ricqlès *et al.*, 2004; Davidson, 2010; Sanchez *et al.*, 2012).

Ao longo dos anos a técnica usada para confeccionar as lâminas vem sendo aprimorada constantemente. A partir dos anos 60, começou a ser utilizado componentes básicos empregados até hoje em dia, como resinas e lâminas com borda diamantadas para as máqui- nas de corte (Chinsamy & Raath, 1992; Wilson, 1994; Ahmed & Voort, 2003). Isso levou ao aprimoramento da técnica, e consequentemente à produção de lâminas mais duradouras e informativas (Lamm, 2013). Stein & Sander (2009) desenvolveram um método alternativo de perfuração de núcleo que é menos destrutivo que o método convencional. Porém, o método de Stein & Sander (2009) possui uma limitação importante que é uma visão restrita do córtex, além de depender da boa preservação dos espécimes.

A grande revolução da paleohistologia veio com os trabalhos dos pesquisadores Donald Enlow e Armand Ricqlès, entre as décadas de 1960 e 1970, que demonstraram em seus traba- lhos o valor expressivo das análises da microestrutura óssea apresentando um extenso estudo histológico comparativo em vertebrados, tanto fósseis como viventes. Ricqlès nos seus trabalhos "*Recherches paléohistologiques sur les os longs des tetrápodes*" (1975–1978), usou a microanatomia do osso para infe- rir o estilo de vida de muitos vertebra- dos basais, como os stegocefalianos do Paleozoico (1981) e de amniotas (1974), além de propor a padronização da nomenclatura dos tecidos ósseos. Essa nomenclatura reflete, principalmente, a densidade e a orientação da vascularização, a orientação das fibras colágenas e a densidade de ósteons secundários (Laurin, 2011). Francillon-Vieillot *et al.* (1990) discutiu detalhadamente o desenvolvimento e evolução da estrutura hierárquica do osso. Esses trabalhos são usados até hoje na classificação e interpretação das estruturas ósseas e seus processos.

A paleohistologia começou oferecendo caracteres diagnósticos, usados para reconhecer e classificar os fósseis em contextos taxonômicos, independentemente de qualquer implicação paleobiológica (Ricqlès, *et al.*, 2004; Ricqlès, 2011). No entanto, durante as últimas três décadas, pesquisas paleohistológicas tem focado principalmente em desvendar as numerosas e complexas causas que explicam os padrões e processos da evolução de vertebrados, demonstrando ser uma poderosa ferramenta para a reconstrução biológica tanto de fósseis como organismos vivos (Ricqlès, 2011).

A taxa relativa de formação do osso é indicada pela textura da matriz fibrilar, enquanto a natureza do osso compacto primário proporciona uma avaliação direta se a sua deposição foi contínua ou interrompida (Chinsamy, 1997). A quantidade de formação óssea secundária descreve o grau de reabsorção óssea primária e subsequente deposição (Chinsamy, 1997; Hall, 2005; Huttenlocker *et al.*, 2013). Além disso, a organização interna do osso indica processos de remodelação e de realocação de crescimento, incluindo adaptações funcionais da morfologia óssea. Assim, a osteohistologia consegue refletir a ontogenia, dinâmica de crescimento, as adaptações biomecânicas, bem como os vários eventos que pontuam a história de vida de um animal (Chinsamy, 1997).

As diferenças histológicas preservadas localmente em um esqueleto, ou entre os ossos homólogos em espécies diferentes, não são apenas as consequências - e expressões - de crescimento, modelagem e remodelamento ósseo (Ricqlès, 2011). Ao contrário, eles nos mostram as causas biológicas reais de especificidade do crescimento em ação e da formação da morfologia local durante o desenvolvimento (Enlow, 1963; Ricqlès, 2011).

A PALEOHISTOLOGIA EM DIFERENTES TETRÁPODES

A histologia está se tornando essencial em várias áreas da paleontologia, como a tafonomia (Jans *et al.*, 2004), determinação de idade (Maat et al., 2006), complementando análises de DNA (Guarino *et al.*, 2006), no estudo de proteínas (Schimidt-Schultz & Schu- ltz, 2004), e em diagnósticos de paleopatologias (De Boer *et al.*, 2013). A paleohistopatologia se tornou uma ferramenta confiável na diferenciação entre tumores, distúrbios metabólicos e doenças infecciosas já que características histo-arquiteturais específicas podem estar ligadas a determinadas doenças (Schultz, 2001; De Boer *et al.*, 2013).

O número de pesquisas que aplicam essa metodologia em diversos grupos fósseis vem aumentando cada vez mais no mundo todo (e.g Chinsamy *et al.*, 2013; Nakajima *et al.*, 2014; Woodward *et al.*, 2014). Dentre os vertebrados, os grupos mais estudados até então foram dinossauros (Horner *et* al., 1999; Horner *et* al., 2000; Sander *et* al., 2006; Stein & Prondvai, 2014; Evans *et al.*, 2018), ictiossauros (Houssaye *et al.*, 2018), pterossauros (Ricqlès *et al.*, 2000; Duque & Barreto, 2018; Cheng *et al.*, 2018), crocodilomorfos (Woodward *et* al., 2011; Andrade & Sayão, 2014; Buffrénil *et al.*, 2015), testudines (Scheyer *et al.*, 2007), cinodontes (Botha & Chinsamy, 2000) e mais recentemente mamíferos (Kolb *et al.*, 2015).

Implicações evolutivas para os osteodermos da tartaruga de casco mole da Família Trionychidae foram descritas por Scheyer *et al.* (2007), onde o padrão histológico do osteodermo indica uma estrutura "*plywood*" que seria a chave adaptativa desse grupo, permitindo o achatamento da carapaça e redução da necessidade mineral sem, no entanto, desestabilizar a biomecânica, aumentando assim a sua agilidade natatória.

Chinsamy *et al.* (2013) demonstraram o poder desta ferramenta ao fazer a primeira identificação de sexo em uma ave do Mesozoico, *Confuciusornis sanctus* Hou et al. 1995, através de análises histológicas em ossos longos, concluído que o espécime em questão era uma fêmea que tinha acabado de pôr ovos antes de morrer. Os autores identificaram a presença de osso medular, um tipo de tecido ósseo exclusivo de aves fêmeas em período reprodutivo. Este trabalho fornece informações importan- tes sobre o início da maturidade sexual e o alcance do tamanho corporal adulto dessa e de outras aves primitivas.

Aureliano *et al.* (2018) realizaram análises histológicas em um espinosauro, um grupo pouco conhecido devido ao seu registro fóssil incompleto, tendo seus resultados contribuído para o conhecimento relacionado à paleobiologia desses animais.

Não só ossos longos são utilizados na paleohistologia. Em geral, todos os tecidos biomineralizados dos vertebrados, como os dentes, também são analisados com o uso desta técnica, como demonstrado por Haridy *et al.* (2017) que aplicaram a histologia para entender os modelos de substituição em dentes acrodontes.

Além de ossos longos e dentes, há também vários trabalhos recentes explorando implicações filogenéticas e funcionais da histologia comparativa de osteodermos em répteis (Scheyer & Sander, 2009; Andrade & Sayão, 2014; Pereira, 2014) e mamíferos xenartros (Hill, 2006; Wolf *et al.*, 2012; Da Costa Pereira *et al.*, 2014).

Woodward *et al.* (2018) fizeram um extensivo estudo paleohistológico em dinossauros hipsilophodontideos da Austrália, sendo estes os primeiros a realizarem uma análise histológica ontogenética em múltiplos espécimes. Este trabalho atenta ainda para as limitações da técnica, principalmente em relação ao número de amostras utilizado nos estudos, enfatizando ainda a importância de combinar análises histológicas com dados morfológicos, geológicos, entre outras linhas de pesquisas.

Além dos estudos descritivos qualitativos, é possível ainda trabalhar com dados histológicos quantitativos através de softwares como *Bone Profiler* (Girondot & Laurin, 2003) e *BoneJ* (Doube *et al.*, 2010), que oferecem dados como taxa de compactação, parâmetros relacionados à trabécula, comprimento das fibras, entre outros. Diversos trabalhos já utilizaram esses softwares (Straeh *et al.*, 2013; Amson *et al.*, 2014; Asakura *et al.*, 2017), principalmente para rela- cionar a taxa de compactação do osso com o estilo de vida da espécie (Laurin *et al.*, 2004; Scheyer & Sander, 2009; Amson *et al.*, 2014)

As considerações de Woodward *et al.* (2018) são importantes também ao lembrar que embora os fósseis venham sendo seccionados por séculos, eles nunca foram amostrados sistematicamente, até pelo menos recentemente. Os espécimes estão sendo sistematizadas para ter uma abordagem explícita e padronizada de amostragem, com o objetivo de identificar e controlar o maior número possível de variáveis (Padian, 2013), já que a capacidade de padronizar as amostras é a maior dificuldade das análises paleohistológicas.

PALEOHISTOLOGIA EM XENATROS CINGULADOS

Os cingulatas alcançaram uma grande diversidade ao longo do Cenozoico, com representantes desde o Eoceno tardio até os dias de hoje, tendo mais de 200 espécies já descritas para os Glyptodontidae (McKenna & Bell, 1997; Carlini & Zurita, 2010; Zurita *el al.*, 2016), constituindo o grupo de mai- or diversidade taxonômica. Dentre este número de espécies descritas, aproximadamente 30 táxons tiveram o padrão histológico de seus osteodermos analisados (Quadro 1), demonstrando que essas características são de extrema relevância para os estudos das relações evolutivas, além de fornecerem caracteres potencialmente diagnósticos a nível de espécie (Hill, 2006; Wolf ,2007; Krmpotic *et al.*, 2009; Wolf *et al.*, 2012; Da Costa Pereira *et al.*, 2014).

O alto grau de diversidade morfológica e histológica nos osteodermos de Xenarthra foi demonstrado por Hill (2006), Krmpotic *et al.* (2009), Wolf *et al.* (2012), Da Costa Pereira *et al.* (2014), Asakura *et al.* (2017) e Araújo & Porpino (2018). As principais carac- terísticas dos osteodermos dos cingula- tas incluem uma estrutura geralmente “diploe-like” (Fig.1), que consiste em uma região do osso trabecular interposta entre camadas superficiais e profundas do osso compacto (Hill, 2006; Wolf, 2007). No entanto, a organização da região central do osteodermo difere dentre os grupos. Pampatérios e gliptodontes apresentam geralmente uma região central marcada por um tecido ósseo esponjoso bem desenvolvido, já os dasipodídeos são caracterizados por um tecido ósseo esponjoso fracamente desenvolvido na região central (Wolf, 2007). Dasipodídeos mais derivados, como *Tolypeutes tricinctus* Lineu, 1758¸ apresentam a região central diferenciada com um tecido esponjoso bem marcado (Wolf, 2007).

Apesar da alta variabilidade interespecífica nos osteodermos, algumas características são comuns a todos gliptodontídeos (Hill, 2006). Estas características (Quadro 2), que incluem um elevado teor de feixes de fibras mineralizadas, linhas de crescimento e camadas superficiais e profundas densas e escassamente vascularizadas (Wolf *et al.*, 2012), também são comuns para os osteodermos de outros vertebrados (Scheyer & Sander, 2004).

Quadro 1 - Listagem dos táxons fósseis com padrões histológicos do osteodermo já descritos.

Táxon		**Referência**
Dasypodidae	*Astegotherium dichotomus*	Ciancio *et al.* (2017)
	Dasypus bellus	Hill (2006); Wolf *et al.* (2012)
	Eutatus sp.	Krmpotic *et al.* (2009)
	Meteutatus lagenaformis	Ciancio *et al.* (2017)
	Parutaetus chicoensis	Ciancio *et al.* (2017)
	Prozaedyus sp.	Wolf (2007)
	Riostegotherium yanei	Victer (2011)
	Sadypus sp.	Ciancio *et al.* (2017)
	Stegosimpsonia sp.	Ciancio *et al.* (2017)
	Stegotherium variegatum	Ciancio *et al.* (2017)
	Pachyarmatherium brasiliense	Da Costa Pereira *et al.* (2014), Oliveira *et al.* (2013)
	Utaetus buccatus	Ciancio *et al.* (2017)
Pampatheriidae	*Holmesina floridanus*	Hill (2006); Wolf *et al.* (2012)
	Holmesina sp.	Chávez-Aponte *et al.* (2008); Wolf *et al.* (2012)
	Kraglievichia sp.	Wolf (2007); Wolf *et al.* (2012)
	Pampatheriidae indet.	Wolf *et al.* (2012)
	Pampatherium humboldtii	Wolf (2007); Wolf *et al.* (2012)
	Pampatherium typum	Wolf *et al.* (2012)
Glyptodontidae	*Cochlops* sp.	Wolf (2007)
	Doedicurus giganteus	Hill (2006)
	Glyptodon sp.	Wolf *et al.* (2012)
	Glyptodon clavipes	Wolf (2007); Chávez-Aponte *et al.* (2008); Wolf et al. (2012)
	Glyptodon reticulatus	Hill (2006)
	Glyptotherium cf. *arizonae*	Hill (2006)
	Glyptotherium floridanum	Hill (2006)
	Glyptotherium sp.	Wolf *et al.* (2012); Da Costa Pereira *et al.* (2014); Araújo & Porpino (2018); Luna *et al.* (2018)
	Neuryurus sp.	Asakura *et al.* (2017)
	Panochthus frezelianus	Hill (2006)
	Panochthus jaguaribensis	Da Costa Pereira *et al.* (2014)
	Panochthus sp.	Hill (2006); Da Costa Pereira *et al.* (2014); Asakura *et al.* (2017); Araújo & Porpino (2018)
	Panochthus tuberculatus	Wolf *et al.* (2012)
	Propalaehoplophorus sp.	Wolf (2007); Wolf *et al.* (2012)
Incertae sedis	*Eocoleophorus glyptodontoides*	Victer (2011)

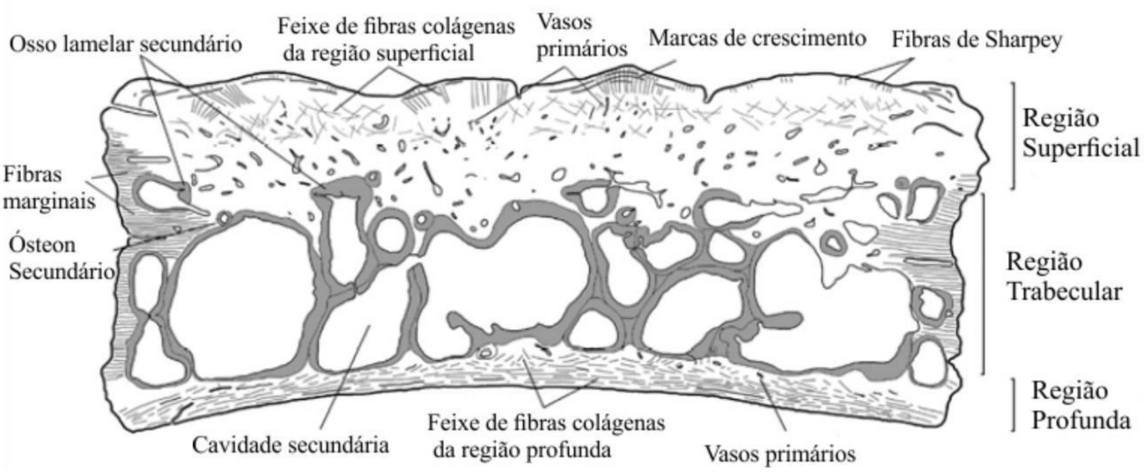

Figura 1 – Representação de um osteodermo padrão de Cingulata (baseado em *Tolypeutes tricinctus*), mostrando a divisão das regiões e as estruturas encontradas em cada região (Adaptado de Wolf, 2007).

No geral, os pampatérios apresentam uma conformação histológica básica similar aos dasipodídeos e gliptodontídeos, porém exibem, diferentemente dos outros grupos, fibras marginais marcadamente organizadas em feixes que se cruzam em vários ângulos (Wolf, 2007). Apesar de aparentemente serem semelhantes aos dasipodídeos, os pampatérios exibem indícios de uma relação mais próxima com os gliptodontes. Por exemplo, o arranjo dos feixes de fibras dentro das regiões superficiais e profundas é similar em gliptodontes e pampatérios (Hill, 2006; Wolf, 2007; Wolf *et al.*, 2012). Embora não proponha uma resposta definitiva ao problema sistemático, Wolf *et al.* (2012) definem como sinapomorfia dos pampatérios esse arranjo incomum das fibras encontrados nas margens dos osteodermos.

Os trabalhos de Hill (2006) e Wolf *et al.* (2012) talvez sejam os mais detalhados, aplicando os dados histológicos em inferências sobre crescimento, sistemática e adaptações biomecânicas. Hill (2006) apresenta dados tanto de osteodermos de cingulatas (gliptodontes, dasipodídeos e pampatérios) como de pilosas, estabelecendo a extensão da variabilidade histológica e morfológica desse elemento ósseo dentro do clado Xenartra. Wolf *et al.* (2012) estudaram osteodermos de pampatérios, discutindo a posição sistemática do grupo e avaliando mecanismos ontogenéticos e biomecânicos. Os autores sugerem que a ossificação metaplástica do tecido conjuntivo dérmico é um dos principais processos de crescimento do osteodermo durante a vida pós-embrionária.

Quadro 2 - Principais características da organização histológica dos osteodermos de gliptodontes (Adaptado de Wolf *et al.*, 2012).

Região	**Tipo de Osso**	**Vascularização**	**Aparência dos feixes de fibras de colágeno**	**Arranjo dos feixes de fibras de colágeno**
Superficial	Esponjoso para compacto; principalmente (em espécimes maduros): secundário; lamelar. Também: primário, lamelar	Ósteons/Cavidades secundário abundantes	Fino	Principalmente: osso lamelar. Também: perpendicular à superfície; fibras de Sharpey do periósteo
Central	Trabecular; secundário; lamelar	Esponjoso totalmente desenvolvido	Típico de osso lamelar	Típico de osso lamelar
Profunda	Compacto; primário; principalmente metaplástico	Moderado a justo	Grosso; frouxamente empacotado, ramificado	Irregular a radial
Margens	Compacto: primário; metaplástico		Fino; paralelo	Perpendicular às superficies marginais

Um exemplo importante que demonstra a necessidade de usar espécimes bem identificados é o caso do gliptodonte *Panochthus* Burmeister, 1886. A ornamentação dos osteodermos das diferentes espécies desse gênero é bastante semelhante, além de ser comumente confundido com o gliptodonte *Neuryurus* Ameghino, 1889, dificultando a sua identificação com precisão. Da Costa Pereira *et. al.* (2014) utilizam um osteodermo que possivelmente é de *Panochthus greslebini* Castellanos, 1942 e um osteodermo de *Panochthus jaguaribensis* Moreira, 1965 que pode ser um fragmento do escudo cefálico. Já Wolf *et al.* (2012) utilizam *Panochthus tuberculatus* Owen, 1845, porém não descreve isoladamente e sim em conjunto com outros gliptodontídeos, apresentando apenas uma tabela de características gerais, não sendo possível definir as características exclusivas dessa espécie. A única espécie bem descrita é *Panochthus frenzelianus* Lydekker, 1895 cuja identificação do exemplar não é ambígua (Hill, 2006). Asakura *et al.* (2017) descrevem as diferenças entre os gêneros *Panochthus* e *Neuryurus*, acrescentando às diferenças histológicas uma análise quantitativa do grau de compactação óssea, o que confirmou a diferença do padrão histológico dos dois táxons. Nesse caso, a definição de um padrão histológico poderia ser utilizada em comparações e análises sistemáticas.

Além desses importantes trabalhos, outras pesquisas inovadoras têm sido conduzidas em cingulatas, como por exemplo Ciancio *et al.* (2017) que utilizaram pela primeira vez microtomografia em osteodermos de dasipodídeos extintos. O resultado permitiu a reconstrução 3D das cavidades internas, possibilitando relacionar a variação do volume e extensão dessas cavidades com as mudanças das condições climáticas e ambientais nas diferentes regiões (Ciancio *et al.*, 2017). Luna *et al.* (2018) descreve pela primeira vez o padrão histológico de osteodermos de indivíduos adultos e juvenis do gliptodonte *Glyptodon* sp. fomentando futuras discussões sobre a ontogenia desse grupo.

