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Comparative paleohistology in osteoderms of Pleistocene *Panochthus* sp. Burmeister, 1886 and *Neuryurus* sp. Ameghino, 1889 (Xenarthra, Glyptodontidae)

Comparaison paléohistologique entre les ostéodermes du Pléistocène Panochthus *sp. Burmeister, 1886 et* Neuryurus *sp. Ameghino, 1889 (Xenarthra, Glyptodontidae). Handled by Lars van den Hoek Ostende*

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ABSTRACT

Xenarthran osteoderms are integumentary bones with high fossilization potential presenting a high degree of morphological and histological diversity. Here, new data on the osteoderms histology of two glyptodonts, *Panochthus* and *Neuryurus* are presented. The poor spatial organization of the mineralized fibers and a large trabecular area in the middle zone identified in *Neuryurus* indicate a different bone pattern than the one found in *Panochthus*, which is mainly characterized by a middle zone with less spongiosa. Through the Bone Profiler program, the degree of compactness of the specimens was obtained, with about 70% for *Neuryurus* sp. and approximately 90% for *Panochthus* sp., showing the difference in bone pattern. These values confirm the visible difference in the histological patterns of these taxa, especially in the middle zone. This work demonstrates the microstructural variation studied in osteoderms and shows the importance of paleohistology as a starting point for a better understanding of extinct taxa.

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RÉSUMÉ

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Les ostéodermes de xénarthres sont des os tégumentaires avec un haut potentiel de fossilisation, présentant un haut degré de diversité morphologique et histologique. Dans cet article sont présentées de nouvelles données sur l'histologie des ostéodermes à partir de deux glyptodontes, *Panochthus* et *Neuryurus*. L'organisation spatiale médiocre des fibres minéralisées et un grand espace trabéculaire dans la zone médiane identifiée dans le *Neuryurus* indiquent une structure d'os différente de celle de *Panochthus*, qui se caractérise principalement par une zone médiane moins spongieuse. Grâce au programme Bone Profiler, on a obtenu le degré de compacité des spécimens, avec environs 70 % pour *Neuryurus* sp. et approximativement 90 % pour *Panochthus* sp., montrant la différence dans la structure de l'os. Ces valeurs confirment la différence visible dans les structures histologiques de ces taxons, en particulier dans la zone médiane. Ce travail démontre la variation de microstructure étudiée dans les ostéodermes, et montre l'importance de la paléohistologie comme point de départ pour une meilleure compréhension des taxons éteints.

1. 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 (Carrol, 1988; Hoffstetter, 1982; Patterson and Pascual, 1968; Paula Couto, 1979; Simpson, 1948). The group includes armadillos, pampatheres and glyptodonts, sloths and anteaters (Fig. 1), divided into two orders: Pilosa Flower, 1882 (sloths and anteaters) and Cingulata Illiger, 1811 (armadillos, pampatheres, and glyptodonts) (McKenna and Bell, 1997; Reis et al., 2011).

The Order Cingulata is a taxonomically distinct and complex group, traditionally divided into two superfamilies: Dasypodoidea (Dasypodidae) and Glyptodontoidea (Pampatheriidae and Glyptodontidae) (McKenna and Bell, 1997). The glyptodonts form a group of cingulates with an extensive stratigraphic distribution (Zurita et al., 2009) ranging from the late Eocene (McKenna and Bell, 1997) to the early Holocene (Cione et al., 2003), reaching a diversity of over 65 genera and 220 species (McKenna and Bell, 1997).

Among the enormous diversity of glyptodonts, we have chosen two problematic Pleistocene taxa: *Neuryurus* Ameghino, 1889 and *Panochthus* Burmeister, 1866. The genus *Neuryurus* is a very problematic taxon since its fossil record is very scarce and because it is difficult to identify due to their poor morphological characterization (Zurita et al., 2009). Therefore, it is commonly confused with *Panochthus* in northeastern Brazil. The only well-characterized species is *Neuryurus rudis* (Gervais, 1878), limited to the Ensenadan Age/Stage (early-middle Pleistocene) of the Pampean region in Argentina (Zurita and Ferrero, 2009; Zurita et al., 2009). Zamorano et al. (2014) recognize six valid species of *Panochthus*, with only two species described from the Brazilian Northeast (Porpino et al., 2014): *P. jaguaribensis* Moreira, 1965 and *P. greslebini* Castellanos, 1942.