Ao mesmo tempo em que os osteodermos dos cingulados têm recebido grande atenção por parte da histologia, as pesquisas com ossos longos de xenartras são escassas (Straehl *et al.*; 2013; Amson *et al*., 2014). Straehl *et al.* (2013) é o primeiro trabalho que utiliza ossos longos dos dois grandes grupos de Xenarthra (Cingulata e Pilosa). Os autores analisaram o padrão histológico e o grau de compactação dos ossos longos (úmero, fêmur, rádio, ulna e tíbia) de 22 táxons. Os resultados apresentados mostram o grande potencial dessa linha de pesquisa, onde os parâmetros de compactação óssea indicaram um padrão no fêmur que separa os dois grupos, com os cingulados tendo um menor grau de compactação que as pilosas e ainda uma relação alométrica entre o grau de compactação do fêmur e úmero.

PERSPECTIVAS PARA O FUTURO

A paleohistologia constitui uma ferramenta de domínio interdisciplinar, onde a colaboração envolvendo biólogos e paleobiólogos contribuirá para o aporte significativo de conhecimento na biologia evolutiva como um todo. Apesar do aprimoramento da metodologia, a prática usual da histologia óssea ainda envolve técnicas de seccionamento destrutivas de ossos completos, dentes, osteodermos ou quaisquer outros tecidos duros preservados.

Por ser um método destrutivo, os estudos paleohistológicos tem sido limitado apenas a espécimes isolados ou fragmentados, impedindo análises comparativas substanciais. O maior desafio dos pesquisadores focados na paleohistologia tem sido convencer os curadores das coleções a doar material para seccionar. Atualmente, todo material escolhido para ser cortado deve ser, antes de tudo, fotografado, moldado e replicado. Assim, todas as feições morfológicas são preservadas para futuros trabalhos.

No entanto, o século XXI trouxe grandes avanços tecnológicos para a ciência, entre eles o uso das tomografias computadorizadas (CT scan) na paleontologia (Tafforeau *et al.*, 2006). Essa técnica não destrutiva permite acessar a estrutura interna dos fósseis e, muito mais que as técnicas histológicas convencionais, permite a reconstrução da arquitetura óssea em três dimensões, porém não alcança uma resolução histológica (Cunningham *et al.*, 2014). Para obter um resultado ainda mais refinado é necessário usar uma fonte de luz síncronton (Tafforeau *et al.*, 2006; Sanchez et al., 2012). A histologia virtual por síncroton é o novo passo para acessar os caracteres histológicos em imagens 3D de alta resolução de uma maneira não-destrutiva (Murdock *et al.*, 2013; Mondéjar-Fernández *et al.*, 2014; Kolb *et al.*, 2015; Ciancio *et al.*, 2017).

Além da inclusão de novas tecnologias, o futuro da paleohistologia de cingulados deverá focar na utilização de espécimes com taxonomia bem definida, buscando-se aplicar as informações histológicas em diversos aspectos biológicos e paleoambientais, incluindo as análises sistemáticas (Scheyer & Sander, 2004; Scheyer *et al.*, 2007). Hill (2005) ressalta o valor sistemático das características histológicas dos osteodermos para reconstruções filogenéticas, mas grande parte dos trabalhos não abordam esse tópico com muitos detalhes (Wolf *et al.*, 2012). Portanto, o conhecimento das características histológicas de osteodermos de cingulados é até agora limitado a espécimes indivi- duais de um número muito pequeno de táxons superiores (Wolf *et al.*, 2012). O avanço das pesquisas paleohistológicas poderia finalmente ajudar a definir as relações filogenéticas de espécies pro- blemáticas que apresentam relações filogenéticas controversas.

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3.2 AN INSIGHT INTO A LARGE CINGULATE PALEOBIOLOGY: THE CASE OF *HOPLOPHORUS EUPHRACTUS* LUND, 1839 (CINGULATA, GLYPTODONTOIDEA)

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Abstract

Osteoderms of *Hoplophorus euphractus* are historically mistaken by other glyptodonts osteoderms, such as *Glyptodon, Glyptotherium* and *Neosclerocalyptus*, which creates enormous confusion in the identification of these materials. In this work we present a morphological description of a carapace fragment with four articulated osteoderms and others post-cranium bones in order to clarify some features that would differentiate those species. For the first time, the microstructure features are presented from an isolated osteoderm and a limb bone, allowing some perception into their paleobiology. The paleohistological analysis of the osteoderm and humerus of the glyptodont *Hoplophorus euphractus* demonstrated how the pattern of bone microstructure, reflected mainly in the arrangement of the collagen fibers and the morphology of the osteocytes lacunae, is useful in the inferences about the paleobiology of extinct species. In the humerus, it is possible to identify a bone derived from dynamic osteogenesis, where elongate osteocytes lacunae and collagen fibers with a preferably transverse orientation are visible. In the superficial and deep layer of the osteoderm, a bone tissue derived from a combination of dynamic and static osteogenesis is seen. In the trabeculae of the osteoderm a lamellar bone tissue is present, more organized than in the other regions of the bone. These characteristics in both bones indicate adaptations to the lifestyle of this large glyptodon.

Keywords: Mammalia; Glyptodontidae; Osteoderm histology; Bone microstructure; Bone profiler; Pleistocene.

Introduction

Glyptodonts form a group of cingulates with an extensive stratigraphic distribution (Zurita et al. 2009), ranging from the Late Eocene (Mustersan age) (McKenna and Bell 1997; Zurita et al. 2016) to the Early Holocene (Lujanian age) (Cione et al. 2003). They differ from the others cingulates, among several characteristics, for having a strongly trilobed dentition, fused vertebral columns and an essentially immobile dorsal carapace covering the top and sides of the trunk (Hoffstetter 1958).

These armored mammals are represented in the Brazilian Intertropical Region -BIR (Cartelle 1999) by the following fossil taxa: *Panochthus greslebini* Castellanos, 1941, *P. jaguaribensis* Moreira, 1965, *Hoplophorus euphractus* Lund, 1839, *Glyptotherium* sp. Osborn, 1903 and *Neuryurus* sp. Ameghino, 1889 (Oliveira et al. 2009; Porpino et al. 2010; Araújo-Júnior and Porpino 2011; Dantas 2012; Silva et al. 2012; Porpino et al. 2014). For the state of Pernambuco, all taxa of glyptodonts of RIB are recorded, except *P. jaguaribensis* (Silva et al. 2006; Oliveira et al. 2010; Silva et al. 2010a; Silva et al. 2012).

Hoplophorus euphractus and *Hoplophorus selloi* Lund, 1839 were the first formally recognized glyptodonts from the Pleistocene-Holocene of Brazil and Uruguay, respectively (Zurita 2007). It is now known that *H. euphractus* is the only well-characterized species of the genus (Zurita 2007), being a medium-sized glyptodont (Porpino et al. 2010) estimated to reach

2.70m in length (Paula Couto 1957), considerably bigger than *Neosclerocalyptus* Paula Couto, 1957 whose length would be about 1.90m (Burmeister 1870-1874; Paula Couto 1957). The taxa *Neosclerocalyptus* is proposed by Paula Couto (1957) to include *Glyptodon ornatus* Owen, 1845, which would belong neither to the genus *Glyptodon* nor to *Hoplophorus*.

Porpino et al. (2010) redescribed the material of Lagoa Santa, presenting new materials that allowed a better identification of the taxon and the differentiation of other glyptodonts, especially in relation to *Neosclerocalyptus*. Zurita et al. (2007) performed a review of the nomenclatural history of the Hoplophorinae, clarifying the confusion about *Hoplophorus*, *Sclerocalyptus* and *Neosclerocalyptus*. The works of Zurita (2007), Zurita et al. (2007) and Porpino et al. (2010) confirm the validity of the taxon *Neosclerocalyptus* and *Hoplophrous* as separated genera.

Rolim (1971) collected material from Pleistocene mammals from a tank deposit, known as Sítio Lagoa Tanque, located in the state of Pernambuco, wrongly reporting the first occurrence of isolated osteoderms from *Hoplophorus* sp for the region. The osteoderms were described as "hexagonal in shape with a circular design in the center surrounded by eight smaller figures". Later, this material was attributed to *Glyptodon* sp. (Silva 2009) and finally reallocated correctly to *Glyptotherium* sp. (Oliveira et al. 2010). Silva et al. (2010b) made the first accurate report for *H. euphractus* in the state of Pernambuco, where they described a carapace fragment with four articulated osteoderms.

Since the osteoderms are the most frequent and abundant fossil elements in the Cingulata record (Carlini and Zurita 2010), the arrangement and morphology of these bones are largely used as a source of taxonomic information (Vickaryous and Hall 2006; Krmpotic et al. 2009), but lately the microstructure has also been an important features to be taken into account (Hill 2006; Wolf 2007; Wolf et al. 2012). Besides the overall morphology of the bone, biological data depends deeply on the microarchitectural features, turnover rate and characteristics of the mineral and collagen phases (density, orientation) of the bone tissue (Stein and Prondvai 2014). Furthermore, several authors have clearly established the high degree of histological diversity in osteoderms of Xenarthra (Hill 2006; Krmpotic et al. 2009; Wolf et al. 2012; Da Costa Pereira et al. 2014; Asakura et al. 2017), demonstrating the incredible range of biological, ecological, biomechanical and systematic evidence that can be provide by this technique.

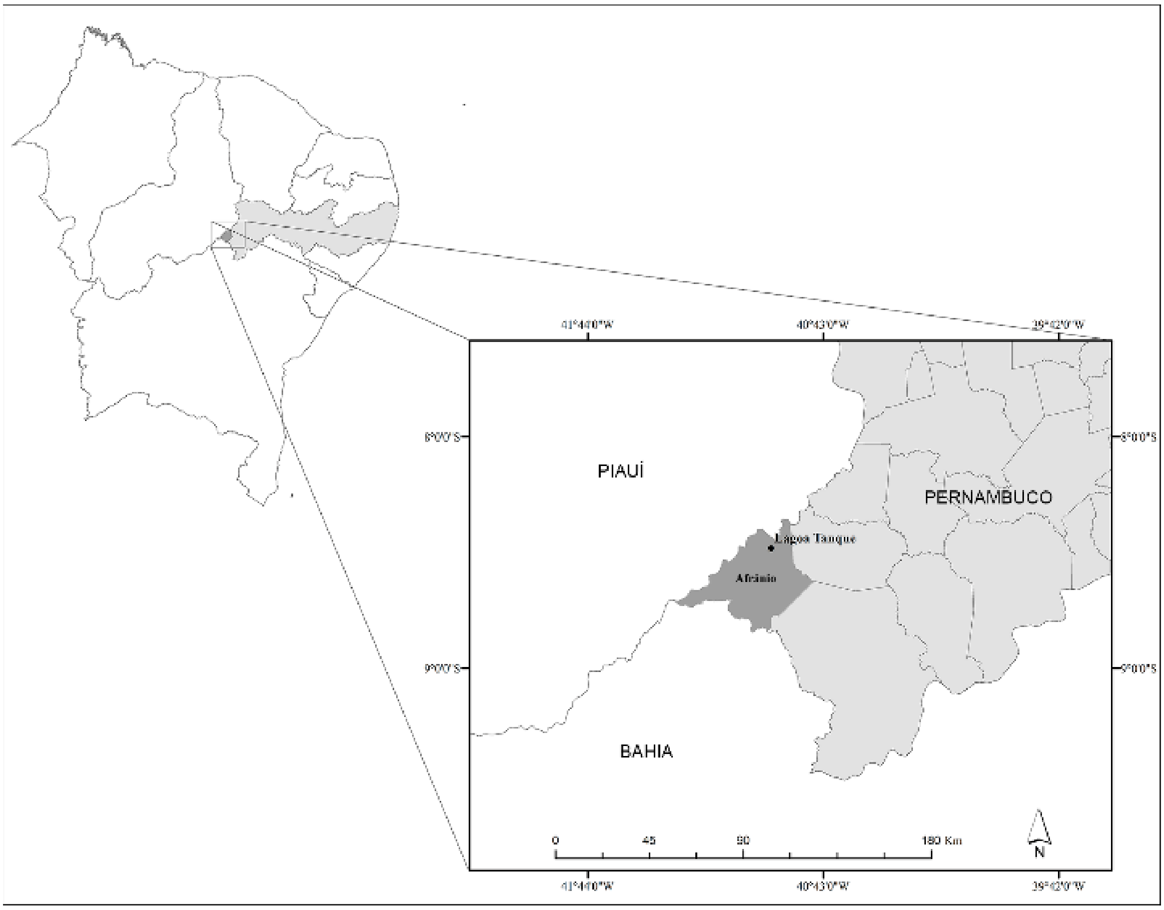
This work aims to discuss the *H. euphractus* material coming from Lagoa Tanque, state of Pernambuco: (1) carapace fragment, presenting morphological details of the osteoderms and as well as the histological pattern; (2) description of tarsal elements, including a redescription of an astragalus previously misidentified as *Holmesina paulacoutoi* (Cartelle & Bohórquez, 1985) by Silva et al. (2010a); (3) description of both morphological and histological pattern of the right humerus.

Regional Setting

The material described here was collected at Lagoa Tanque Site (08º28,904'S / 041º56,482'W), located in the Caboclo Village, Afrânio municipality, westernmost of the State of Pernambuco (Fig. 1). The locality of Caboclo presents a set of lagoon deposits associated with the Caboclo Creek drainage, among them the Lagoa Tanque (Silva et al. 2010a). In this region, it is possible to find other deposits with great fossiliferous potential, including Lagoa Comprida, Redonda, Estrada and Caveira (Silva 2009).

The Lagoa Tanque Site was formed in a natural thank depression in crystalline rocks with a granulometry including fine and coarse sediments (Silva et al. 2010a). The deposit has a granitic and syenitic basement, filled by sand and silt with a mean thickness of 125 cm (Silva et al. 2010a). Optically Stimulated Luminescence (OSL) analysis of the sediment withdrawn from the deposit indicates an age of 18.500 ± 2.200 AP. (Silva 2009), assigning the material as belonging to the Late Pleistocene.

Figure 1 - Location map of Lagoa Tanque Site, Afrânio municipality, State of Pernambuco, Brazil



Materials and Methods

Part of the specimens used in this work were previously described by Silva et al. (2010a) and the other was recently collected by the staff of the Laboratory of Paleontology (PALEOLAB), Department of Geology, Federal University of Pernambuco. The material was part of the private collection of Mr. Albino Lopes and was later donated to the Federal University of Pernambuco, where they are listed in the Macrofossil Collection (DGEO-CTG-UFPE). Taxonomic classification and morphological description used in this study was largely based on studies from Burmeister (1870-1874), Paula Couto (1947, 1957); Porpino et al. (2010) and Zurita (2007).

For the histological analysis, thin sections were performed according to the methodology described by Lamm (2013). The osteoderms were impregnated with polyester resin and sectioned using a diamond disc on a metallographic saw. The samples were fixed on glass slides and ground until the microstructure became evident. The thin sections were analyzed in a Zeiss Axioskop 40 and Zeiss Axio Vision Imager A2 optical microscope with an attached camera, using Axion Vision Release 4.8 software for photomicrographs. The histological terminology used follows the conventional concept seen in the reference literature in bone histology (Francillon-Vieillot et al. 1990; Hill 2006; Huttenlocker et al. 2013).

Bone compactness analysis was carried out for the humerus to be compared with the work of Straehl et al. (2013) . The compactness profile was analyzed quantitatively through the Bone Profiler program version 4.5.8 (Girondot and Laurin 2003) using a binary image transformed from the actual thin-sections in Adobe Photoshop CC 2017. From this function, the following parameters are extracted by the program: global compactness (Cg); the relative distance from the section center to the point of most abrupt change in compactness, i.e., transition from medulla to cortex (P); the reciprocal of the slope at the inflexion point (S).

All material was previously measured, photographed, and the samples used in the histological analysis where also molded and replicate, hence the original form and external morphology were recorded. The material was photographed with a Nikon Coolpix P520 camera and edited in Adobe Photoshop CC 2017 and Adobe Illustrator CC 2017.

Systematic Paleontology

Order CINGULATA Illiger, 1811

Superfamily GLYPTODONTOIDEA Gray, 1869

Family GLYPTODONTIDAE Gray, 1869

Subfamily HOPLOPHORINAE Huxley, 1864

HOPLOPHORUS Lund, 1839

(= *Sclerocalyptus* Ameghino, 1891)

HOPLOPHORUS EUPHRACTUS Lund, 1839

Morphological Remarks

Carapace Fragment and Isolated Osteoderm

The carapace fragment (DGEO-CTG-UFPE 6523) belongs to the dorsal region and is composed of four osteoderms with a subcircular shape (Fig. 2A). The ornamentation of the osteoderms consists of a main figure surrounded by peripheral figures in numbers between 9 and 10. Both figures are rounded, have a rough surface and are separated by narrow and very shallow grooves. Some extra peripheral figures are present forming a double row, particularly at the point of articulation between the four osteoderms. Around the main figure, in the central sulcus and between each peripheral figure, there is a hair follicle. Some capillary holes are also distributed randomly between the peripheral figures, externally to the central figure. The sutures between the four osteoderms is partially seen on the superficial face, whereas they are completely closed at the deep cortex, indicating it probably belonged to an adult individual. The deep surface is flattened, smooth and has at least one neurovascular foramen in each osteoderm.

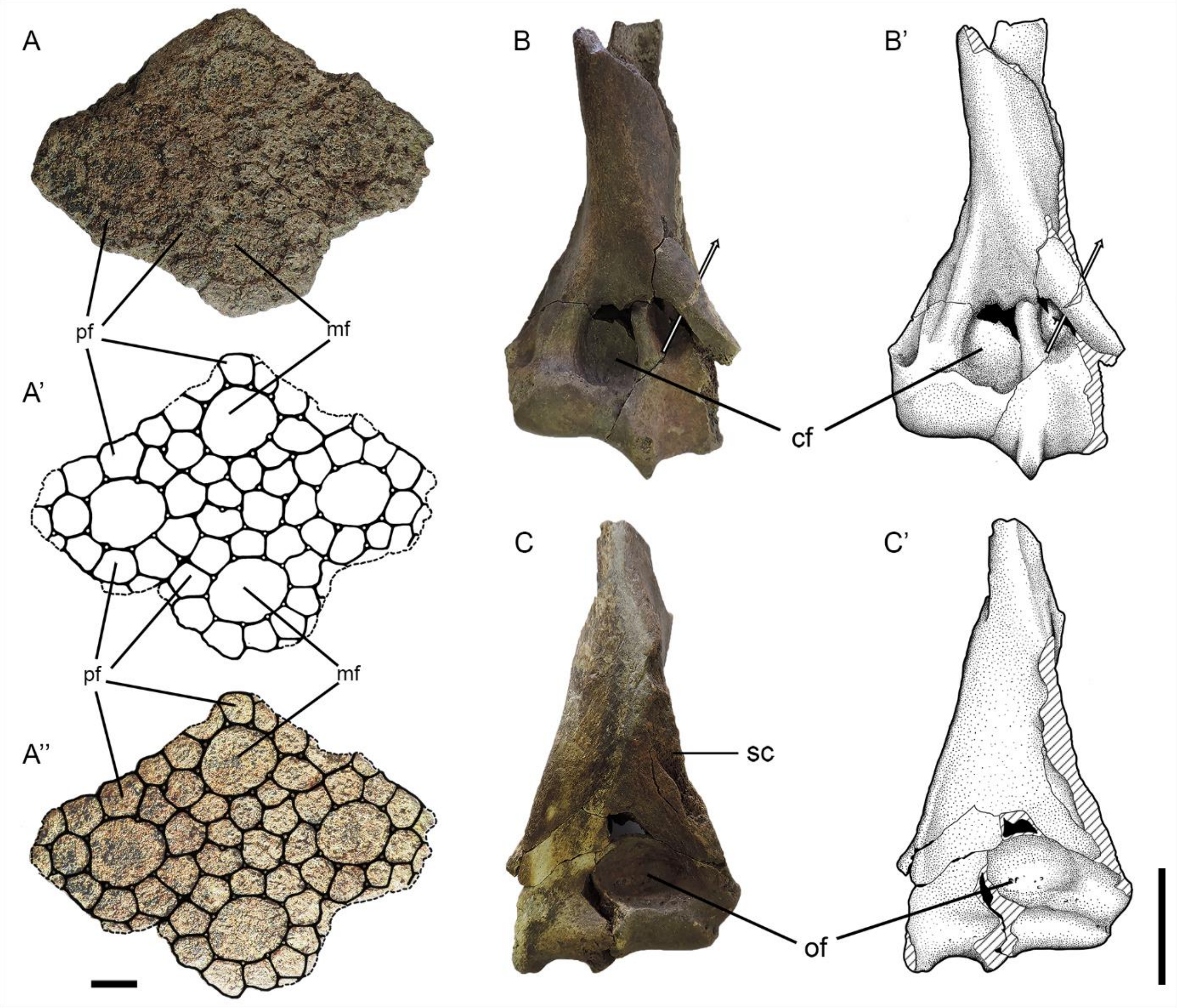
The isolated osteoderm (DGEO-CTG-UFPE 6543) is partially fragmented and was collected in association with the carapace fragment. It bears a main figure surrounded by smaller figures; it was not possible to count the total number of peripheral figures because the fragmentation of the material. The figures are separated by narrow and shallow sulci with piliferous holes. The deep surface is smooth and has one neurovascular foramen. Both specimens have 15mm of thickness and 16mm in diameter of the main figure, the peripheral figures of DGEO-CTG-UFPE 6523 have between 6 and 8mm of diameter of the peripheral figure.

Right Humerus

The humerus (DGEO-CTG-UFPE 8623) presents part of the distal epiphysis until the end of the deltoid crest, which is prismatic-triangular (Fig. 2B-C). The completely fused distal epiphysis of the humerus could indicate, as the carapace fragment, that it belongs to an adult individual. The entire proximal region is lacking. The supinator crest begins at the mid-posterior part of the diaphysis and ends at the slightly fragmented ectepicondyloid.

The distal articular surface is well developed, the articular face for ulna is present and the articular face for the radius is partially fragmented. The articular surface for the ulna is wider than the articular face for the radius, both are convex in the anterior-posterior direction. While the articular face to the radius is well preserved, only half of the ulna facet is present. A slight median depression separates the two facets. The coronoid fossa has a nearly triangular shape and has a small fracture in the upper portion. The entepicondyloid foramen is present and oriented vertically, as well as the bony bridge above the foramen is well preserved. The fossa for the olecranon is deep and oval. The bony septum that divides the two fossa is rather thin. Table 1 for measurements.

Figure 2 - a, Carapace fragment of *H. euphractus* (DGEO-CTG-UFPE 6523); a', Schematic drawing; a'', Schematic drawing superimposed on the carapace image showing the boundaries of each peripheral and main figure; Schematic draws (b', c') and accompanying images (b, c) of the right humerus in cranial (b) and caudal (c) view. Abbreviations: mf, main figure; pf, peripheral figure; cf, coronoid fossa; ol, olecranon fossa; sc, supinator crest; the arrow pass through the entepicondyloid foramen. Scale bar equals 1cm in a, 5cm in b and c



Left Astragalus

The specimen (DGEO-CTG-UFPE 6512) has only a few slightly worn edges and fragmentation (Fig. 3A-C). The trochlea is asymmetrical, exhibiting a well-developed lateral crest with a small fragmented portion. It is remarkable the presence of a longitudinal valley that separates the crest of the trochlea. The ectal facet is flat and triangular with rounded edges, the distal portion is elongated at an angle of approximately 45 °. The sustentacular facet is oblong and practically the same length as the ectal facet. Both facets are separated by the sulcus tali, measuring approximately 11mm wide and 5mm deep. The facet for the navicular is convex, subtriangular and exhibits a navicular tuberosity on the proximal portion of the dorsal border. This tuberosity is partially fragmented. Measurements can be seen in table 1.

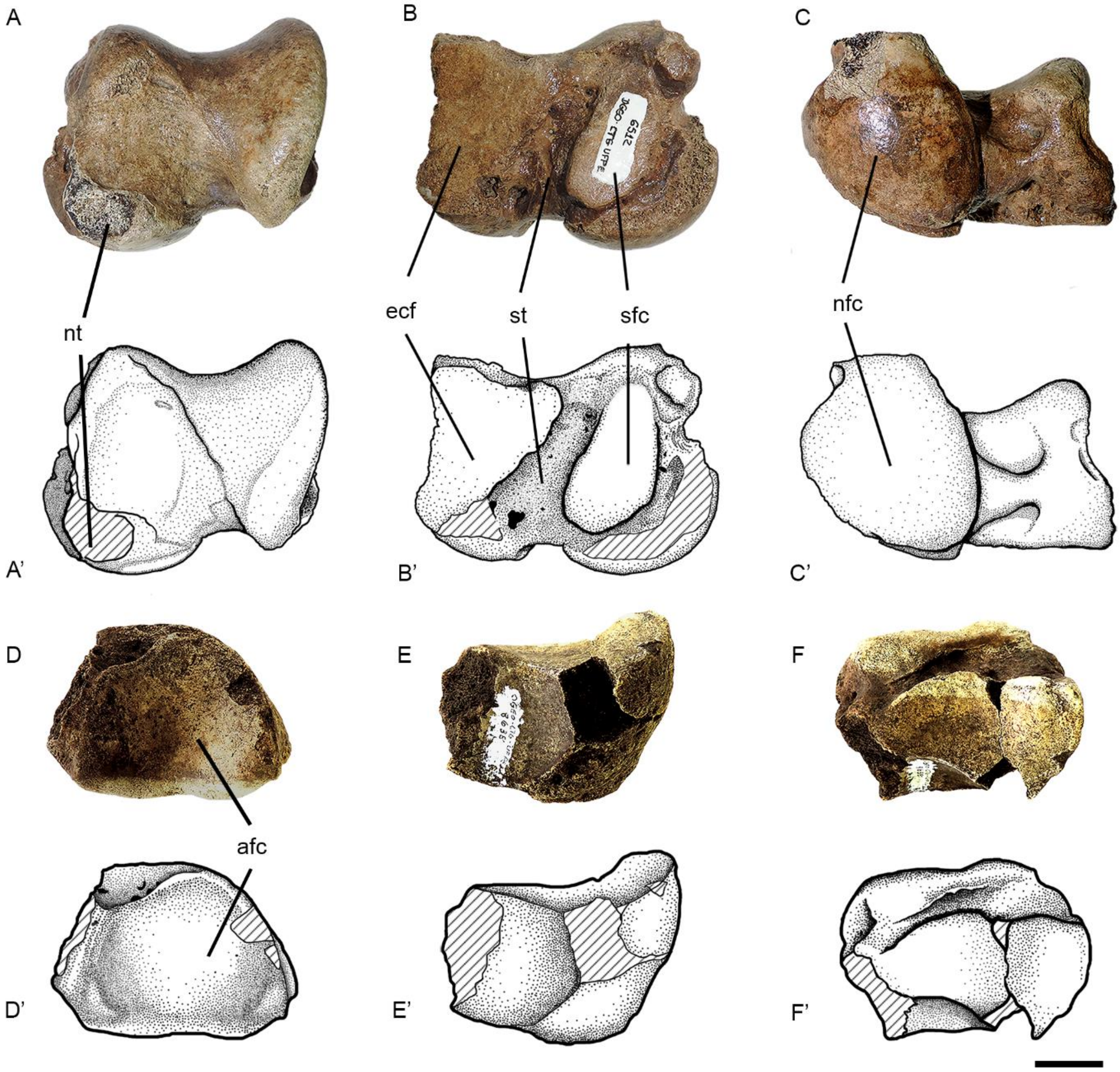
Table 1 - Comparative measurements in mm of the right humerus and the left astragalus of Hoplophorus euphractus, based on Porpino et al. (2010).

Measurements	Specimens		
Right humerus	*DGEO-CTG-UFPE 6512*	*Porpino et al. 2010 (MHN 987)*	*Porpino et al. 2010 (MHN 996)*
Length	192	332	330
Distal width	91	99	100
Width of distal articular surface	61	62	62
Length of deltopectoral shelf	-	167	168
Left astragalus	*DGEO-CTG-UFPE 6512*	*Porpino et al. 2010 (MCL 21.107)*	
Length	61	70,8	
Width	73	79,6	
Length of ectal facet	41	51,4	
Length of sustentacular facet	35	41,6	

Right Navicular

The navicular (DGEO-CTG-UFPE 8638) is partially fragmented in the medial portion lacking the plantar processes (Fig. 3D-F). This tarsal bone is quite flat in the proximodistally axis. The facet for the talus, in the proximal region, is concave and triangular. The dorsal region is convex and rather rough. In the distal region are located the articular facets for the cuneiforms (entocuneiform, ectocuneiform and mesocuneiform), which despite the fragmentation of the material, appear to be contiguous with each other. The facet to the cuboid is smoothly convex and elongated latero-medially, is located plantolaterally, where the tuberosity for the insertion of tendons of the tibial muscle is also located.

Figure 3 - Schematic drawings (a'-f') and accompanying images (a-f) of the tarsal elements of H. euphractus. Left astragalus (DGEO-CTG-UFPE 6512) in dorsal view (a), plantar view (b) and distal view (c). Right navicular (DGEO-CTG-UFPE 8638) in proximal view (d), plantar view (e) and distal view (f). Abbreviations: afc, astragalar facet; cfc, cuboid facet; ecf, ectal facet; nfc, navicular facet; nt, navicular tuberosity; sfc, sustentacular facet; sut, sulcus tali. Scale bar equals 2cm

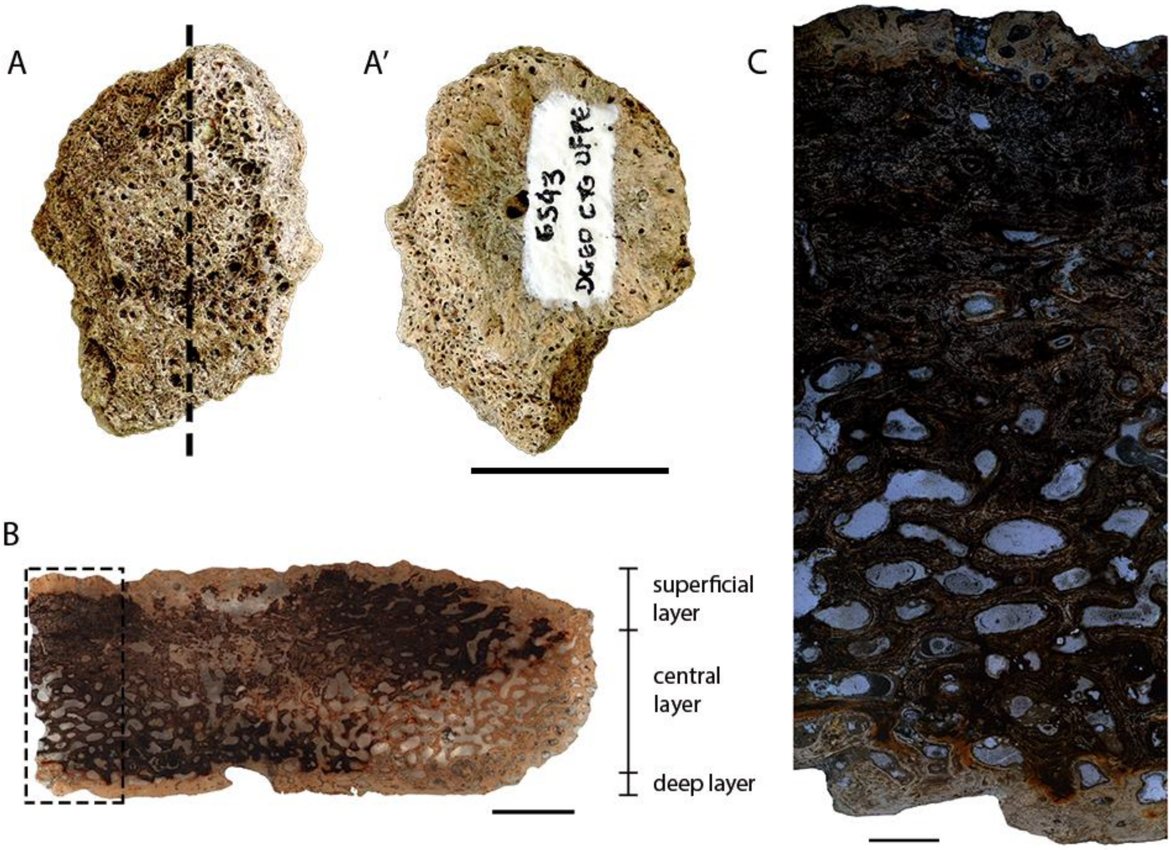


Histological Description

Isolated Osteoderm

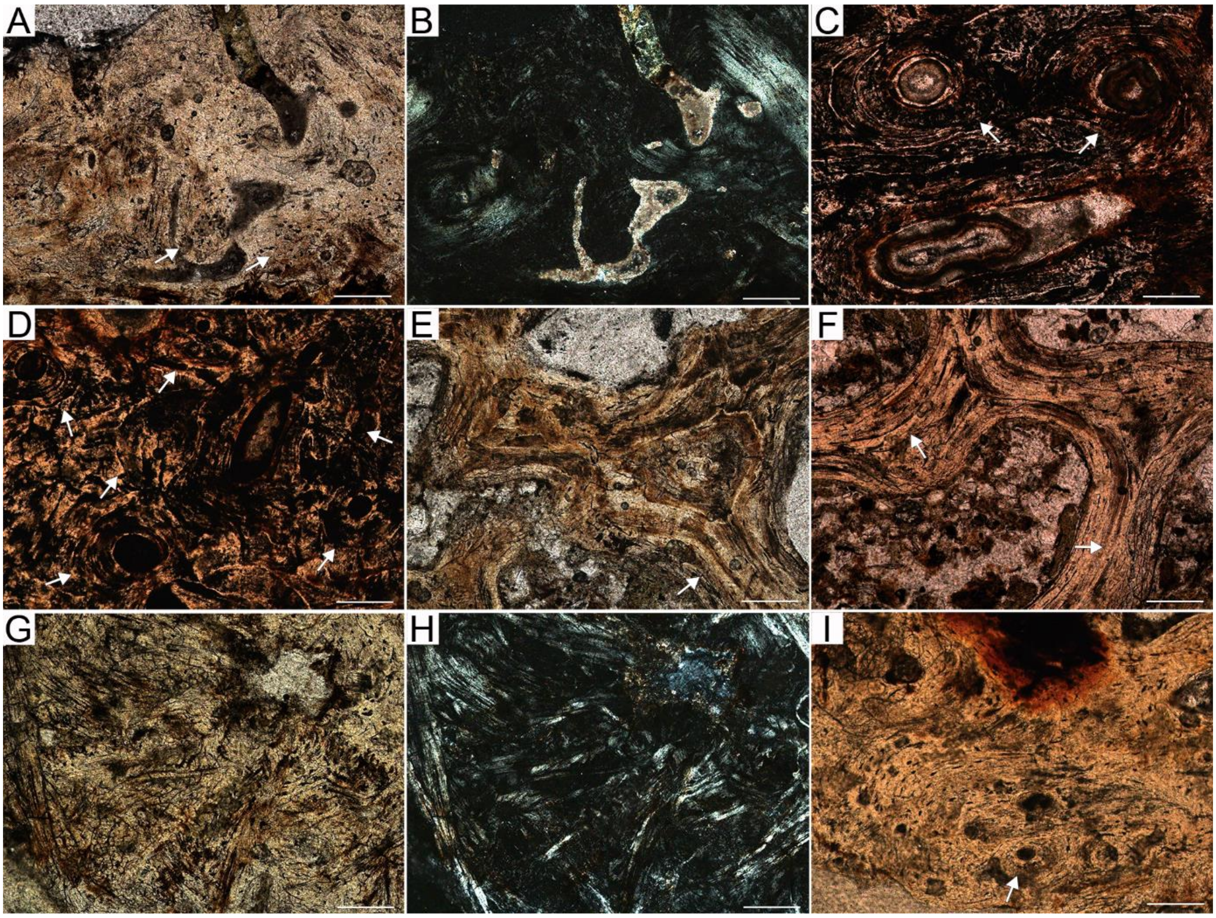
Histological sections from the longest axis of the specimen DGEO-CTG-UFPE 6543 (Fig. 4A) reveals the diploe-like structure already expected for flat dermoskeleton bones such as osteoderms, with a central zone of cancellous bone sandwiched between two layers of compact cortex (Fig. 4B). The presence of opaque minerals, probably manganese oxide, throughout the bone tissue obscures some of the microstructure pattern. The thin sections show a relatively compact microstructure, with only moderate expansion of trabecular bone in the deep half of the specimen, with a very thin compact deep cortex (Fig. 4C).

Figure 4 - a, Isolated osteoderm of *H. euphractus* (DGEO-CTG-UFPE 6543) in superficial (a) and deep face (a’), dashed line indicates the section plane; b, Overview of the osteoderm thin section (several merged images); c, Close-up of the section area marked by the black rectangle in b (several merged images). Scale bar equals 5mm in a and a’, 5mm in b, 1mm in c



The very superficial layer presents a predominantly fibro-lamellar bone tissue (Fig. 5A-B) with several microfractures (probably occasioned during the fossildiagenesis). Both the superficial and deep layer exhibits a combined static and dynamic osteogenesis (SO-DO) characteristics (but mostly SO), like the predominance of rounds and irregular osteocytes lacunae randomly arranged, some of them with preserved canaliculi, and more rarely elongate osteocytes lacunae. It's possible to see a woven bone matrix associated with some lamellar bone matrix of primary osteons, traversed by longitudinal primary osteons and more rarely by reticular canals (Fig. 5C). Towards the central region, it is notable a haversian bone replacing the primary cortex, with several generations of secondary osteons (Fig. 5D). The central layer is characterized by a well-developed cancellous bone tissue, with moderately sized inter-trabecular spaces. The trabeculae are formed by a lamellar zonal bone pattern (Fig. 5E-F), with low vascularization, each lamella showing rows of flattened osteocyte lacunae with few canaliculi. The very thin deep compact layer exhibits a fibro-lamellar pattern, with a relatively undisturbed primary woven-fibered bone matrix associated with lamellar matrix of primary longitudinal osteons, and fewer reticular canals are seen in this region (Fig. 5G-I). Intersecting marginal Sharpey's fibers have not been observed.

Figure 5 - Osteoderm histology of H. euphractus (DGEO-CTG-UFPE 6543); a, The superficial layer shows numerous rounds and irregular osteocytes lacunae with a random arrange (white arrows) and an intercalated woven bone matrix and lamellar bone matrix, normal light; b, The same as a in cross polarized light; c, Longitudinal (white arrows) and more rarely reticular canals are present in the superficial layer; d, The most inner region of the superficial layer shows a Haversian bone with several overlapping secondary osteons (white arrows); e and f, The central layer presents trabeculae formed by a lamellar zonal bone pattern (white arrows); g, The deep layer exhibits the same pattern as the superficial layer, with a predominance of a woven bone matrix, normal light; h, The same as g in cross polarized light; i, Primary osteon (white arrow) and DO-derived osteocyte lacunae can also be found in the deep layer. Scale bar equals 200 µm.



Right Humerus

A transversal section was made from the midshaft of the humerus, in the very beginning of the supinator crest (Fig. 6A). It reveals an overall compact cortex and a dense network of thick trabeculae in the medullary region (Fig. 6B), even though some of the trabeculae had been destroyed by diagenetic process and/or damaged during bone sectioning. The presence of opaque minerals, probably manganese oxide, throughout the bone tissue obscures some of the microstructure pattern.

The cortex is a haversian tissue, heavily remodeled with overlapping longitudinal secondary osteons (Fig. 6D-E), the outermost periosteum layer also shows a lamellar parallel-fibered bone tissue, which indicates a dense haversian bone replacing a fibro-lamellar primary cortex.

The endosteum region is basically a bone tissue derived of dynamic osteogenesis (DO) with a prevailing transversely alignment of a nonlamellar parallel-fibered bone and very few stripes of woven bone. A strong organization of the lacunocanalicular network (LCN) is seen, with a mutual alignment of several elongate and flattened osteocytes lacunae and long cytoplasmatic process running mainly perpendicular to the long axis of the cell lacunae (Fig. 6F-G). Since the elongate and flattened osteocytes lacunae is the long axis of the DO-derived osteocyte, it indicates a parallel alignment to the predominant orientation of collagen fibres, hence the transversely alignment of the fibres.