Since osteoderms have been the most frequent and abundant fossil elements in the record of Cingulata (Carlini and Zurita, 2010), the arrangement and morphology of their osteoderms have also been used as a source of taxonomic information (Krmpotic et al., 2009; (Vickaryous and Hall, 2006). Studies such as Hill (2006); Wolf (2007); Chávez-Aponte et al. (2008); Krmpotic et al. (2009); Wolf et al. (2011) and Da Costa Pereira et al. (2014) show that the characteristics of osteoderm anatomical microstructure are also relevant to the study of evolutionary relationships and could provide diagnostic characters to differentiate species.

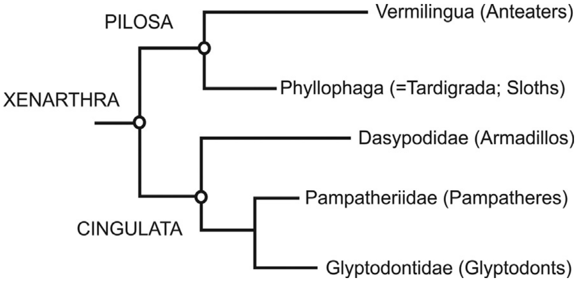

Fig. 1. Cladogram showing hypothesized phylogenetic relationships among major groups of Xenarthra (Modified from Hill, 2006).

Fig. 1. Cladogramme montrant les relations phylogénétiques hypothétiques entre les principaux groupes de xénarthres. (Modifié d'après Hill, 2006.)

Nowadays, there is a significant increase in microanatomical and ultrastructural research applied to fossil groups (Chinsamy et al., 2013; Nakajima et al., 2014; de Ricqlès, 2006, 2011; Straehl et al., 2013; Vickaryous and Sire, 2009; Woodward et al., 2014). Methodological advances, such as the Bone Profiler program (Girondot and Laurin, 2003), have supported the paleohistology research also in the quantitative aspect. The Bone Profiler program provides a possibility to standardize the method used to analyze bone segments and can model a bone compactness profile to test biological hypotheses.

Paleohistological studies of the Cingulata osteoderms have appeared regularly in the last decade (Chávez-Aponte et al., 2008; Da Costa Pereira et al., 2014; Hill, 2006; Wolf, 2007; Wolf et al., 2011); however, some taxa, such as *Neuryurus*, remain unstudied demonstrating the need for more paleohistological data of armored mammals. Here, we use paleohistological techniques, including

Table 1
List of specimens and their respective localities.
Tableau 1
Liste des spécimens et leurs localisations respectives.

Specimens examined		Localities
Panochthus sp.	SP/MHN/UFAL 1061-V	Paraíba
	SP/MHN/UFAL 1062-V	Lagoa de Inhapi, Inhapi, Alagoas
	DGEO-UFPE 5828	Poço das Trincheiras, Alagoas
	DGEO-UFPE 5830	
	DGEO-UFPE 6921	Santa Cruz do Capibaribe, Pernambuco
	DGEO-UFPE 6528/A	Povoado Caboclo, Afrânio, Pernambuco
	DGEO-UFPE 6528/B	
Neuryurus sp.	DGEO-UFPE 5939	Fazenda Nova, Brejo da Madre de Deus, Pernambuco
	DGEO-UFPE 5919	
	MCPU-PV 153	Arroio Garupá, Quaraí, Rio Grande do Sul
	MCPU-PV 154	