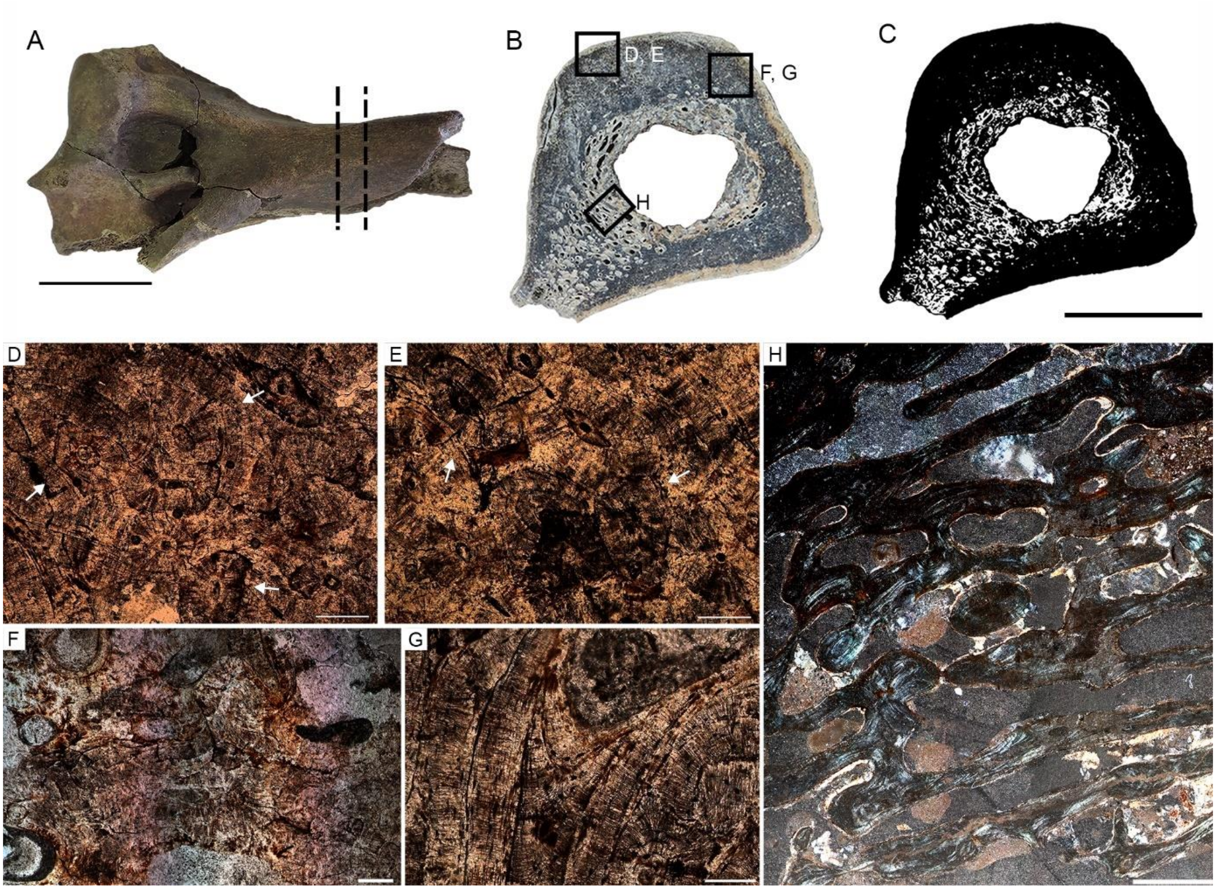
The medullary region is only partially preserved and it’s thicker in the supinator crest region. The trabeculae are very elongate, circularly aligned and with a highly organized bone matrix (Fig. 6H).

The analysis of the binary image of the midshaft of the humerus indicates a bone global compactness of 75% for *H. euphractus*. All the parameters values and comparison with other glyptodonts taxa can be seen in Table 2.

Table 2 - Bone profiler outputs for glyptodonts humeri, based on Straehl et al. (2013).

Taxa/Parameter	*S value*	*P value*	*Cg*
Glyptodon clavipes	0,040	0,636	0,583
Glyptodon clavipes	0,043	0,567	0,671
Lomaphorus ornatus	0,069	0,535	0,696
Hoplophorus euphractus	0,050	0,500	0,753

Figure 6 - a, Right humerus of H. euphractus (DGEO-CTG-UFPE 8623) in cranial view, dashed line indicates de section plane; b, Overview of the humerus section, showing the cancellous and compact bone tissue distribution, with black boxes indicating where the related images were taken.; c, Binary image produced for the compactness analysis; d-h, Humerus histology of H. euphractus; d and e, The cortex presents a dense harversian tissue, with several overlapping longitudinal secondary osteons (white arrows) and many interstitial lamellae throughout the region; f, Several merged images demonstrating the spatial alignment of the elongated DO-derived osteocytes lacunae; g, Close-up showing the strong organization of the lacunocanalicular network.; h, Several merged images of the cancellous bone tissue, showing the very elongate and circularly aligned trabeculae, with a highly organized bone matrix. Scale bar equals 5 cm in a, 2 cm in b and c, 200 µm in d and e, 1 mm in f, 100 µm in g, 1 mm in h.



Morphological Considerations

Paula Couto (1957) comments that osteoderms from *H. euphractus* are relatively thin, as in *Neosclerocalyptus* and different from other glyptodonts in general, such as *Panochthus* and *Glyptodon*. Like Paula Couto (1957), Zurita (2007) reports that there are no significant differences in the ornamentation of *Hoplophorus* and *Neosclerocalyptus* osteoderms.

However, some characteristics appears to be essentially different in both taxa (Paula Couto 1957; Porpino et al. 2010), including: (a) the number of peripheral figures in *H. euphractus* varies between 9 and 12, whereas in *Neosclerocalyptus* it varies from 8 to 10 and are slightly larger; (b) *H. euphractus* presents more follicular foramina than *Neosclerocalyptus* on the superficial face; (c) the external surface of the *Hoplophorus* osteoderm is more punctuated and rough than in *Neosclerocalyptus*, which has a smoother surface; (d) the dorsal osteoderms of *H. euphractus* are rounded in outline, whereas those of *Neosclerocalyptus* are pentagonal or hexagonal; and finally (e) *H. euphractus* presents in some dorsal osteoderms a double row of peripheral figures, which does not occur in the osteoderms of *Neosclerocalyptus*.

The rough surface of *H. euphractus* is like others common glyptodonts in the BIR, such as *Glyptotherium* and *Panochthus*. The peripheral figures of other glyptodonts vary as follows: *Panochthus* has no difference between main and peripheral figures at the dorsal region (Porpino et al. 2014), *Glyptodon* ranges from 5 to 11, *Glyptotherium* ranges from 6 to 10 (Zurita et al. 2018). Finally, peripheral sulci are deeper in *Glyptodon* and *Glyptotherium* than in *H. euphractus* and *Neosclerocalyptus* (Oliveira et al. 2010; Porpino et al. 2010).

From a general perspective, the *H. euphractus* humerus closely resembles that of *Neosclerocalyptus* and *Panochthus*. In the studied specimen the entire proximal portion was lost and only a part of the diaphysis and the distal portion remained. *Hoplophorus* humerus is thinner and longer than *Neosclerocalyptus*, but smaller in length than in *Panochthus*, *Glyptodon* and *Glyptotherium* (Gillette and Ray 1981; Paula Couto 1957; Porpino et al. 2010). It differs from *Neosclerocalyptus*, mainly by the less marked deltoid crest and pectoral crest (Porpino et al. 2010). The entepicondyloid is more salient in *Neosclerocalyptus* than in *H. euphractus* (Paula Couto 1957). The supinator crest begins at the central axis of the diaphysis in *Neosclerocalyptus*, unlike in *H. euphractus*, *Panochthus* and *Glyptodon*, where the crest begins more distally (Paula Couto 1957; Porpino et al. 2010). The entepicondyloid foramen is present in both *Panochthus* and *Neosclerocalyptus*, but absent in *Glyptodon* and *Glyptotherium* (Gillette and Ray 1981; Porpino et al. 2010).

Proximal tarsal elements (astragalus and calcaneus) in xenarthrans have been considered of great phylogenetic importance since the late nineteenth century (Kraglievich 1928; Cifelli 1983). The irregular structure of these bones provides many characters that can be used in the interpretation of mammalian systematics and biology (Berqvist 1996; Muizon et al. 1998; Bergqvist et al. 2004). For the Xenarthra clade, the following tarsal apomorphies are considered: transversely broad astragalar body with greater lateral arc, and calcaneal facets aligned virtually on the same axis of rotation (Bergqvist et al. 2004). Burmeister (1870-1874), in his monograph on the glyptodonts, describes that the foot of *Hoplophorus* is very similar to that of *Panochthus*, differing only in the smaller size and thickness of the *Hoplophorus* bones. It differs from *Glyptodon* because it presents the longitudinal valley that separates the crest of the deeper and wider trochlea, and also a deeper sulcus tali (Porpino et al. 2010). The navicular tuberosity is flattened in *H. euphractus*, *Panochthus* and *Neosclerocalyptus*, yet in *Glyptodon* is cylindrical (Porpino et al. 2010). The length of the ectal and sustentacular facets is equivalent, just as in the *Glyptodon* astragalus, and inequivalent in *Panochthus* and *Neosclerocalyptus* astragalus (Porpino et al. 2010).

Cartelle and Bohorquez (1983) briefly described the astragalus of the pampathere *Holmesina paulacoutoi*. A more detailed description was presented by Cartelle et al. (1989), who compared the bones of the hand and foot of *H. paulacoutoi* and *Pampatherium humboldti*. The astragalus of *H. paulacoutoi* presents distinct characteristics in relation to that of *H. euphractus*: (a) significantly shorter length, ~ 55mm; (b) the presence of a marked sulcus at the distal edge of the trochlea; (c) the lateral-medial axis of the facet to the navicular more prominent than the dorsal-ventral axis, unlike that of *H. euphractus*. Finally, these characteristics allow the differentiation of the astragalus of these species, identifying correctly the material studied here as belonging to the *Hoplophorus euphractus*.

Porpino et al. (2010) listed the subtle differences of the navicular between *H. euphractus* and the other glyptodonts, among them: (a) facet to astragalus more elongated than in *Glyptodon*, *Panochthus* and *Neosclerocalyptus*; (b) facet to cuboid more elongated mid-laterally than in *Neosclerocalyptus*; (c) the facet to the ectocuneiform is not in contact dorsally with the facet to the mesocuneiform, as it happens in *Neosclerocalyptus*.

Paleohistological Considerations

The initial contribution on the bone paleohistology of mammals was performed by Quekett (1849) as part of comprehensive studies dealing with the bone cortex of not only mammals (which also included fossils of xenarthrans such as *Megatherium*) but also fish, reptiles, and birds (Kolb et al. 2015) and later continued by Enlow and Brown (1958). Thenceforth, since the carapace is an outstanding feature of xenarthrans, osteoderms has been the major focus of the xenarthrans paleohistology (Vickaryous and Hall 2006; Hill 2006; Wolf 2007; Chávez-Aponte et al. 2008; Wolf 2008; Krmpotic et al. 2009; Vickaryous and Sire 2009; Wolf et al. 2012; Da Costa Pereira et al. 2014; Asakura et al. 2017; Araújo and Porpino 2018).

Hill (2006) stated that high content of mineralized fiber bundles, growth lines and dense, sparsely vascularized superficial and deep layers are features common in both xenarthran and other vertebrates osteoderms. The histological pattern described for *H. euphractus* osteoderm correspond to the overall pattern reported for derived glyptodonts osteoderms (Hill 2006; Wolf et al. 2012), differing mostly with the central trabeculae bone occupying about half or less of the osteoderm volume, not more than half as described in Hill (2006). The osteoderms of Dasypodinae contrast mostly by generally appear to present a comparatively more compact central region with only poorly developed cancellous bone (Vickaryous and Hall 2006). The degree of secondary remodeling presented in *H. euphractus* is not as intense like in *Panochthus frenzelianus*, *Doedicurus giganteus* (Hill 2006), *Neuryurus* sp (Asakura et al. 2017), *Glyptotherium* sp. (Araújo and Porpino 2018) and in the derived glyptodons studied by Wolf et al. (2012), but rather more cancellous like in *Panochthus* sp. (Asakura et al. 2017; Araújo and Porpino 2018). No growth marks were seen in the specimen studied here. The presence of a fibro-lamellar bone tissue complex indicates a highly organized secondary parallel-fibred bone replacing the more disorganized primary woven matrix, which can be interpreted to represent a mature ontogenetic stage of an adult individual.

H. euphractus also presents some features described by Main et al. (2005) as typical of metaplastic ossification, like low vascularization and osteocyte lacunae aligned with the fiber bundles (as in parallel-fibered bone). Osteoderms are largely associated with a metaplastic ossification (Hill 2006), but signs of different process of development has similarly been observed in cingulate osteoderms, like periosteal bone formation process (Wolf et al. 2012) and intramembranous ossification (Vickaryous and Sire 2009). These observations imply that other modes of ossification may contribute to osteoderm growth besides the metaplastic process, at least in different phases of development (Vickaryous and Sire 2009; Wolf et al. 2012).

Luna et al. (2018) recently provide new criteria for the analysis of ontogenetic variation within the subfamily Glyptodontinae by describing the osteoderm microstructure from a juvenile Glyptodontidae. As expected, the histological pattern shows numerous features related to its early stage of development. The microstructure consists entirely of a rather simple, homogeneous woven fibred matrix, which is expected for an early stage of development in bone tissues (Vickaryous and Hall 2006; Vickaryous and Sire 2009; Stein and Prondvai 2014). The absence of periosteal lamellar and parallel fibred matrices, as well as secondary lamellar bone in both cancellous and compact bone, is also typical of bones tissue newly formed (Luna et al.

2018). This new data implies a necessary review of the previous studies, since it is possible that several microstructural variations reported in Glyptodontidae (and other xenarthrans) may be due to ontogenetic variation, as already suggested by Wolf et al. (2012). This problem was also approached by Zurita et al. (2011) regarding the use of morphological features of isolated osteoderms in glyptodonts taxonomy. The authors drew attention to the fact that variations on osteoderms ornamentation could also be related to the different regions of the carapace, different ontogenetic stages and the effects of taphonomic processes and not exclusively due to interspecific variation. An extensive review including both morphological and histological features and considering factors like ontogenetic stages could have a great impact on the systematic of glyptodonts.

In general, xenarthran long bone microstructure is highly consistent with other placental mammals (Singh et al. 1974; Enlow and Brown 1958; Hurum and Chinsamy-Turan 2012; Kolb et al. 2015). The most comprehensive study with xenarthran long bone histology in both qualitatively and quantitatively approach was performed by Straehl et al. (2013). They sampled several long bones mid-diaphyseal across extinct and extant xenarthrans (including adult and juvenile specimens) and presented a fine-scale insight into xenarthran bone histology. The study revealed a size effect on secondary remodeling that could be caused by increasing of loading in extinct xenarthran, also the extend of remodeling could be a feature in which extinct and extant both cingulate and pilosa differ. Furthermore, an allometric relationship between humerus and femur compactness in armadillos was considered as possibly indicative of their fossorial habits, this deduction is in accordance to the morphological adaptations described by Vizcaíno and Milne (2002).

Bone profiler values for the parameters *Cg* (global compactness), *S* (reciprocal of slope at inflexion point) and *P* (relative distance from the section center to inflexion point) of the humerus compactness profiles of *H. euphractus* are within the range described by Straehl et al.

(2013). In comparison with other glyptodont's taxa, *H. euphractus* presents a slightness high global compactness value (75%) align with a smaller *P* value, which means that the relative distance from the center of the bone to the transition point from cancellous medullary region to cortical compacta is small, indicating that the cortex occupies a bigger proportion of the cross-section area, therefore explaining 75% of compact area.

Since the work of Straehl et al. (2013) does not describe the histological features for each taxa used and each skeletal element (instead they separate in body mass categories), only a general comparison is possible. Overall, long bones of large cingulates are characterized by a dense haversian bone in the cortex with a thin layer of parallel-fibered and/or lamellar bone tissue surrounding the exterior; a dense network of thick trabeculae in the medullary region with parallel-fibered and/or lamellar bone tissue organization; a varied orientation of the vascular canals (longitudinal, radial or reticular) with an irregular arrangement (Straehl et al. 2013). All those characteristics above are seen in the thin section of the *H. euphractus* humerus, and beside that it was also seen a preferentially transversely alignment of the fibres in the DO-derived highly organized bone tissue.

Current biological studies indicate that the presence of DO-derived bone tissues provides the mechanical stability of the growing skeleton while infilling the extensive vascular spaces left from the SO-derived woven, which provides rapidly skeletal growth (Stein and Prondvai 2014). The pattern described for the humerus also can be interpreted as a mature ontogenetic stage of an adult individual development.

Biomechanical Implications

Biomechanical studies performed on large glyptodonts and ground sloths has led to interesting results on the capacity of the limb bones to withstand bending forces, forearm extension and velocity, bipedalism or digging abilities (Farinã 1995; Fariña et al. 1998; Milne et al. 2009). Although xenarthrans limbs have been largely studied in a functional morphology approach, with functional analyses of postcranium including the use of qualitative bone morphology, length measurements and related indices (Farinã 1995; Bargo et al. 2000; McDonald 2007; Milne et al. 2009; Vizcaíno et al. 2011; Amson and Nyakatura 2017), only very few gliptodonts taxa has been studied (Farinã 1995; Milne et al. 2009; Vizcaíno et al. 2011) and *H. euphractus* indices were never analyzed. So far, only a body mass estimate was made for the genus, which is thought to reach about 280 kg (Smith et al. 2003). Since the post cranium material in this study includes only an incomplete humerus and tarsal bones, a proper limb function analysis cannot be provided.

In sum, the analysis of limb proportions in glyptodonts indicates that both the hindlimb and the forelimb have proportions that correlate strongly with body mass (Milne et al. 2009; Vizcaíno et al. 2011), possibly indicating that the structure of the forelimb in glyptodonts (based on index of fossorial ability) is directly related to body support and movement (Vizcaíno et al. 2011) and not to digging behavior as though for living armadillos (Vizcaíno and Milne 2002).

The fact that the femora have higher strength indicators than the humerii shows that sometimes glyptodonts' hindlimbs are required to bear an even greater proportion of the body weight, probably when rising to a bipedal posture (Farinã 1995; Vizcaíno et al. 2011). The bone compactness paraments (*Cg*) analyzed by Straehl et al. (2013) in the glyptodont *Lomophotus ornatus* limb bones shows a global compactness much higher in the femur than the humerus, which fits with the conclusion from Vizcaíno et al. (2011) that the majority of the glyptodont's weight is borne by the hindlimbs.

In this complex system, the mineral constituent of the bone tissue is responsible for the stiffness of the bone, whereas collagen provides toughness by assuring flexibility and absorbing the energy of forces acting on the bone (Martin et al. 2015). Although DO-derived bone is less mineralized than woven bone, it has improved mechanical properties, since the collagen fibres are highly organized, which has been proved to be much more important than the degree of mineralization in determining the mechanical properties of bone (Martin and Ishida 1989; Marotti 2010). As body mass increases, the proportional amount of woven bone decreases, and DO-derived highly organized bone tissue will dominate the cortex of the limb elements (Stein and Prondvai 2014). The low percentage of woven bone in favor of parallel-fibered bone throughout the cortex of large cingulate limb, like in *H. euphractus* humerus, is therefore to be expected.

Several studies have suggested that collagen fiber orientation can be used to infer main loading regimes on the bone (Ascenzi and Bonucci 1967, 1968; Bromage et al. 2003; Skedros et al. 2003; Skedros et al. 2007; Martin et al. 2015), concluding that in a limb bone, longitudinal fiber orientation is found in the cortical regions under tension, whereas transversely running fibres characterize the areas under compressive loading. Consequently, the preferentially transversely fiber alignment found in *H. euphractus* bone could be an indicative of good resistance of compressive forces. However, a more extensive research with a broader sample is necessary for more definitive conclusions.

As for the osteoderms biomechanics, little is known and can be properly inferred. The diploe structure and the overall thickness of cingulate osteoderms are interpreted as strengthening adaptations in the context of the protective function of these bones (Wolf et al. 2012; Amson and Nyakatura 2017). Even considering that the presence of trabecular bone could provide a low resistance against crushing, the incorporation of structural organized fibers bundles might counteract this weakening effect and still provide a weight-reducing effect (Wolf et al. 2012; Stein and Prondvai 2014). Hence, the lamellar zonal bone patter found in the central layer of *H. euphractus* would compensate the well-developed trabeculae area that provides a weight-reducing effect and ensure the osteoderm protective function.

Final Remarks

The morphological characteristics observed in the material of the tank deposit in the Municipality of Afrânio, allowed to correctly assign the material to *Hoplophorus euphractus*. The astragalus (DGEO-CTG-UFPE 6512) previously assigned to *Holmesina paulacoutoi*, belongs in fact to the taxon *H. euphractus*. For the first time, histological features of the humerus from the glyptodont *H. euphractus* were described and combining with morphological features, thus enabling the inference of new information regarding functional morphology. The osteoderm presents a fibro-lamellar bone tissue complex, with a cancellous bone occupying about half of the thickness. The humerus thin section presents a mostly haversian bone surrounded by a lamellar parallel-fibered bone tissue and followed by a prevailing transversely alignment of a nonlamellar parallel-fibered bone. This particularly fibers alignment indicates areas of the bone under compressive loading. Both histological and morphological features of the osteoderm and humerus can be interpreted to represent a mature ontogenetic stage of an adult individual.

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3.3 ELUCIDATING CINGULATE'S OSTEODERMS HISTOLOGY – IMPLICATIONS FOR SYSTEMATICS, OSTEODERM GROWTH, AND BIOMECHANICAL ADAPTATION

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ABSTRACT

Bone is a stunning example of a structural modulations, as a tissue shows complex and highly variable structural patterns which need to be recognized and classified with regard to biological significance. The main purpose of paleohistology is to understand this complexity, extrapolating these results into a phylogenetic context to infer evolutionary patterns of biological strategies. Osteoderms are integumentary bones with high fossilization potential and their importance for functional morphology and phylogenetic research have been proven for years. Xenarthran osteoderm histology pattern has been recently explored and have provide many data regarding cingulate paleobiology and phylogenetic relationship. Here we analyze six fossil cingulates to add new information on their osteoderm histology pattern, inferring functional and development adaptation. *Pachyarmatherium brasiliense* osteoderms histology shows close resemblance to what is expected for Dasypodidae, indicating some affinity with this group. A combination of several features acting together can be interpreted as a biomechanical adaptation for glyptodonts osteoderms, such as the increase of thickness of cancellous bone in the middle zone; the presence of both SO and DO-derived bone tissues and the level of organization of the mineralized collagen fibers. Our analysis also corroborates with the hypothesis of multiple growth processes acting throughout the ontogenetic stages, with the classic idea of a metaplastic ossification also taking part of the osteoderm development.

Keywords: Cingulata; Glyptodontidae; Pampatheriidae; Osteoderm histology; Systematics; Biomechanics; Osteoderm growth.

INTRODUCTION

Xenarthra is one of the four major clades of placental mammals and has a rich fossil record throughout the Cenozoic era, comprising many fossils and extant forms (Carroll, 1988; Hoffstetter, 1958; Paula Couto, 1979; Simpson, 1945). The group includes armadillos, pampatheres and glyptodonts, sloths and anteaters, divided into two orders: Pilosa Flower, 1882 and Cingulata Illiger, 1811 (McKenna & Bell, 1997). The Order Cingulata is a distinct group, traditionally divided into two superfamilies: Dasypodoidea (Dasypodidae) and Glyptodontoidea (Pampatheriidae and Glyptodontidae) (McKenna & Bell, 1997).

Armadillos are the xenarthrans with the greatest temporal and geographical distribution (Krmpotic *et al.*, 2009). The oldest fossils date back to the early Eocene of Brazil (Bergqvist *et al.*, 2004; Bergqvist *et al.*, 2019; Oliveira & Bergqvist, 1998). One of their most distinctive anatomical features is the presence of a protective dorsal carapace formed by a flexible mosaic of dermal scutes covered by a layer of keratinous epidermal scales (Gaudin & Wible, 2006; Krmpotic *et al.*, 2009). The glyptodonts also have an extensive distribution (Krmpotic *et al.*, 2009), ranging from the late Eocene (Zurita *et al.*, 2016) to the early Holocene (Cione *et al.*, 2003), reaching a diversity of over 65 genera and 220 species (McKenna & Bell, 1997). These animals reached more than a ton, and their main characteristic was to have a rigid bony shell covering almost the entire body (Carlini & Tonni, 2000). The pampatheriids are considered close relatives of the glyptodonts and are recorded from the Miocene to Pleistocene (Scillato-Yané *et al.*, 2005). They present many characteristics like armadillos (mobile bands in the carapace) and also characteristics of glyptodonts as nuclei of osteodentine (Gaudin & Wible, 2006; Wolf *et al.*, 2012).

The phylogenetic position of the three constituent groups of Cingulata has been difficult to resolve (Delsuc *et al.*, 2016; Gibb *et al.*, 2015). Gaudin & Wible (2006) have shown that glyptodonts and pampatheriids are sister-taxa, forming a monophyletic group. Whereas this view was already widely accepted, taxonomy and relationships among individual pampathere taxa are still discussed (Wolf *et al.*, 2012). Gaudin & Wible (2006) additionally suggested a close relationship between glyptodonts and pampatheres with the armadillo subfamily Euphractinae, which contradicts the well-established view that glyptodonts must have diverged from other cingulates at a very early point in their phylogenetic history (Hoffstetter, 1958). This hypothesis is based on a restricted set of cranio-dental characters. Even more recently, Delsuc *et al.* (2016) and Mitchell *et al.* (2016) presented a phylogenetic reconstruction based on mitochondrial genome also establishing that glyptodonts are deeply nested within the armadillo crown-group, but unlike what was suggest by Gaudin & Wible (2006), glyptodonts would represent a distinct subfamily (Glyptodontinae) within family Chlamyphoridae.

The presence of a carapace consisting of innumerable dermal shields (osteoderms) disposed in regular arrangements provides one of the most useful diagnostic features of the group, both armadillos and glyptodonts, and it has traditionally been the basis for most systematic schemes of this group (Krmpotic *et al.*, 2009; Vickaryous & Hall, 2006). These osteoderms constitute a tegumentary bone with high fossilization potential (Hill, 2006), with the most frequent and abundant fossil elements in the Cingulata fossil record (Carlini & Zurita, 2010).

Several recent works have explored the phylogenetic and functional implications of the comparative histology of osteoderms in xenarthran mammals. Studies such as Hill (2006), Wolf

(2007), Chávez-Aponte *et al.* (2008), Krmpotic *et al.* (2009), Wolf *et al.* (2012), Da Costa Pereira *et al.* (2014), Asakura *et al.* (2017), Araújo & Porpino (2018), Ciancio *et al.* (2019) and Bergqvist *et al.* (2019) use several different taxa and approach some of their biological significance. They clearly show that characteristics of the anatomical microstructure of the osteoderms are also relevant for the study of evolutionary relationships.

The ontogenetic variation of the osteoderm morphology, the variability within populations and taphonomic processes may not be correctly evaluated and, in some cases, even underestimated, leading to a complex nomenclature problem (Zurita *et al.*, 2011). Dermal ossifications could provide information on soft tissue structures that have not been preserved in fossils; Such studies also shed light on the phylogenetic relationships within the group and on the functional morphology of xenarthrans (Da Costa Pereira *et al.*, 2014; Hill, 2006; Straehl *et al.*, 2013; Wolf, 2007; Wolf *et al.*, 2012).

Inference on ontogenetic and physiological aspects, recognition of fossil-genetic features, diagnosis of paleopathologies, improvement of systematic and anatomical studies has been the main points addressed by paleohistological studies (de Ricqlès, 2011). Including the discovery of new characters for taxonomic differentiation, demonstrating great potential in phylogenetic analysis (Hill, 2006).

In this contribution, we present data on the microstructure of some fossil xenarthran osteoderms, approaching the histological variability within the clade and the biological significance of their pattern.

MATERIALS AND METHODS

The material consists of isolated osteoderms from the scientific collections of the Laboratório de Paleontologia of Departamento de Geologia, Universidade Federal de Pernambuco (DGEO-UFPE), the Museu Coronel Tancredo Fernandes de Mello (MCTFM-PV), the Laboratório de Paleontologia of Universidade Federal de Sergipe (LPUFS) and the Laboratório de Geologia of the Universidade Estadual do Sudoeste da Bahia (UESB 318PV).The fossil specimens studied here all came from Late Pleistocene deposits of Brazil, South America. Table 1 summarize the list of specimens used in this studied and their respective localities. All material was previously measured and photographed.

The thin sections were made at the Laboratório de Laminação of Departamento de Geologia, Universidade Federal de Pernambuco and Seção de Laminação of Instituto de Geociências, Universidade de São Paulo. The methodology used to prepare the specimens and produce the thin sections follow Lamm (2013). The standard petrographic slides (normally 27 x 46 mm) was used for most of osteoderms, for the biggest ones was used 50 x 80 mm poly (methyl methacrylate) slices. The thin sections analysis was carried out in a Zeiss Axioskop 40 and Zeiss Axion Vision Imager.A2 petrographic microscope with an attached camera (AxioCam MRc), using Axion Vision Release 4.8 software for photomicrographs. The specimens were photographed with a Nikon Coolpix P520 camera and edited in Adobe Photoshop CC 2017 and Adobe Illustrator CC 2017.

The terminology used follows the reference literature on bone histology (Francillon-Vieillot *et al.*, 1990; Hill, 2006; Huttenlocker *et al.*, 2013; Prondvai *et al.*, 2014).

Table 1 - List of specimens used in this studied and their respective localities.

Family	Taxon	Reference No.	Locality
Pampatheriidae	***Pampatherium humboldti***	DGEO-CTG-UFPE 8476 A; B; C; D; E	Jacobina - Bahia
	Holmesina paulacoutoi	DGEO-CTG-UFPE 5964	Brejo da Madre de Deus - Pernambuco
		DGEO-CTG-UFPE 7029	Bom Conselho - Pernambuco
		DGEO-CTG-UFPE 6552	Afrânio - Pernambuco
		DGEO-CTG-UFPE 8807	Anagé - Bahia
?Dasypodidae	***Pachyarmatherium brasiliense***	DGEO-CTG-UFPE 7768 A; B; C	Brejo da Madre de Deus - Pernambuco
		DGEO-CTG-UFPE 8814	Poço Redondo - Sergipe
Glyptodontidae	***Glyptodon* sp.**	DGEO-CTG-UFPE 8817; 8818; 8819; 8820; 8821	Santa Vitória do Palmar – Rio Grande do Sul
	***Glyptotherium* sp.**	DGEO-CTG-UFPE 5938; 6992	Brejo da Madre de Deus - Pernambuco
		DGEO-CTG-UFPE 6507	Afrânio - Pernambuco
		DGEO-CTG-UFPE 7754	Congo - Paraíba
		DGEO-CTG-UFPE 8809	Toca da Raposa, Simão Dias - Sergipe
	Doedicurus clavicaudatus	DGEO-CTG-UFPE 8815; 8816	Santa Vitória do Palmar – Rio Grande do Sul

RESULTS

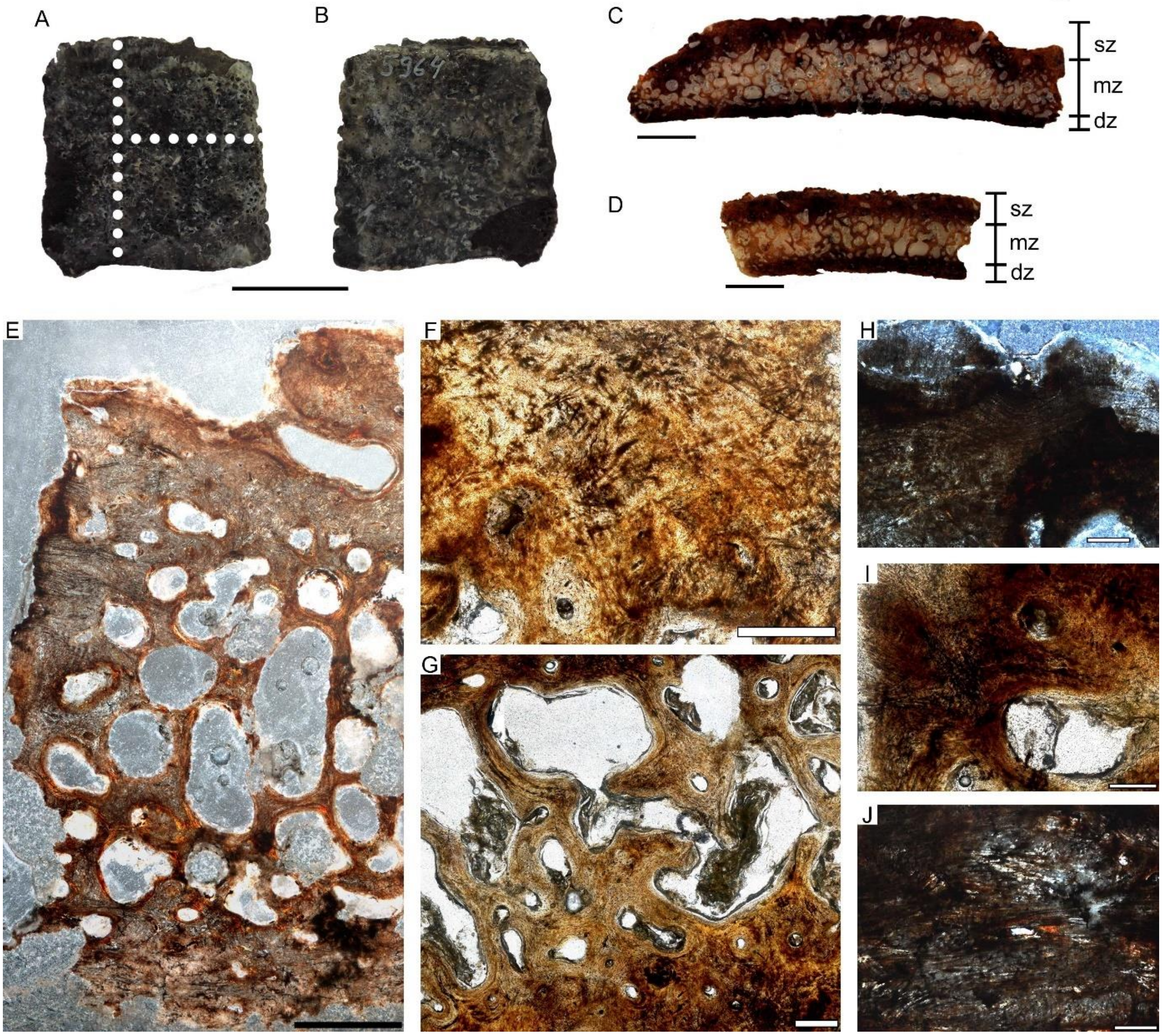
Holmesina paulacoutoi

The DGEO-CTG-UFPE 5964 and 318PV198 specimens are the most complete ones, slightly fragmented, it presents a subrectangular shape, while DGEO-CTG-UFPE 6552 and DGEO-CTG-UFPE 7029 is more fragmented, ranging from subrectangular to subquadrangular. Only the totality of the shape is fragmented, the ornamentation of the external surface is present. The osteoderms presents a very rugose external surface with delicate punctuation and a wide marginal area around the elevate principal figure. All the specimens belong to the dorsal carapace. The average thickness of the osteoderms is 0,9 cm.

All the specimens present a histological structure as classic diploe conformation, but unlike the heavily remodeled osteonal tissues in the osteoderms of large glyptodonts, *H. paulacoutoi* presents a mainly primary compact bone in both the superficial and deep zones. Vascularization is generally low within the superficial and deep zones, consisting of a few primary osteons and some rare secondary osteons (Fig 1).

The superficial zone is observed to contain several growth lines in the most superficial extent, followed by a layer of woven bone with rare strips of parallel-fibered bone tissue. Osteocyte lacunae appear mostly rounded in this woven bone layer. The deep zone is very thin and presents a predominance of parallel-fibered bone tissue, with very few strips of a disorganizes woven bone tissue. The compact bone at the osteoderm margins is marked by abundant crossing Sharpey’s fibers arranged in thick but rather loose bundles. The central trabecular zone exhibits extensive remodeling characterized by numerous large trabecular areas, surrounded by concentric bone lamellae with abundant and evenly spaced osteocyte lacunae.

Figure 1 - Isolated buckler osteoderm of *Holmesina paulacoutoi* (DGEO-CTG-UFPE 5964) in superficial (A) and deep face (B), dashed line indicates the section plane; C and D, Overview of the osteoderm thin section in both section plane (several merged images); E, Close-up of the marginal area showing the three distinct layers of compact and trabecular bone (several merged images); F, The superficial layer shows a woven bone matrix with numerous rounds and irregular osteocytes lacunae with a random arrange; G, The central trabecular zone exhibits numerous large trabecular areas, surrounded by concentric bone lamellae with abundant and evenly spaced osteocyte lacunae; H, Several growth lines in the most superficial layer; I, The marginal area is marked by abundant crossing Sharpey's fibers; J, The deep zone presents a predominance of parallel-fibered bone tissue. Scale bar equals 2cm in A, B; 5mm in C,D; 4mm in E; 500 µm in F, G; 200 µm in H, I, J. All histological images taken under polarized light. Abbreviations: sz, superficial zone; mz, middle zone; dz, deep zone.



Pampatherium humboldti

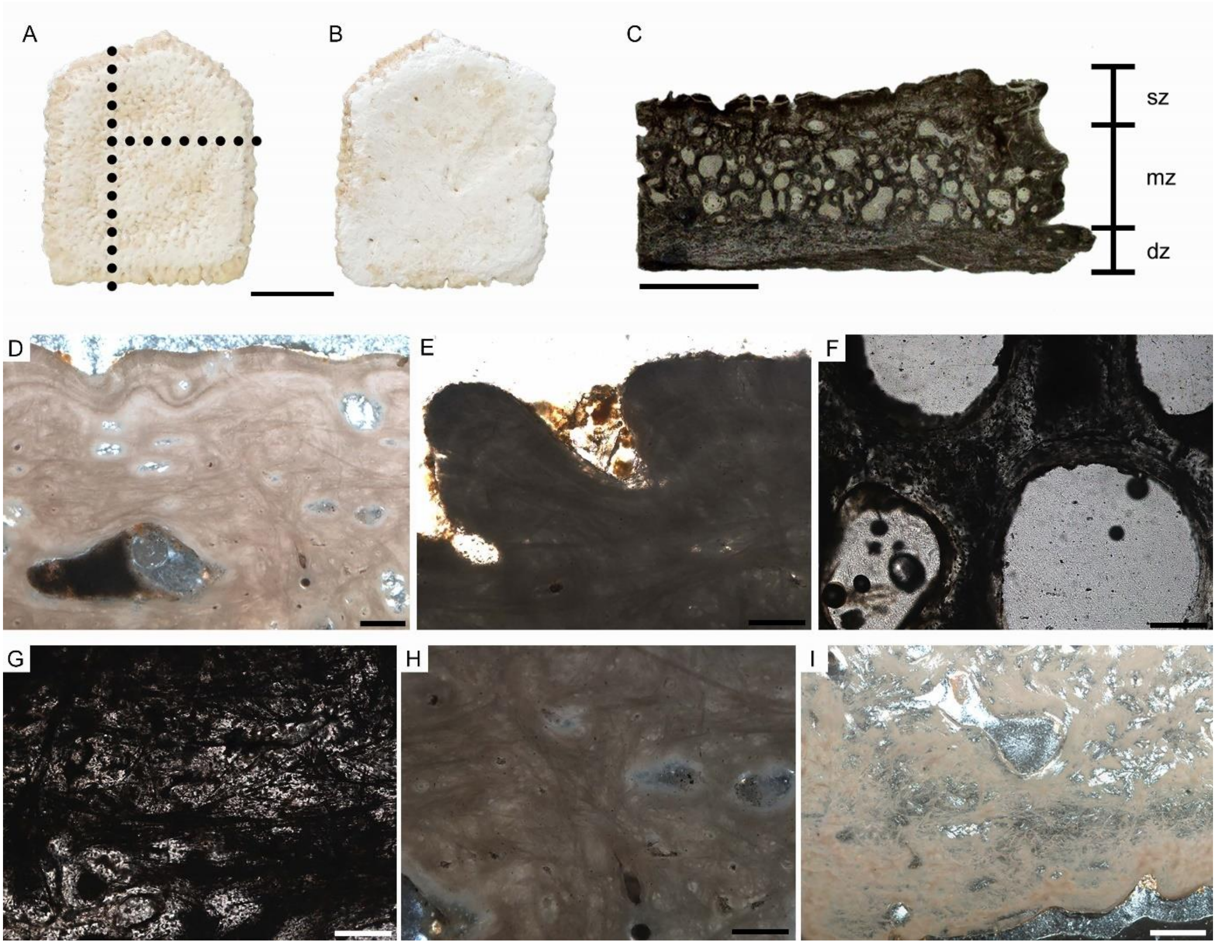
The specimens DGEO-CTG-UFPE 8476 A; B; C; D are isolated buckler osteoderms and DGEO-CTG-UFPE 8476 E is a movable osteoderm slightly fragmented, with the ornamentation a little worn. All of the specimens were found in association. The buckler osteoderms has the external surface very punctuated by small pits, the marginal area is very narrow with no large difference between the marginal area and the principal figure. The principal figure is wide and very low, and the longitudinal elevation is elongated and delimited laterally by two very shallow sulci. The imbricate band osteoderm presents both the anterior end with the raised table and the posterior end with the ornamented part fragmented, it has an ornamented surface similar to the fixed osteoderms, with a smooth longitudinal central elevation in the posterior end. The average thickness of the buckler osteoderms is 0,7 cm.