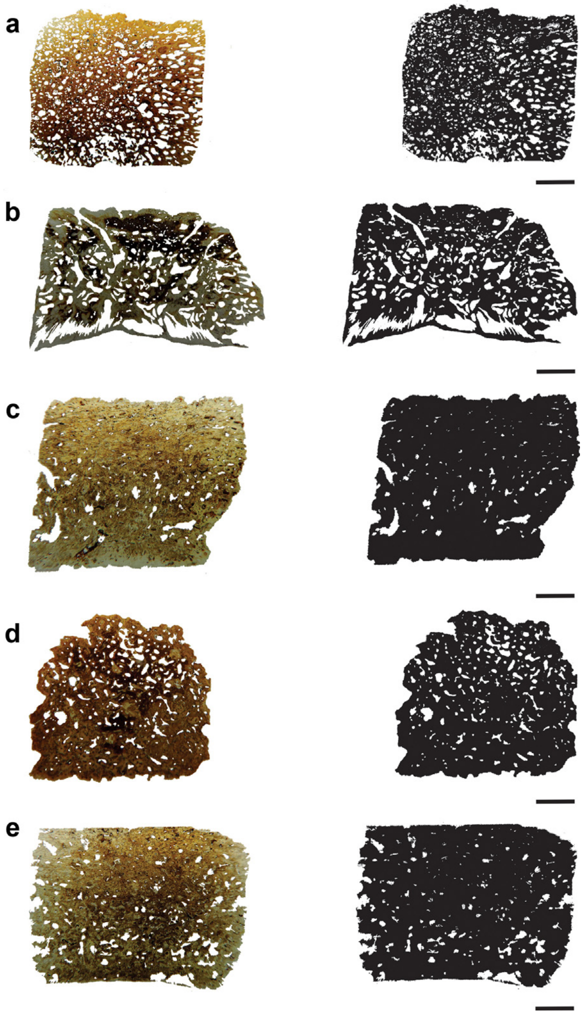

Fig. 2. Selection of binary images produced for the compactness analysis. *Neuryurus* sp. a: MCPU-PV 153; b: DGEO-UFPE 5919; *Panochthus* sp.; c: DGEO-UFPE 5830; d: DGEO-UFPE 6528/A; e: DGEO-UFPE 6921. Scale bars: 5 mm.

Fig. 2. Sélection d'images binaires produites pour l'analyse de compacité. *Neuryurus* sp. a : MCPU-PV 153 ; b : DGEO-UFPE 5919 ; *Panochthus* sp. ; c : DGEO-UFPE 5830 ; d : DGEO-UFPE 6528/A ; e : DGEO-UFPE 6921. Barres d'échelle : 5 mm.

the application of the Bone Profiler program, to analyze osteoderm histology of *Panochthus* and *Neuryurus* in order to differentiate the two genera.

2. Material and methods

The studied specimens belong to the *fossil collection from the Departamento de Geologia, Universidade Federal de Pernambuco* (DGEO-UFPE), to the *paleovertebratres collection from Setor de Paleontologia, Museu de História Natural, Universidade Federal de Alagoas* (SP-MHN-UFAL) and to the *paleovertebrate collection from Museu de Ciências, from PUCRS* (MCPU-PV) (Table 1). The material comprises seven osteoderms of *Panochthus* and four of *Neuryurus*; these represent isolated osteoderms found in association with other osteoderms and, in some cases, postcrania elements.

The osteoderms were impregnated with epoxy resin and sectioned using a diamond disc on a metallographic saw. The samples were fixed with ultraviolet resin on glass slides and ground on a lap wheel until the microstructure became evident. The slides were analyzed in a Zeiss Primo Star and Zeiss Axion Vision Imager A2 optical microscope with an attached camera, using Axion Vision Release 4.8 software for photomicrographs.

In both taxa, *Neuryurus* and *Panochthus*, the compactness profile was analyzed quantitatively through the Bone Profiler program version 4.5.8 (Girondot and Laurin, 2003). To use the program, black and white schematic drawings (Fig. 2) showing bone in black and vascular spaces (i.e. medullary cavity, vascular canals, osteons and resorption areas) in white were produced from the actual thin-sections using Photoshop CC 2014 software (Adobe Systems Inc.). Compactness profiles were calculated mainly using the automatic bone center function of the program. However, in a few cases, it was necessary to manually adjust the center to maximize the scanned bone area.

Considering that osteoderms occur in the animal's integument and can also cover the lateral region of the carapace, it was decided to use the term "surface" for the environment face and "deep" for the interior face of the animal, following the proposal of Hill (2006). These terms correspond to "external" and "internal" used by Scheyer & Sánchez-Villagra (2007) and Wolf (2007) and to "outer" and "inner" used by Krmpotic et al. (2009). Terminology used here to describe specific bone microstructures largely follows that of Hill (2006). For aspects of the macroscopic morphology, the terms "external" and "internal" were used, as is usual in this type of description.