In all osteoderms some of the histological details have been obscured by diagenetic processes, but most of the major features can still be observed. Both the buckler and the imbricate osteoderms presents the diploe feature, where superficial and deep layers of compact bone encloses a central region of trabecular bone.

The buckler osteoderm presents in the most external area of some osteoderms several growth lines that run parallel to the external surface (Fig 2). This area is followed by a distinguishable zone of collagen fibers bundles without clear orientation. The woven bone tissue in the lower area of the superficial zone presents several randomly distributed rounded osteocytes lacunae. The non-lamellated parallel fibered bone also presents some elongate osteocyte lacunae, very organized and arranged in layers parallel to each other. Sharpey's fibers in this region are abundant, and cross perpendicularly the superficial surface of the osteoderm. The deep zone is considerable bigger than the superficial zone, it contains a fibro-lamellar bone tissue with a well-developed woven bone matrix. Several rounded osteocyte lacunae are present in this area. The deep zone is enclosed by a very thin parallel fibered bone tissue that becomes thickened toward the periphery, with collagen fibers running parallel to the surface.

The middle zone exhibits a trabecular bone with small to moderate trabecular areas, except DGEO-CTG-UFPE 8476 D which presents the largest trabecular areas. This zone is made by a fibro-lamellar bone tissue. The marginal zone of the osteoderm is very compact and is marked by abundant Sharpey's fibers. These poorly mineralized collagen fibers are arranged in thick but rather loose bundles, running obliquely to the marginal surface. The buckler osteoderm presents in general a low vascularization with rare primary and secondary osteons throughout the bone tissue, except by DGEO-CTG-UFPE 8476 A which shows a bigger concentration of secondary osteons in the superficial zone.

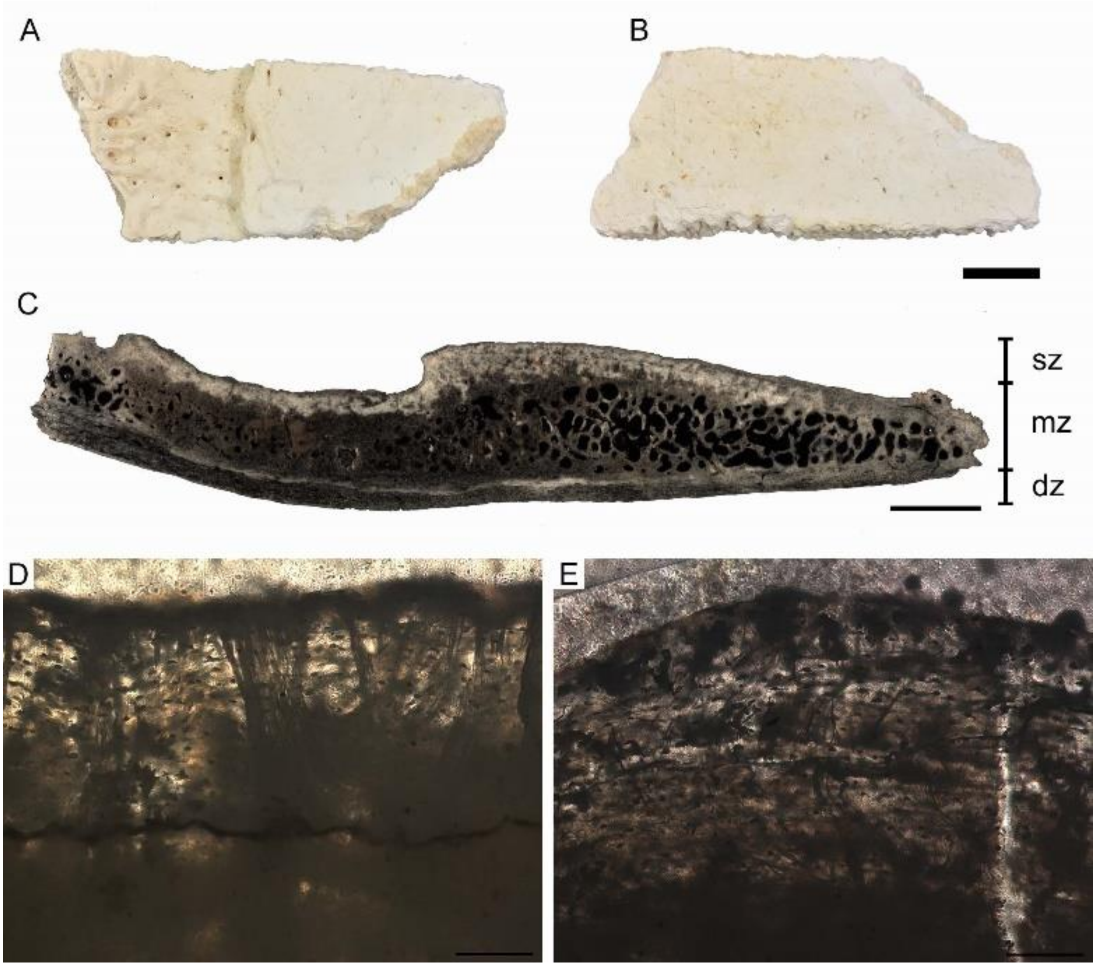
Figure 2 - Isolated buckler osteoderm of Pampatherium humboldti (DGEO-CTG-UFPE 8476A) in superficial (A) and deep face (B), dashed line indicates the section plane; C, Overview of the osteoderm thin section (several merged images under polarized light); D, The superficial layer shows a non-lamellated parallel fibered bone tissue followed by a woven bone, cross polarized light; E, Several growth lines and Sharpey's fibers in the most superficial layer, polarized light; F, The central trabecular zone exhibits a fibro-lamellar bone tissue, polarized light; H, The deep zone present a well-developed woven bone matrix, cross polarized light; I, The fibro-lamellar complex of the deep zone, cross polarized light. Scale bar equals 1cm in A, B; 5mm in C; 1mm in D; 200 µm in E to H; 2mm in I. Abbreviations: sz, superficial zone; mz, middle zone; dz, deep zone.



The imbricate osteoderm is largely comparable to that of the buckler osteoderms, such as the generally low vascularization, but nevertheless shows some noticeable differences (Fig

3). The superficial zone at the anterior region present a non-lamellated parallel bone tissue with a longitudinal orientation whereas the posterior region presents a more lamellar parallel bone tissue with intercalated layers of elongated and rounded osteocytes lacunae. Near the intermediate region, in the end of the anterior regions, is the biggest concentration of surface-perpendicular bundles (Sharpey's fibers), the posterior region also bares some stripes throughout the region. The central zone of the imbricate osteoderm is very spongiosa. The middle of the anterior portion is the most porous area, with large trabeculae areas. Towards the posterior portion the trabeculae areas become more moderate in size, the middle of the posterior region being the less porous area of the central zone. The deep zone is similar to the buckler osteoderm, presenting a fibro-lamellar complex with a well-developed woven bone matrix, enclosed by a parallel fibered bone tissue with fibers oriented parallel to the surface. The deep zone is much larger in the posterior region. The marginal zone is not preserved due to fragmentation.

Figure 3 - Isolated imbricate osteoderm of *Pampatherium humboldti* (DGEO-CTG-UFPE 8476E) in superficial (A) and deep face (B); C, Overview of the osteoderm thin section (several merged images); D, The superficial zone at the anterior region present a non-lamellated parallel bone tissue with several Sharpey's fibers; E, the posterior region presents a more lamellar parallel bone tissue. Scale bar equals 1cm in A, B; 5mm in C; 100 µm in D, E. All histological images taken under polarized light. Abbreviations: sz, superficial zone; mz, middle zone; dz, deep zone.



Pachyarmatherium brasiliense

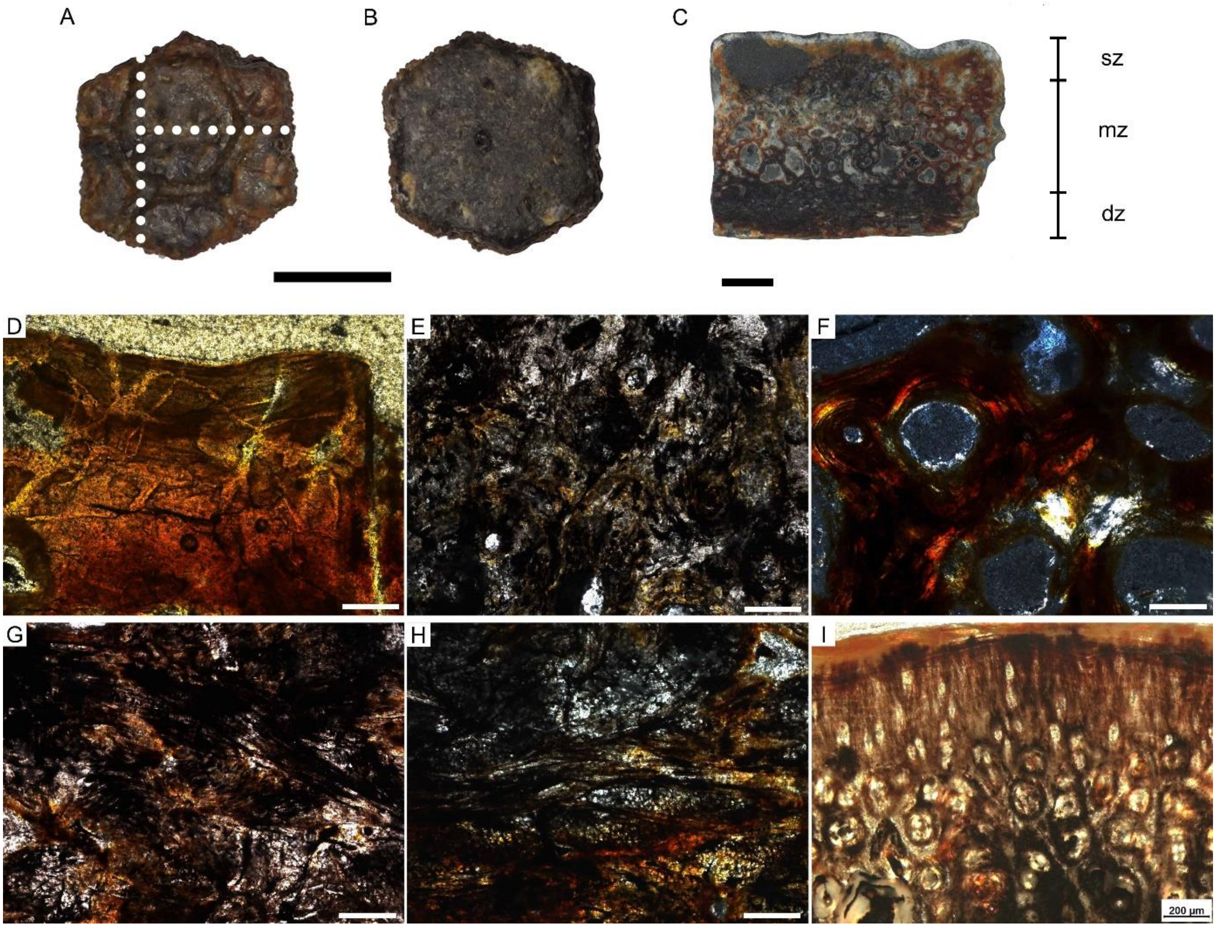
The specimens are isolated osteoderms from the dorsal region, all presenting a hexagonal shape. The main figure is rounded to sub-rounded, displaced toward the posterior edge, nearly flat and accompanied by five or six peripheral figures. The external surface is moderately wrinkled. Most osteoderms present foramens at the intersection of the radial grooves with the main groove. The internal osteoderm surface is flat and presents a striated texture, showing some small neurovascular foramina. The thickness ranges from 0,8 cm to 1,1 cm.

The thin sections of the osteoderms reveals a relatively compact microstructure, with only moderate development of the trabecular bone in the central layer, also enclosed by two layers of compact bone (Fig 4). The superficial zone presents a thin parallel fibered bone layer in the outmost periosteum, followed by a heavily remodeled area of haversian bone. The dense haversian bone consists of overlapping secondary osteons with remaining interstitial lamellae. DGEO-CTG-UFPE 8814 have the least remodeled superficial layer. In the most external area of some specimens are seen growth lines parallel to the osteoderm surface.

The middle zone is a trabecular bone with small to moderate trabeculae areas and thick trabeculae bordered by parallel fibered bone enclosing a woven bone. The PFB exhibits several aligned elongated osteocytes lacunae, whereas the woven bone presents several disorganized rounded osteocytes lacunae. This zone has a low vascularization, with some secondary osteons seen in the trabeculae. The deep zone is thinner than the superficial zone. It reveals a fibro-lamellar complex with mineralized collagenous fibers deposited in multiple directions followed by an almost avascular parallel-fibred periosteal bone in the deepest extent.

Interestingly, DGEO-CTG-UFPE 7768A presents a rather different histological conformation. Between the outmost periosteum and the haversian bone, this specimen has a layer of mineralized collagenous fibers perpendicular to the surface with simple longitudinal canals, indicating the presence of a periosteal reactive bone tissue. It is also the specimen with the highest degree of remodeling, and the middle zone appears to be completely remodeled.

Figure 4 - Isolated buckler osteoderm of *Pachyarmatherium brasiliense* (DGEO-CTG-UFPE 7768 B) in superficial (A) and deep face (B), dashed line indicates the section plane; C, Overview of the osteoderm thin section (several merged images under polarized light); D, Superficial zone presents parallel fibered bone layer in the outmost periosteum, polarized light; E, Haversian bone showing overlapping secondary osteons with remaining interstitial lamellae in the superficial zone, polarized light; F, Middle zone showing the predominance of parallel fibered bone in the trabeculae, cross polarized light; G, Deep zone reveals a fibro-lamellar complex with mineralized collagenous fibers deposited in multiple directions, polarized light; H, Parallel-fibred bone in the deepest extent of the deep zone, cross polarized light; I, Mineralized collagenous fibers perpendicular to the surface with simple longitudinal canals in the superficial zone, cross polarized light (DGEO-CTG-UFPE 7768 A). Scale bar equals 1cm in A, B; 2mm in C; 200 µm in D to H. Abbreviations: sz, superficial zone; mz, middle zone; dz, deep zone.



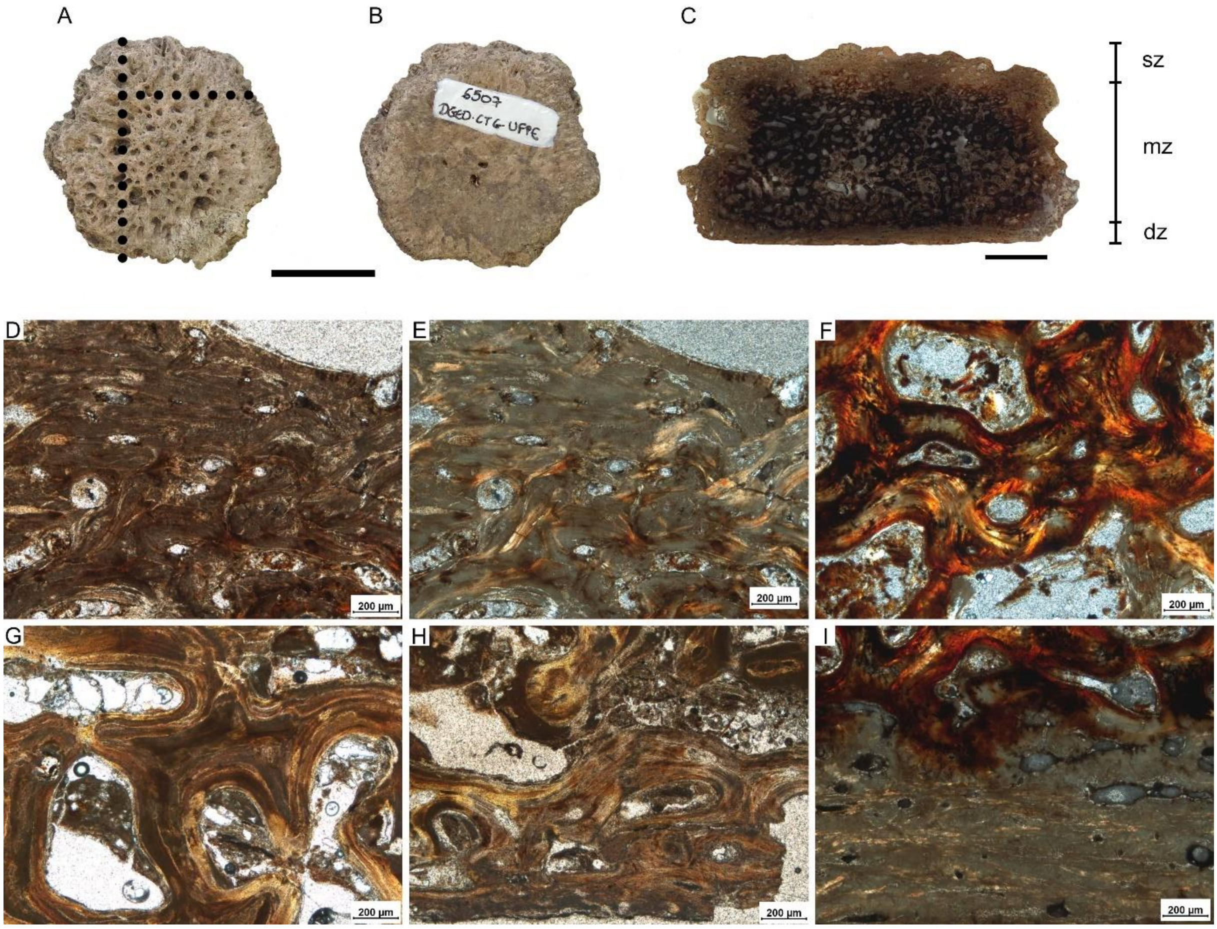
Glyptotherium

The isolated osteoderms examined here belong to the dorsal region, most of them are partially fragmented, except for DGEO-CTG-UFPE 6507 which is complete and clearly hexagonal in superficial view. All specimens have the external surface very punctuated by small orifices presenting a rugose aspect. The central and radial sulci are very shallow. The thickness of the osteoderms range from 1,7 cm to 4,4 cm.

In histological sections, the osteoderms reveals a highly remodeled microstructure and no growth marks is visible (Fig 5). Both the superficial and deep zones are very thin compact area and is predominantly occupied by a secondary non-lamellated parallel fibered bone tissue. The superficial layer shows very few straps of disorganized woven bone between the parallel fibered bone, which is organized parallel to the surface. This zone is poorly vascularized, exhibiting scarce secondary osteons. The superficial zone of DGEO-CTG-UFPE 6992 have the highest occurrence of disorganized mineralized collagenous fibers, but even so the parallel fibered bone tissue is predominant. The deep zone is slightly thicker than the superficial zone and presents a parallel to the surface organized mineralized collagenous fibers bundles. Hardly any woven bone is seen in this area. Is the most vascularized zone, with some longitudinal and reticular simple canals throughout the layer.

The middle zone is the most remarkable area of the osteoderm. It is very large and occupies most of the osteoderm entire thickness. The microstructure of this zone consists of a coarse cancellous bone with moderately sized trabeculae areas and thick trabeculae. The trabeculae consist of a lamellated parallel-fibered bone tissue and almost no primary bone can be seen. Mostly of the preserved osteocytes lacunae are elongated and organized with a dense lacunocanalicular network, but it is still possible to see some rounded osteocytes lacunae between the organized line. The degree of vascularization is moderated, with some longitudinal simple canals and secondary osteons presents.

Figure 5 - Isolated buckler osteoderm of *Glyptotherium* sp. (DGEO-CTG-UFPE 6507) in superficial (A) and deep face (B), dashed line indicates the section plane; C, Overview of the osteoderm thin section (several merged images under polarized light); D, Superficial zone presents a non-lamellated parallel fibered bone tissue E, The same as D in cross polarized light; F and G, Middle zone consist of a lamellated parallel-fibered bone tissue, polarized light; H, Deep zone showing a parallel to the surface organized mineralized collagenous fibers bundles, polarized light; I, Deep zone with some longitudinal and reticular simple canals, cross polarized light. Scale bar equals 2 cm in A, B; 5mm in C. Abbreviations: sz, superficial zone; mz, middle zone; dz, deep zone.



Glyptodon

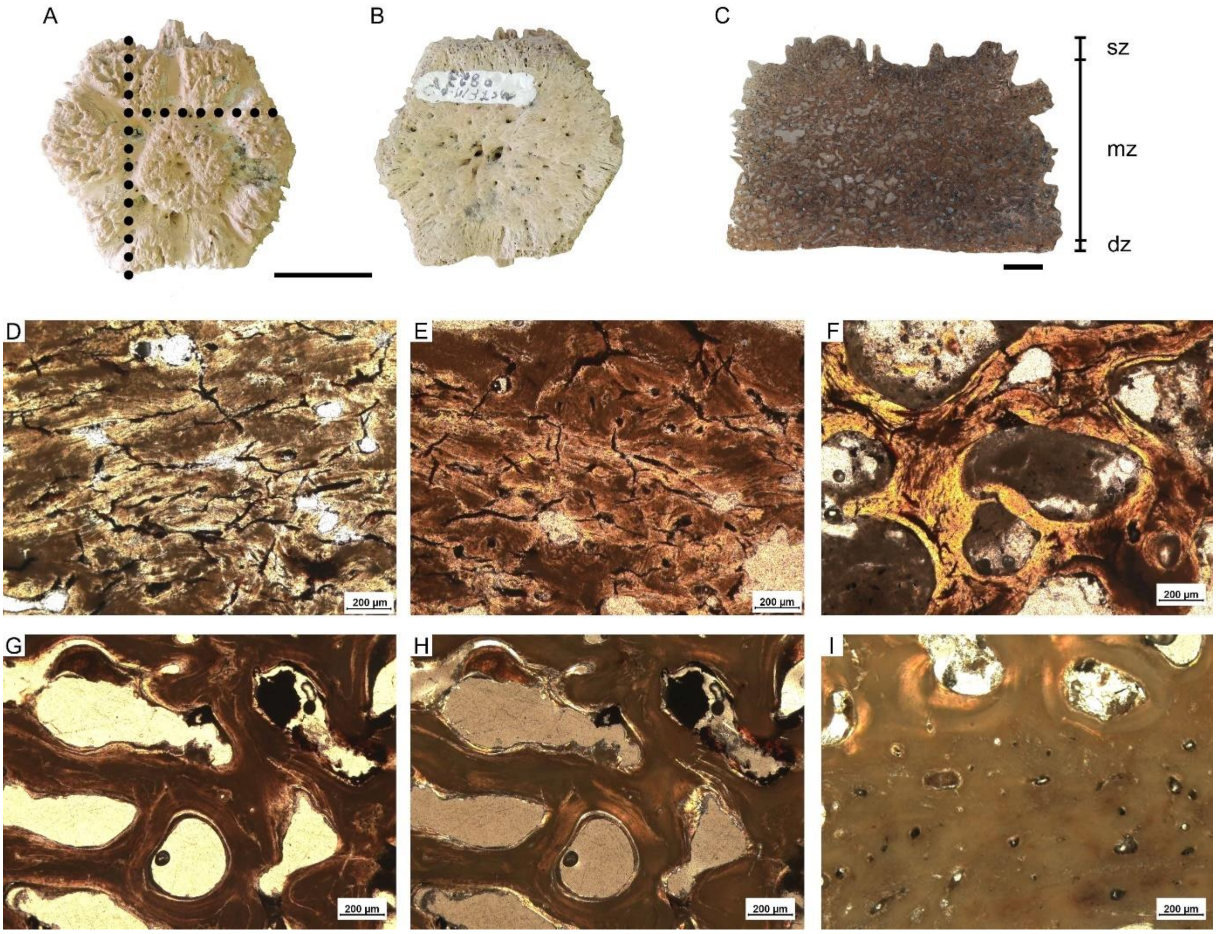
We analyzed osteoderms from the dorsal region, which present a hexagonal shape and a rugose aspect in the external surface. The principal figure is surrounded by a single row of peripheral figures, which are separated by very deep and large central and radial sulci. The thickness ranges from 1,9 cm to 2,7 cm.

In general, the superficial and deep zone consists of a heavily remodeled bone tissue and small sized compared to the middle trabecular zone thickness (Fig 6). Both zones present in the outermost layer a pattern of mineralized fibered bundles parallel to the surface with very few disorganized straps within the matrix; towards the center they are followed by a haversian bone with several secondary osteons.

The middle zone has moderately sized trabecular areas with trabeculae thickness ranging from thin to moderate. All specimens have some trabeculae areas filled with fine sediment and some quartz fragments. The spongy bone take place of most of the osteoderm configuration, representing almost 2/3 of the total thickness. The microstructure in this zone consists of a parallel fibered bone tissue with several elongated osteocytes lacunae displayed in a very organized pattern. Secondary osteons are present along the middle zone. In the lateral area, mineralized fiber bundles are oriented perpendicular to the lateral surface. Throughout all the zones in every osteoderm, the amount of osteocyte lacunae preserved is enormous.

The specimens DGEO-CTG-UFPE 8818 and 8820 presents a peculiar histologic pattern, with some important differences from the others. DGEO-CTG-UFPE 8818 has a predominantly woven bone tissue in the most superficial layer, where the several random and rounded osteocytes lacunae indicate a disorganized display of the collagenous fiber bundle. Between the fibers of the woven matrix are abundant primary osteons. This layer is also followed by a secondary haversian bone. On the other hand, DGEO-CTG-UFPE 8820 shows the highest degree of remodeling in the superficial and deep zone, with almost no primary bone present. Some fiber bundles running parallel to the surface is seen, but the matrix is made predominantly by longitudinal secondary osteons overlapping each other with remnants interstitial lamellae. Secondary osteons in a reticular orientation and parallel to the surface are also seen.

Figure 6 - Isolated buckler osteoderm of Glyptodon sp. (MCTFM–PV 0823) in superficial (A) and deep face (B), dashed line indicates the section plane; C, Overview of the osteoderm thin section (several merged images under polarized light); D, Superficial zone with mineralized fibered bundles running parallel to the surface, polarized light; E, Haversian bone with several secondary osteons in the bottom of the superficial zone, polarized light; F, Several elongated osteocytes lacunae displayed in a very organized pattern in the trabecular bone, polarized light; G, Middle zone consists of parallel fibered bone tissue, polarized light; H, The same as G in cross polarized light; I, Deep zone is a very compact layer with parallel fibered bone tissue, cross polarized light. Scale bar equals 2 cm in A, B; 5mm in C. Abbreviations: sz, superficial zone; mz, middle zone; dz, deep zone.



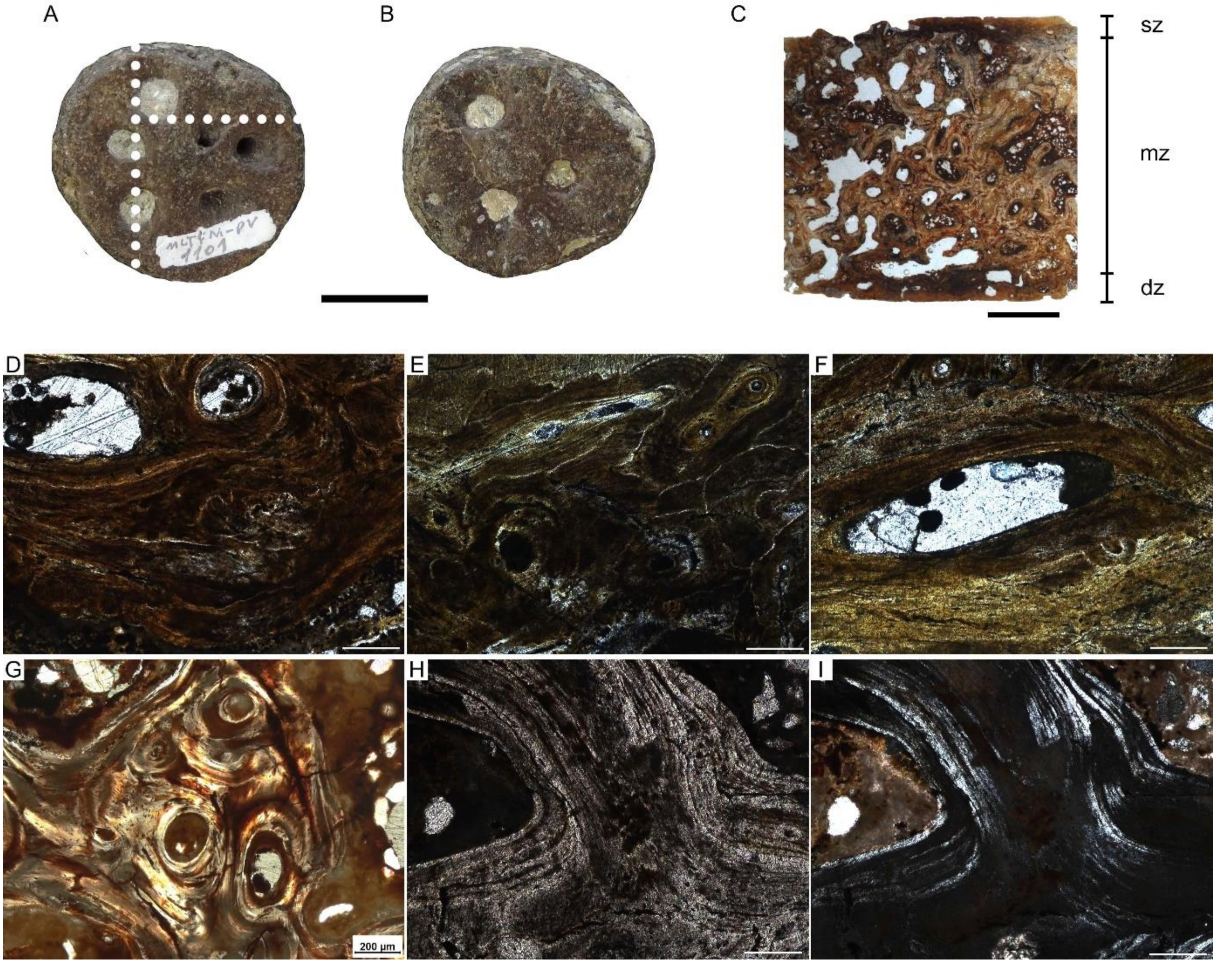
Doedicurus clavicaudatus

The osteoderms have an almost round shape and lack ornamentation in the form of figures and sulci. Both external and internal surface are smooth and characterized by the presence of several large canals, which are trans-osteodermal canals passing entirely through the osteoderm from one side to another. The average thickness of the osteoderms is 2,2 cm.

The specimens show in the thin section three distinct histological regions, two compact bone layers enclosing a very large layer of cancellous bone (Fig 7). The superficial and deep zone is both thin and very similar. These zones are mostly a secondary parallel-fibered bone tissue with several longitudinal secondary osteons distributed along the layer and a few reticular ones. Elongated and rounded osteocytes lacunae are well preserved, they are seen in a distinct pattern of organization, following the mineralized fibers branches of the parallel-fibered bone tissue.

The middle zone is big, it occupies most of the osteoderm thickness and is largely remodeled. The microstructure of this zone consists of a coarse cancellous bone with thick trabeculae and trabeculae areas size ranging from moderate to large. The trabeculae consist of a lamellated parallel-fibered bone tissue. The degree of vascularization is moderated, with several secondary osteons presents. The entire zone exhibits several elongated osteocytes lacunae very organized and evenly distributed; a few rounded osteocytes lacunae are also preserved. The trabeculae areas are filled with fine sediment and some quartz fragments.

Figure 7 - Isolated buckler osteoderm of Doedicurus clavicaudatus (DGEO-CTG-UFPE 8815) in superficial (A) and deep face (B), dashed line indicates the section plane; C, Overview of the osteoderm thin section (several merged images under polarized light); D, The most superficial zone showing parallel-fibered bone tissue, polarized light; E, Longitudinal and reticular secondary osteons in the superficial zone, polarized light; F, Reticular secondary osteon and parallel-fibered bone tissue in the deep zone; G, The middle zone presents thick trabeculae and moderate trabeculae areas filled with fine sediment and some quartz fragments, cross polarized light; H, The trabeculae consist of a lamellated parallel-fibered bone tissue, polarized light; I, The same as H in cross polarized light. Scale bar equals 2cm in A, B; 5mm in C; 200 µm in D to I. Abbreviations: sz, superficial zone; mz, middle zone; dz, deep zone.



DISCUSSION

As already stated by several authors (Araújo & Porpino, 2018; Asakura *et al.*, 2017; Bergqvist *et al.*, 2019; Chávez-Aponte *et al.*, 2008; Ciancio *et al.*, 2019; Hill, 2006; Wolf *et al.*, 2012), cingulate osteoderms presents an considerable level of histological diversity. Although quite a few taxa already have their osteoderm histological pattern described and compared

(Asakura & Oliveira, 2019), it’s important to recognize that the biggest difficult in comparative histology, besides the isolation condition they are generally found, is the variety of histological nomenclature used by the authors, which makes it problematic to assess evolutionary patterns through comparison.

Prondvai *et al.* (2014) stated that the main sources of misinterpretation of bone histology are the description of matrix fibers and osteocyte lacunae and they combined appearance of which reflects the general pattern of the bone tissue. This also includes the correct use of methodologies such as the plane of section (Prondvai *et al.*, 2014) and linear or cross polarized light (Bromage *et al.*, 2003). Prondvai *et al.* (2014) and Stein & Prondvai (2014) discussed the importance of standardized the paleohistology terminology and call attention to the importance of correct interpretation of biological significance of the described features. In this work, we largely follow their proposed terminology combined with Francillon-Vieillot *et al.* (1990).

Comparative histological pattern

Cingulate osteoderms are highly derived with respect to the small, isolated ossicles found in mylodontid sloths (Wolf *et al.*, 2012). They are characterized by a number of morphological and histological traits. the major features include polygonal, suturally articulating osteoderms and a generally diploe-like structure (Asakura & Oliveira, 2019; Hill, 2006; Wolf, 2007; Wolf *et al.*, 2012). Asakura & Oliveira (2019) presents a table with cingulate taxa with their osteoderm histology described so far with the related reference.

All the specimens studied here present the expected diploe-like disposition, where the cancellous bone in the middle zone is enclosed by two layers of compact bone. In this work, we have considered compact and cancellous/spongy bone as macroscopic categories related only to the overall bone porosity (Francillon-Vieillot *et al.*, 1990). The most varying feature of this conformation is the thickness of each layer in each species. The osteoderms from glyptodonts and P. *brasiliense* presents the widest middle zone, at least more than half of the total thickness, where the pampatheres osteoderms present a more even distribution. Even though *P. brasiliense* presents a distinctly large middle zone, the trabecular bone is not very well developed and shows only a moderate degree of porosity. This observation matches the pattern described in Hill (2006) and Wolf *et al.* (2012).

The overall Dasypodinae osteoderm microstructure is a comparatively compact central region with only poorly developed cancellous fibrolamellar bone with moderate degree of secondary remodeling (Bergqvist *et al.*, 2019; Krmpotic *et al.*, 2015; Vickaryous & Hall, 2006; Wolf *et al.*, 2012). In contrast, glyptodonts and pampatheres share a relatively homogeneous distribution of a well-developed trabecular bone and in some taxa reaching a heavily remodeling degree (Araújo & Porpino, 2018; Asakura *et al.*, 2017; Da Costa Pereira *et al.*, 2014; Hill, 2006; Wolf *et al.*, 2012).

The osteoderm microstructure of the pampatheres analyzed largely match the description of Wolf *et al.* (2012), particularly regarding the array of collagen fibers that seems to contain bundles sectioned at various angles with respect to the plane of section. This less regular meshwork described in Wolf *et al.* (2012) was interpreted here as a woven bone. Also, marginal Sharpey's fibers are present and are arranged in broad, often diverging or converging bundles, frequently cross each other at various angles as described by Wolf (2007) and Wolf *et al.* (2012).

Da Costa Pereira *et al.* (2014) have previously described the *Pachyarmatherium brasiliense* osteoderm histology as being composed of well-developed thick superficial and deep layers of compact bone with no visible fiber bundles enclosing a central region of thin trabecular bone. Our samples demonstrate a rather different pattern, showing a more uniform distribution of the diploe structure, with a clear parallel fibered bone tissue layer ate the most superficial zone. This differences could be due diagenetic alterations in Da Costa Pereira *et al.*

(2014) samples, since they reported the presence of dark superficial zones throughout the osteoderm, or simply intraspecific variations. Remarkably, DGEO-CTG-UFPE 7768A presents a layer of mineralized collagenous fibers perpendicular to the surface with simple longitudinal canals, indicating the presence of a periosteal reactive bone tissue. This type of bone tissue could be a pathological indicative but further analyzes should be made to confirm.

Glyptodonts osteoderms have their histology been largely described, with all taxon from the Brazilian Intertropical Region (Cartelle, 1999) analyzed (Asakura & Oliveira, 2019): *Panochthus* sp. (Araújo & Porpino, 2018; Asakura *et al.*, 2017; Da Costa Pereira *et al.*, 2014; Hill, 2006; Wolf *et al.*, 2012), *Hoplophorus euphractus* (Asakura & Oliveira, in press), *Glyptotherium* sp. (Araújo & Porpino, 2018; Da Costa Pereira *et al.*, 2014; Hill, 2006; Luna *et al.*, 2018; Wolf *et al.*, 2012) and *Neuryurus* sp. (Asakura *et al.*, 2017).

The general and specific pattern of the several glyptodonts taxa is already starting to be well studied, but three imperative features needs to be taken more into account in these descriptions, the ontogenetic stage (Luna *et al.*, 2018), pathology and the variation on osteoderms from distinct regions of the carapace (Araújo & Porpino, 2018). This is in fact valid for all histological analyzes in any taxa.

Luna *et al.* (2018) by analyzing a juvenile *Glyptotherium* sp. and comparing with a juvenile *Glyptodon* sp. demonstrated that the histological differences with previously studied Glyptodontidae are mainly due to ontogenetic variation occurring during osteoderm development. Araújo & Porpino (2018) have analyzed osteoderms from distinct regions of the carapace from *Glyptotherium* sp. and *Panochthus* sp. proven that the histological pattern for distinct parts of the exoskeleton can exist and the position of the osteoderm must be taken into consideration before taxonomic differentiations based on histology pattern can be proposed.

As for the comparison between *Panochthus* sp. osteoderms with *ante mortem* alteration (Araújo & Porpino, 2018) with *Neuryurus* sp. osteoderms (Asakura *et al.*, 2017), both works seems to have described an immature bone tissue since the most remarkable feature is the presence of fibres bundles with no preferential alignment at the superficial and deep zone. *Panochthus* sp. material described as ‘osteoderms with *ante mortem* alteration’ doesn’t appear to have any histological indicative of pathological tissue, it is more likely to be a young specimen with taphonomic alterations. Even so, a detail review of both materials should be considerate.

All of the specimens studied presents characteristics of mature bone tissue, like the degree of remodeling and the predominance of dynamic osteogenesis derived bone tissue; excepted the *Glyptodon* sp. DGEO-CTG-UFPE 8818 specimen which seems to belong to an young individual due to the predominantly woven bone tissue in the most superficial layer and abundant primary osteons. Moreover, only the *Pachyarmatherium brasiliense* DGEO-CTG-UFPE 7768A presented a possible pathological structure. Unfortunately, all the material used in this study was isolated osteoderms and a precise location of the carapace region wasn’t possible.

Systematic considerations

It is well accepted that glyptodonts are members of the monophyletic group Xenarthra, one of the four major placental mammal lineages (Ciancio *et al.*, 2019; Gaudin & Wible, 2006; Gillette & Ray, 1981; Hoffstetter, 1958; McKenna & Bell, 1997; Zurita *et al.*, 2016). Nevertheless, they have fascinated evolutionary biologists for a long time because of their notable skeletal adaptations and apparently isolated phylogenetic position even within their natural group (Delsuc *et al.*, 2016; Fernicola & Porpino, 2012; Gaudin & Wible, 2006; Mitchell *et al.*, 2016; Zurita *et al.*, 2016).

Phylogenetic reconstructions based on mitochondrial genome and cranial morphological characters indicated that glyptodonts are in fact deeply nested within the armadillo crown-group, representing a distinct subfamily (Glyptodontinae) within family Chlamyphoridae (Billet *et al.*, 2011; Delsuc *et al.*, 2016; Gaudin & Wible, 2006; Mitchell *et al.*, 2016). Interestingly, the monophyletic Glyptodontoidea (glyptodonts and pampatheres) remains strongly supported (Billet *et al.*, 2011).