3. Results

Family GLYPTODONTIDAE Gray, 1869
Subfamily HOPLOPHORINAE Huxley, 1864

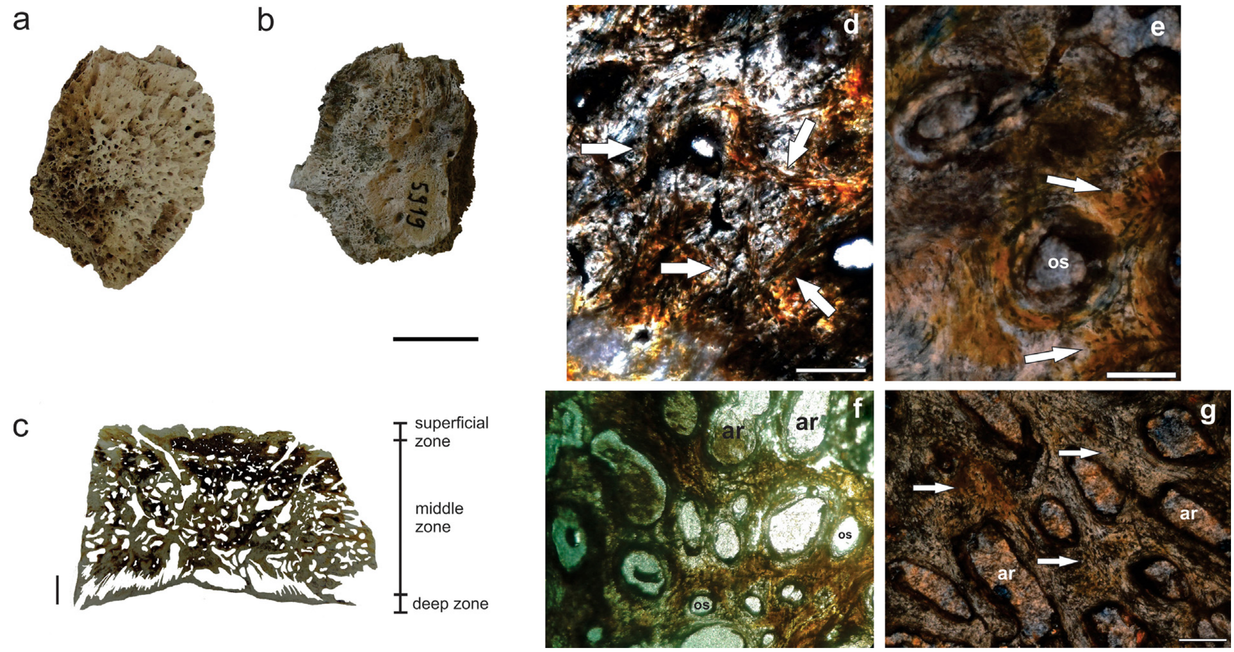

Fig. 3. *Neuryurus* sp., DGEO-UFPE 5919. Isolated carapacial osteoderm. (a) External view and (b) internal view, (c) internal structure of the osteoderm. Paleohistological sections: superficial layer of osteoderm (d,e), note arrows indicating mineralized fiber bundles (d) and osteocytes (e); the middle zone showing the trabecular area (f) and the deep zone with arrows indicating numerous osteocytes. Scale bars: (a,b) 20 mm; (c) 5 mm; (d,e,g) 200 μm; (f) 2,5 ×. Abbreviations: ra, resorption area; so, secondary osteon.

Fig. 3. *Neuryurus* sp., DGEO-UFPE 5919. Ostéodermes isolés de la carapace. (a) Vue externe et (b) vue interne, (c) structure interne des ostéodermes. Sections paléohistologiques : couche superficielle de l'ostéoderme (d,e), noter les flèches indiquant les faisceaux de fibres minéralisées (d) et les ostéocytes (e) ; la zone médiane montrant la zone trabéculaire (f) et la zone profonde avec des flèches indiquant de nombreux ostéocytes. Barres d'échelle : (a,b) 20 mm ; (c) 5 mm ; (d,e,g) 200 μm ; (f) 2,5 ×. Abréviations : ra : zone de résorption ; so : ostéone secondaire.

Genus *Neuryurus* Ameghino, 1889
Neuryurus sp.

3.1.1. Morphological description

The specimens DGEO-UFPE 5939 and DGEO-UFPE 5919 are fragments belonging to the dorsal region of the carapace, measuring between 15 and 19 mm thick, respectively. The articulation surface has a denticulate aspect. The external surface is ornate with conical tubercles uniformly punctured and a roughened appearance. It features many foramina arranged at an angle of approximately 45° to the osteoderm surface (Fig. 3a). The internal surface appears slightly concave, partially preserved and exhibits numerous foramina and a smooth roughness (Fig. 3b).

The specimens MCPU PV-153 and MCPU PV-154 are isolated fragments, both measuring 19 mm thick. The external surface has small, poorly defined figures at an oblique angle, showing a rough surface. The internal surface is slightly concave and presents some vascular foramina.

3.1.2. Histological description

3.1.2.1. Specimens DGEO-UFPE 5919 and DGEO-UFPE 5939

The histology of these buckler osteoderms conforms to the description of a diploe-like structure (Fig. 3c). The microstructure consists of a superficial and a deep zone of compact bone, enclosing a middle zone of trabecular bone. These small compact bone layers are characterized by having collagen fibers with no preferential orientation (Fig. 3d).

The more superficial zone is a darker area with an absence of microstructure details, which may be due to some mineral impregnation during the fossilization process. In this area, many collagen fibers with no preferential orientation, few resorption areas and numerous osteocytes lacunae (Fig. 3e) can be observed. Several primary osteons are identifiable in the superficial zone, as well as some secondary osteons.

The middle zone is extremely spongy, presenting a trabecular bone layer that occupies most of the osteoderm, with large and elongated resorption areas (Fig. 3f). In this area, the concentration of primary osteons and osteocytes lacunae decrease, while the quantity of secondary osteons increases.

In the deep zone (Fig. 3g), it is still possible to observe a very cancellous bone with large resorption areas, multiple secondary and some primary osteons, with a final band of approximately 1 mm of compact bone in the deepest zone. In all osteoderms, it is possible to see osteocytes lacunae.

3.1.2.2. Specimens MCPU-PV 153 and MCPU-PV 154

The osteoderms from the Arroio Garupá locality, State of Rio Grande do Sul, have a darker color which appears in the histological slides as a dark yellow, making it difficult

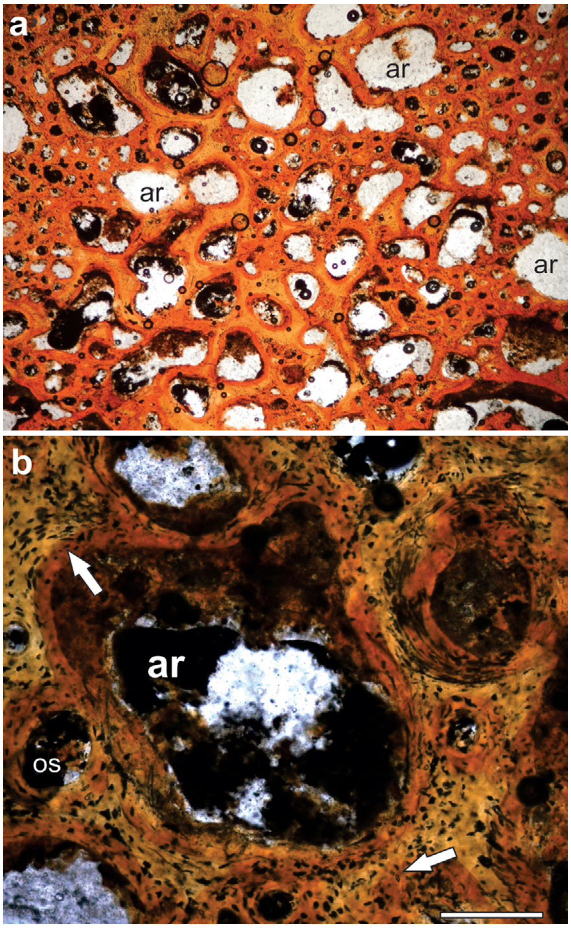

Fig. 4. *Neuryurus* sp., MCPU-PV 154. (a) Middle zone showing multiple small areas of resorption; (b) Middle zone showing the cavities filled by sediment. White arrows indicate the numerous osteocytes present throughout the osteoderm; air absorption area. Scale bar: 200 μm. Abbreviations: ra: resorption area; so: secondary osteon.

Fig. 4. *Neuryurus* sp., MCPU-PV 154. (a) Zone médiane montrant de multiples zones de résorption ; (b) zone médiane montrant les cavités remplies par les sédiments. Les flèches blanches indiquent les nombreux ostéocytes présents tout au long de l'ostéoderme ; zone d'absorption d'air. Barres d'échelle : 200 μm. Abréviations : ra : zone de résorption ; so : ostéone secondaire.

to view the microanatomical structures (Fig. 4). This color is probably due to some dark mineral impregnation during the fossilization process. In all osteoderms, it is possible to see some resorption areas and osteons channels filled with sediment (Fig. 4a, b).

Just as in the specimens from Fazenda Nova, State of Pernambuco, MCPU-PV 153 and MCPU-PV 154 are in cross section predominantly composed of trabecular bone (Fig. 4a) enclosed by thin superficial (2 mm long) and deep (1.5 mm long) layers of compact bone. The middle zone has multiple small areas of resorption. Numerous osteocytes lacunae are found throughout the osteoderm (Fig. 4b).

Family PANOCHTHIDAE Castellanos, 1927
Subfamily PANOCHTHINAE Castellanos, 1927
Genus *Panochthus* Burmeister, 1866
Panochthus sp.

3.1.3. Morphological description

All studied material corresponds to isolated osteoderms from the dorsal carapace, polygonal in shape (hexagonal and pentagonal) with thickness ranging between 19 and 25 mm. DGEO-UFPE 6921 has possible pits for hair follicles in the external surface and SP/MNH/UFAL 1061-V (Fig. 5a) has a rough internal surface with neurovascular foramen; both osteoderms exhibit a pentagonal shape. The largest fragment is SP/MNH/UFAL 1062-V, 25 mm thick, and shows fairly well preserved ornamentation on the external surface in the form of small polygonal figures, and scarce pits for hair follicles between these figures. The specimens DGEO-UFPE 6528/A and DGEO-UFPE 6528/B (Fig. 6a) are hexagonal and only small perforations can be observed in the external surface (Fig. 5b, c). The osteoderms have a homogeneous ornamentation in a reticular pattern without a distinguishable central figure and exhibit small polygonal figures ranging in size from 3 to 7 mm, separated by shallow reticular grooves, containing small pits (Fig. 5).