These studies strongly support the idea that imbricating bands of osteoderms was present in the cingulate ancestor and lost in glyptodonts, highlighting the derived nature of glyptodonts osteoderms. Hill (2006) suggests this idea when he pointed out that the imbricating osteoderms occur in many other extant and fossil vertebrates (e.g. crocodilians, aetosaurs, and squamates).

Unfortunately, the comparison of pampathere, glyptodont and dasypodid osteoderm histology pattern still doesn't provides enough evidence to elucidate some of the systematic problems of each group within Cingulata (Wolf *et al.*, 2012). Besides the nomenclature standardization, ontogenetic, taphonomic and pathological features must be carefully considered so that systematic inferences can be properly made. However, as briefly commented by Wolf (2007), osteoderms microstructure indicates that dasypodids lack clear synapomorphies and there is evidence that both pampatheres and glyptodonts form monophyletic taxa.

Hill (2006) described the histology pattern of pampathere and glyptodont osteoderms as "relatively homogenous distribution of trabecular bone that usually accounts for more than half of the osteoderm volume". Wolf *et al.* (2012) also suggested that "osteoderms belonging to derived glyptodonts generally show a highly secondarily- reconstructed and, in most cases, largely trabecular cancellous bone structure".

The commonalities of pampathere and glyptodont osteoderms include the similar arrangement of collagen fiber bundles within the deep cortex (parallel-fibered bone tissue), the overall distribution of cancellous bone thickness and the predominance of DO-derived bone tissue. Until now, decussating marginal Sharpey's fiber have only been observed in pampatheres, and thus is consider a synapomorphy of Pampatheriidae (Wolf *et al.*, 2012).

Pachyarmatherium brasiliense is a cingulate with uncertain affinities and with his inclusion within glyptodonts very questioned (Oliveira *et al.*, 2013). Some microstructure features have been used to comment this taxon systematic position (Da Costa Pereira *et al.*, 2014; Oliveira *et al.*, 2013) but the problematic haven't been elucidated yet. On the contrary of what was described by Da Costa Pereira *et al.* (2014), our specimens presents a relatively thin superficial and deep zone with a moderate-developed trabecular bone in the middle zone. The remodeling is indeed not extensive but moderated and the decussating marginal Sharpey's fibers are absent, but the superficial and deep zone present a well distinguished parallel fibered bone tissue.

These features would exclude this taxon from the pampatheres but isn't enough to correctly differentiate between dasypodid and glyptodonts. On the other hand, it is also expected for the osteoderms of Dasypodidae to present a comparatively more compact central region with only poorly developed cancellous bone (Vickaryous & Hall, 2006), very similar to the pattern identified for *P. brasiliense*. Combining previously analysis with ours and the character osteoderms described by Oliveira et al. (2013), we tentatively regard *P. brasiliense* as Dasypodidadae. However, further inferences can't be precisely made since the lack of remodeling could be due to an early ontogenetic stage of the specimens used, therefore more samples should be analyzed.

Osteoderm growth

Metaplastic ossification is largely described for cingulates osteoderm's (Hill, 2006; Vickaryous & Hall, 2006; Wolf *et al.*, 2012). However, Vickaryous & Hall (2006) and Vickaryous & Sire (2009) have discussed the possibility of multiple modes of ossification throughout the developmental phases. Since the identification of metaplastic bone in many studies does not consider developmental information, the possibility of multiple modes of ossification is very plausible (Vickaryous & Hall, 2006; Vickaryous & Sire, 2009; Wolf *et al.*, 2012), other process such as intramembranous ossification and periosteal growth may contribute to osteoderm growth (Vickaryous & Hall, 2006; Wolf, 2007).

Features of metaplastic bone was identified by Hill (2006) in his samples, he observed a generally low vascularity, a meshwork of large, intersecting fiber bundles of relatively uniform diameter, osteocyte lacunae aligned with fiber bundles and lacking well-developed canaliculi. This is a very general description and could be interpreted as a woven bone matrix with straps of parallel-fibered bone tissue, but indeed these features are present separately in some level in all of ours samples.

In the material studied here it is observed a predominance of dynamic osteogenesis (DO) derived bone tissues, characterized mostly by highly organized fiber bundles and elongate osteocyte lacunae. The predominance of DO-derived bone tissues, especially in the glyptodonts osteoderms, could be another indicative of metaplastic ossification at least in a postembryonic phase, since these types of bone are exclusively formed in pre-existing surfaces (Prondvai *et al.*, 2014). The osteoderms from *P. brasiliense* and pampatheres also shows presence of static osteogenesis (SO) derived bone tissues and a complex of both types (SO-DO), which could be due the ontogenetic phase of the osteoderm or have another biological significance. SO-derived bone is commonly, but not exclusive, found in young growing bone tissues (Prondvai *et al.*, 2014; Stein & Prondvai, 2014). Sharpey’s fibers is also a feature considerable indicative of metaplastic bone (Wolf *et al.*, 2012) and they were also seen in our samples.

All specimens presented parallel-fibered bone tissue at the most superficial layer of the osteoderm, which is also an indicative of periosteal bone growth in later ontogenetic stages.

Biomechanical adaptations

Although cingulate osteoderm lacks specific tissue-level adaptation, like the plywood arrangements of mineralized collagen fibers in the carapace of soft-shelled turtles (Scheyer *et al.*, 2007) or a more compact central region found in the osteoderms of ankylosaurids, several features acting together can be interpreted as adaptations to compensate stresses and offer strong resistance (Wolf *et al.*, 2012). We recognize the importance of these features: (1) the thickness of cancellous bone in the middle zone; (2) the presence of both SO and DO-derived bone tissues; (3) the presence of decussating marginal Sharpey's fibers and (4) the level of organization of the mineralized collagen fibers.

The increase of thickness of cancellous bone in glyptodonts osteoderms makes a lot of sense since these bones elements had to increase size as these species got bigger but also had to reduce weight to be bearable without being weakened. This pattern has been interpreted as strengthening adaptations in the context of the protective function of these bones (Amson & Nyakatura, 2017; Wolf *et al.*, 2012). Pampatheres and dasypodids have a comparatively smaller and thinner osteoderm and body size, presenting no need for a weight-reducing adaptation and therefore a much more compact middle zone (specially the dasypodids osteoderms). du Plessis *et al.* (2018) analyzed osteoderms from *Glyptotherium arizonae* using micro-CT, to simulate mechanical testing in reverse-engineered models. Their results largely agree with ours, showing that the combination of compact layers and a thick porous core contribute to the strength of the structure. We corroborate with the idea that glyptodonts osteoderms might be indeed biomechanically optimized structures.

Wolf *et al.* (2012) comment that the unusual decussating arrangement of the marginal Sharpey's fibers may have stabilized the sutural zones of the carapace, thus strengthening the armor at the weak position of the osteodermal joint. This feature is so far exclusively to pampatheres.

The presence of SO and DO-derived bone tissue and the distribution of organized mineralized collagen fibers are intrinsically connected. Static osteogenesis results in a woven bone tissue with disorganized collagenous fibers bundles and dynamic osteogenesis produces highly organized bone tissues, like all types of parallel-fibered bone tissues (Marotti, 2010; Prondvai *et al.*, 2014). Woven bone is more mineralized (Marotti, 2010) and could presumably compensated for stresses from more than one spatial directions and therefore provided some stability (Wolf *et al.*, 2012). On the other hand, DO-derived bone tissue has improved mechanical properties even if less mineralized, since the collagen fibers are much highly organized, which has been proved to be much more important in determining the mechanical properties of bone (Marotti, 2010; Martin *et al.*, 2015; Martin & Ishida, 1989).

The biological significance of static osteogenesis lies in its capacity to quickly form new bone tissue thereby ensuring fast growth. SO-derived accounts for the rapid volume expansion, whereas DO-derived guarantees mechanical stability of the growing skeleton (Marotti, 2010; Stein & Prondvai, 2014). In many vertebrates, the most important evolutionary innovation that allows fast growth rates is most likely the development of a SO-DO complex in their limb bone tissue (de Margerie *et al.*, 2002; Stein & Prondvai, 2014) and this could also be true for cingulate osteoderms.

Wolf *et al.* (2012) point out that some histological features in the imbricate osteoderm of *Pampatherium humboldtii* and *Holmesina paulacoutoi* would indicate a reinforcing function, like the presence of collagen fiber bundles in the superficial zone of the posterior region crossing each other at acute to almost right angles and the regular surface-parallel alignment of the fibers in the more superficial parts of the superficial zone of the posterior region. In addition to these structures, we also identified for the imbricate osteoderm of *P. humboldtii* the presence of a non-lamellate parallel-fibered bone tissue in the superficial zone of the posterior region. This kind of bone tissue is more mineralized and has a more complex meshwork of collagen fibers bundles, which might be considerate as an adaptation to particularly strong forces acting lengthwise the long (anteroposterior) axis of the osteoderm (Mitchell & van Heteren, 2016).

CONCLUSIONS

For osteoderm histology pattern help elucidate some of the systematic problems of Cingulata, a nomenclature standardization must be made. Moreover, ontogenetic, taphonomic and pathological features must be carefully considered so that systematic inferences can be properly made. For now, our analysis shows that *Pachyarmatherium brasiliense* osteoderms histology shows close resemblance to what is expected for Dasypodidae, indicating some affinity with this group.

Considering that no sophisticated biomechanical adaptations have been described for glyptodonts osteoderms so far, we understand that a set of features acting together can be interpreted to compensate stresses and offer strong resistance. The increase of thickness of cancellous bone in the middle zone, the presence of both SO and DO-derived bone tissues and the level of organization of the mineralized collagen fibers are important characters for osteoderm biomechanics. In addition, we also identified for the imbricate osteoderm of *P. humboldtii* the presence of a non-lamellate parallel-fibered bone tissue in the superficial zone of the posterior region, which can be considerate as a reinforcing function.

Our analysis also identified signs of both metaplastic ossification and periosteal growth in all samples. This corroborates with the hypothesis of multiple growth processes acting throughout the ontogenetic stages, with metaplastic ossification and periosteal growth acting specially in postembryonic life.

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4 CONCLUSÃO

Os osteodermos de cingulatas demostram uma grande diversidade histológica, tendo diversas espécies seu padrão microestrutural já descrito. Com este trabalho, todas as espécies de gliptodontes registradas para a Região Intertropical Brasileira tiveram seus osteodermos analisados do ponto de vista da paleohistologia, sendo a osteohistologia do osteodermo e do úmero de *Hoplophorus euphractus* descrito pela primeira vez aqui.

É importante salientar que para uma análise comparativa entre esses diversos trabalhos seja propriamente realizada, algumas observações devem ser feitas. A variedade da nomenclatura utilizada, o plano de seção feito no material, a utilização de espécimes isolados sem definição precisa da região da carapaça, a utilização de espécimes de diversos estágios ontogenéticos e a presença de patologias são fatores que devem ser cuidadosamente considerados durante a análise paleohistológica e que até o momento não tem sido amplamente discutida. Essas questões tornam difícil inferir padrões evolutivos através da comparação dos dados na literatura, dificultando principalmente questões relacionadas a sistemática do grupo.

Por enquanto, é possível inferir que a histologia dos osteodermos do *Pachyarmatherium brasiliense* mostra grande semelhança com o esperado para Dasypodidae, corroborando com a hipótese de uma possível afinidade com esse grupo.

No geral, os espécimes de gliptodontes apresentam um padrão compatível com o descrito na literatura. Características como uma região trabecular mais espessa e bem desenvolvida, osso mais remodelado, predominância de osteogênese dinâmica, são marcantes no osteodermos dos gliptodontes e indicam uma posição mais derivada em relação aos dasipodídeos. Todos os espécimes estudados apresentam características de um tecido ósseo maduro, exceto por DGEO-CTG-UFPE 8813 que aparenta pertencer a um indivíduo jovem. Em todas as amostras também foram identificados sinais de ossificação metaplástica e crescimento periosteal. Isso corrobora com a hipótese de diversos processos de crescimento atuando ao longo dos estágios ontogenéticos, com ossificação metaplásica e crescimento perióseo agindo especialmente na vida pós-embrionária.

A seção delgada do úmero de *Hoplophorus euphractus* apresenta um osso principalmente haversiano cercado por um tecido ósseo paralelo-fibroso lamelar e seguido por um alinhamento predominante transversal de um tecido paralelo-fibroso não-lamelar. A presença de um alinhamento particularmente transversal das fibras de colágeno indica que essas áreas do osso estariam sob carga compressiva. As características histológicas e morfológicas do osteodermo e do úmero de *H. euphractus* podem ser interpretadas como um estágio ontogenético maduro de um indivíduo adulto.

Em relação às adaptações biomecânicas, foi identificado algumas estruturas que agindo em conjunto compensaria o estresse ao qual o osso estaria submetido, são elas: o aumento da espessura do osso trabecular na zona do meio; a presença de osteogênese estática e dinâmica; o alto grau de organização das fibras de colágeno e a presença de fibras de Sharpey’s que se intercalam (exclusivo dos pampatérios). A presença dessas estruturas indica que os osteodermos são de fato estruturas biomecanicamente otimizadas.

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APÊNDICE A – Lista dos espécimes de osteodermos utilizados no trabalho e suas respectivas localidades.

Família	Taxon	Número de Tombo	Localidade
Pampatheriidae	***Pampatherium humboldti***	DGEO-CTG-UFPE 8476 A; B; C; D; E	Jacobina - Bahia
	Holmesina paulacoutoi	DGEO-CTG-UFPE 5964	Brejo da Madre de Deus - Pernambuco
		DGEO-CTG-UFPE 7029	Bom Conselho - Pernambuco
		DGEO-CTG-UFPE 6552	Afrânio - Pernambuco
		DGEO-CTG-UFPE 8807	Anagé - Bahia
?Dasypodidae	***Pachyarmatherium brasiliense***	DGEO-CTG-UFPE 7768 A; B; C	Brejo da Madre de Deus - Pernambuco
		DGEO-CTG-UFPE 8814	Poço Redondo - Sergipe
Glyptodontidae	***Glyptodon* sp.**	DGEO-CTG-UFPE 8817; 8818; 8819; 8820; 8821	Santa Vitória do Palmar – Rio Grande do Sul
	***Glyptotherium* sp.**	DGEO-CTG-UFPE 5938; 6992	Brejo da Madre de Deus - Pernambuco
		DGEO-CTG-UFPE 6507	Afrânio - Pernambuco
		DGEO-CTG-UFPE 7754	Congo - Paraíba
		DGEO-CTG-UFPE 8809	Toca da Raposa, Simão Dias - Sergipe
	Doedicurus clavicaudatus	DGEO-CTG-UFPE 8815; 8816	Santa Vitória do Palmar – Rio Grande do Sul
	Hoplophorus euphractus	DGEO-CTG-UFPE 6523; 6543	Afrânio - Pernambuco

ANEXO A – Carta de aceite da Revista Estudos Geológicos

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Recife, 27 de março de 2019

Prezados Yumi e Co-autroes

É com satisfação que informamos que o artigo intitulado:
A HISTOLOGIA COMO UMA FERRAMENTA NO ESTUDO DOS FÓSSEIS: CONSIDERAÇÕES SOBRE A PALEOHISTOLOGIA EM MAMÍFEROS COM CARAPAÇA ÓSSEA".de autoria de Yumi Asakura e Edison Vicente Oliveira; foi aceito para publicação no Estudos Geológicos vol. 29(1) 2019. Informamos que o artigo estará disponível para em pdf no site da revista https://periodicos.ufpe.br/revistas/estudosgeologicos) a partir do final de Janeiro de 2019.

Atenciosamente

Gorki Mariano
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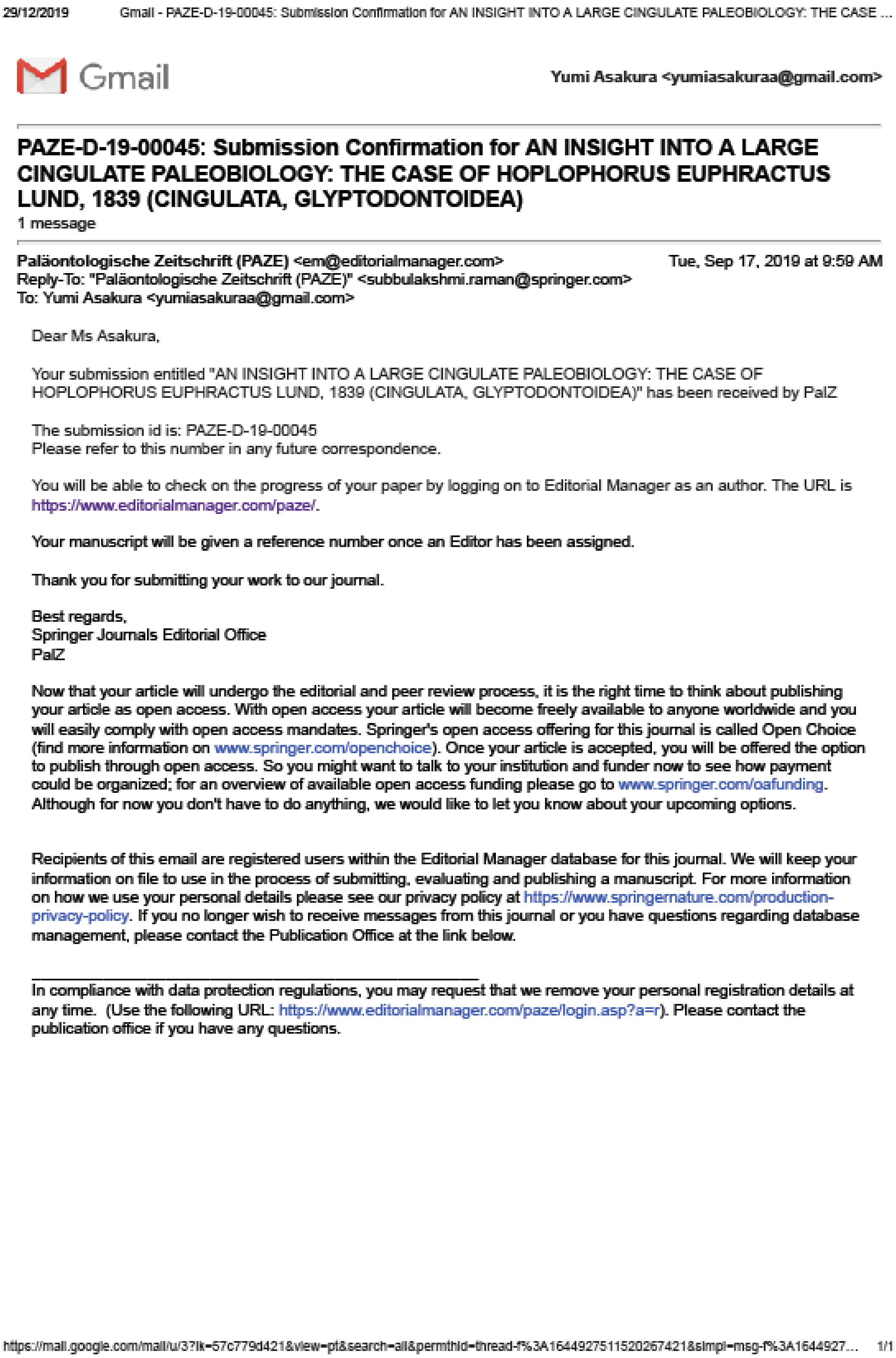
ANEXO B – Comprovante de submissão à revista Paläontologische Zeitschriff



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Yumi Asakura <yumiasakuraa@gmail.com>

PAZE-D-19-00045: Submission Confirmation for AN INSIGHT INTO A LARGE CINGULATE PALEOBIOLOGY: THE CASE OF HOPLOPHORUS EUPHRACTUS LUND, 1839 (CINGULATA, GLYPTODONTOIDEA)

1 message

Paläontologische Zeitschrift (PAZE) <em@editorialmanager.com> Tue, Sep 17, 2019 at 9:59 AM
Reply-To: "Paläontologische Zeitschrift (PAZE)" <subbulakshmi.raman@springer.com>
To: Yumi Asakura <yumiasakuraa@gmail.com>

Dear Ms Asakura,

Your submission entitled "AN INSIGHT INTO A LARGE CINGULATE PALEOBIOLOGY: THE CASE OF HOPLOPHORUS EUPHRACTUS LUND, 1839 (CINGULATA, GLYPTODONTOIDEA)" has been received by PalZ

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