3.1.4. Histological description

All osteoderms studied here have a similar histological pattern (Fig. 6). The microstructure consists of superficial and deep zones of compact bone enclosing a middle zone of trabecular bone (Fig. 6b). The superficial zone displays an initial range of compact bone, with mineralized fiber bundles oriented parallel to the osteoderm surface (Fig. 6c).

DGEO-UFPE 5830 has the largest superficial zone with heavily compact bone, measuring 10 mm, whereas other specimens show a variation from 5 to 8 mm. In this layer, there is almost no primary bone due to remodeling caused by osteoclasts, with few primary osteons. The transition between the superficial and middle zone is quite compressed, similar to an intermediate type, which cannot be characterized as either compact or trabecular.

The middle zone is spongy, varying in the degree of porosity in some specimens. DGEO-UFPE 5830, for example, shows a little more than 1/4 of a visibly spongy area; DGEO-UFPE 6528b and SP-MHN-UFAL 1062 presents a spongy area of approximately 1/3 of osteoderm. Resorption areas begin to appear more often, with the presence of vascular channels enclosed by concentric lamellae also more frequent, indicating bone remodeling (Fig. 6d, e). In the transition between the middle zone and the deep zone, some collagen fibers with no preferential orientation can be observed.

Secondary osteons are more numerous than primary osteons in the deep zone (Fig. 6f). This zone is also characterized by the presence of some mineralized fibers parallelly oriented to the osteoderm surface and resorption areas. Few osteocytes lacunae are present in all zones; these are slightly flattened and arranged randomly (Fig. 6f). The last band of compact bone from the deep zone ranges from 4 to 5 mm in all specimens.

DGEO-UFPE 5828 displays some secondary osteons that are larger than in the other specimens. DGEO-UFPE-6921 has a greater concentration of secondary osteon distributed throughout all of the osteoderm.

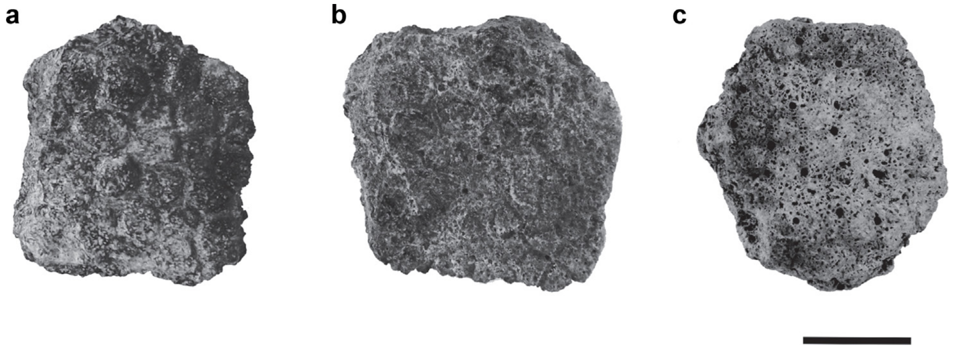

Fig. 5. Isolated carapacial osteoderms from *Panochthus* sp., external view. (a) SP/MHN/UFAL1061-V; (b) DGEO-UFPE 6921; (c) DGEO-UFPE 6528/A. Scale bar: 20 mm.

Fig. 5. Ostéodermes isolés de la carapace du *Panochthus* sp., vue externe. (a) SP/MHN/UFAL1061-V ; (b) DGEO-UFPE 6921 ; (c) DGEO-UFPE 6528/A. Barres d'échelle : 20 mm.

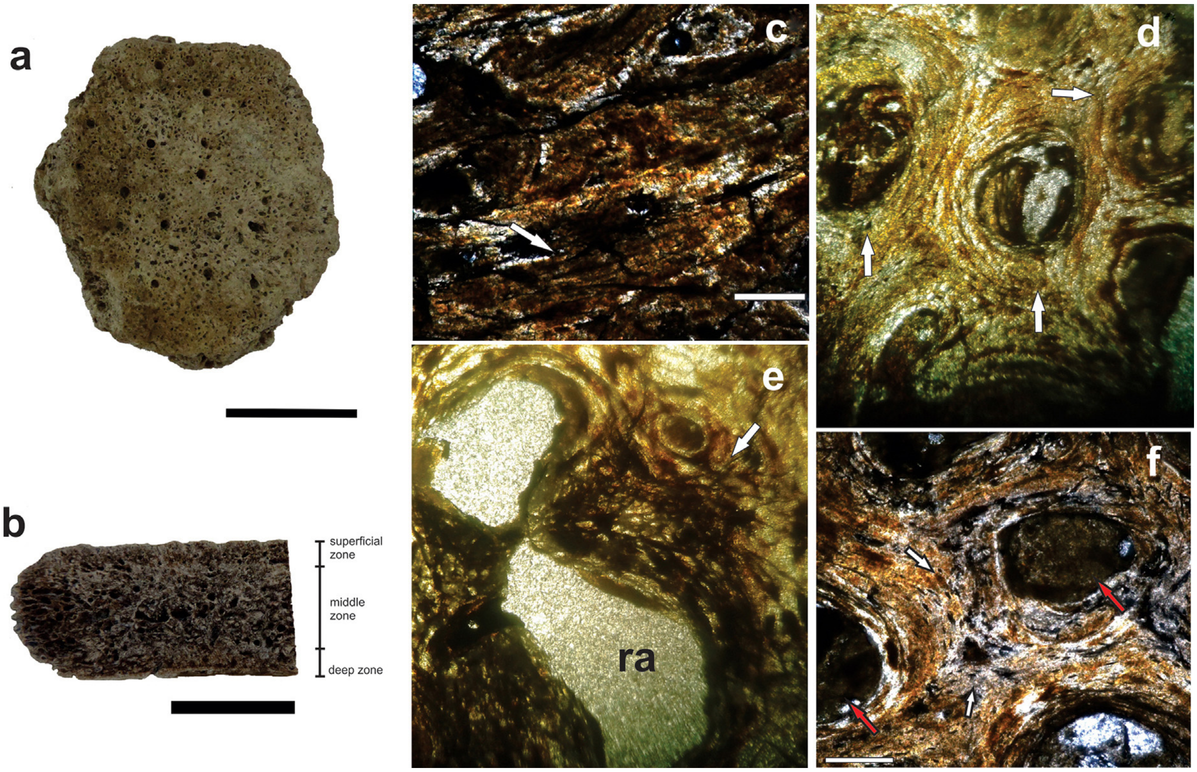

Fig. 6. *Panochthus* sp., DGEO-UFPE 6528/A. Isolated carapacial osteoderm. (a) external view and (b) internal structure of the osteoderm. Paleohistological sections: DGEO-UFPE 5828, superficial zone, showing a compressed bone range and arrow indicating mineralized fiber bundles (c); DGEO-UFPE 6528/A, middle zone, with white arrows indicating vascular channels enclosed by concentric lamellae (d) and mineralized fiber bundles (e); DGEO-UFPE 5828, deep zone with some oversized secondary osteons, red arrows indicate secondary osteons with the filled cavity; white arrows indicating osteocytes (f). Scale bars: (a,b) 20 mm; (c,f) 200 μm; (d,e) 2,5 ×. Abbreviations: ra: resorption area.

Fig. 6. *Panochthus* sp., DGEO-UFPE 6528/A. Ostéodermes isolés de la carapace : (a) vue externe et (b) structure interne des ostéodermes. Section paléohistologique : DGEO-UFPE 5828, zone superficielle, montrant un ensemble d'os comprimés et une flèche indiquant les faisceaux de fibres minéralisées (c) ; DGEO-UFPE 6528/A, zone médiane, avec des flèches blanches indiquant les canaux vasculaires entourés par des lamelles concentriques (d) et les faisceaux de fibres minéralisées (e) ; DGEO-UFPE 5828, zone profonde avec certaines ostéones secondaires surdimensionnées, les flèches rouges indiquent les ostéones secondaires avec la cavité remplie ; les flèches blanches indiquant des ostéocytes (f). Barres d'échelle : (a,b) 20 mm ; (c,f) 200 μm ; (d,e) 2,5 ×. Abréviations : ra : zone de résorption.

Table 2
Compactness values from *Neuryurus* sp. and *Panochthus* sp. specimens.
Tableau 2
Valeurs de compacité des spécimens *Neuryurus* sp. et *Panochthus* sp.

Specimens examined		Compactness values (%)
Neuryurus sp.	DGEO-UFPE 5939	62.2
	DGEO-UFPE 5919	76.3
	MCPU-PV 153	78.5
	MCPU-PV 154	70.2
Panochthus sp.	SP/MHN/UFAL 1061-V	87.4
	SP/MHN/UFAL 1062-V	88.2
	DGEO-UFPE 5828	95.8
	DGEO-UFPE 5830	96.6
	DGEO-UFPE 6921	93
	DGEO-UFPE 6528/A	91.3
	DGEO-UFPE 6528/B	86.7

In addition to the organized mineralized collagen fiber bundles in superficial and deep zones, it is also possible to observe in the osteoderm some collagen fibers randomly positioned. In some specimens, such as DGEO-UFPE 5828, some osteon channels and resorption areas are filled with sediment (Fig. 6f).

3.2. Bone compactness profiles using bone profiler

To independently verify our observations of tissue vascularization and bone compactness above, we obtained bone compactness profiles of the glyptodont osteoderms using Bone Profiler. At first glance, it seems inappropriate to use the program to analyze osteoderms, which are dorsolaterally compressed bones, exhibiting a strong relief and ornamentation, large resorption areas, and foramen of large diameter. However, the Bone Profiler program was able to provide suitable results to evaluate the overall degree of compactness of the samples, as evidenced in Scheyer and Sander (2009).

In some cases, such as the *Neuryurus* slices, minor changes have been made in the schematic drawings of the specimens. As suggested by Scheyer and Sander (2009), thin black lines have been added to close larger channels connecting the main chambers of the osteoderm with the external surface of the bone.

The osteoderms of *Neuryurus* sp. have an average of 71.8% compactness. The material from *Panochthus* sp. exhibits a high degree of compactness that varies between 86.7% and 96.6%, with an average of 91.93%. Table 2 lists all specimens with their respective compactness values.

4. Discussion

4.1. Comparisons

4.1.1. Neuryurus sp.: MCPU-PV 153/154 × DGEO-UFPE 5919/5939

Regarding *Neuryurus* sp., both specimens from Pernambuco (DGEO-UFPE 5919/5939) and Rio Grande do Sul (MCPU-PV 153/154) have a large trabecular bone area in the middle zone and compact bone bands in the superficial and deep zone measuring about 2 mm and 1 mm, respectively. The concentration of primary osteons gradually decreases over the osteoderm, with higher concentration in the superficial zone. These small compact bone ranges are characterized by having poorly organized mineralized fiber bundles and few osteons.

In the specimens from both localities, the middle zone is very spongy, showing generally a trabecular bone layer with resorption areas without an organized pattern and a greater concentration of secondary osteons. This trabecular area presents itself in a different configuration for the osteoderms of both localities. In the Pernambuco specimens, the resorption areas are large and elongated, while the specimens from Rio Grande do Sul present small and rounded resorption areas. Herein, these differences are interpreted as resulted of geographical variation, taking into account the great geographic distance of the two samples studied. Nevertheless, we consider that both specimens belong to the same generic taxon *Neuryurus*, due to the similarity of microanatomical structures (Figs. 3 and 4) and external surface ornamentation.

Analysis on the Bone Profiler program indicates that all samples have a similar degree of compactness. The material from Rio Grande do Sul, MCPU PV-153 and MCPU PV-154, displays a global compactness value of 78.5% and 70.2%, respectively, while the specimens from Pernambuco DEGEO-UFPE 5919 and 5939 DEGEO-UFPE, have a global compactness value of 76.3% and 62.2%, respectively. The pattern of microanatomical structures found in specimens from Pernambuco coincides with the pattern described for specimens from Rio Grande do Sul.

4.1.2. Neuryurus sp. vs. Panochthus sp.

The histologic pattern of *Panochthus* sp. is characterized by having a large range of compact bone in the superficial and deep zone measuring from 5 to 10 mm and 4 to 5 mm, respectively. These bands are much larger than those found in *Neuryurus* sp.

The spatial organization of the collagen fibers is also a differential factor between the two taxa. The poor spatial organization of the collagen fibers and the large trabecular area in the middle zone on *Neuryurus* sp. osteoderms show a different pattern than that found in *Panochthus* sp., which is characterized by a middle zone much less spongy, as indicated by quantitative analysis by the Bone Profiler program.

Quantitatively, a strong difference in the compactness values is observed between *Neuryurus* sp. and *Panochthus* sp. The compactness values from *Neuryurus* sp. specimens are around 70%, while in *Panochthus* sp. this is about 90%. These values confirm the visible differences in histological patterns of both taxa, where the respective middle zones have trabecular areas of different sizes. The histological patterns found for both taxa show that any similarities in the external morphology of osteoderms from *Neuryurus* sp. and *Panochthus* sp. is not reflected in their microstructural pattern, where significant differences were found.

4.2. Comparison with other glyptodonts

Several authors demonstrated the high degree of morphological and histological diversity in osteoderms of Xenarthra (Chávez-Aponte et al., 2008; Da Costa Pereira et al., 2014; Hill, 2006; Krmpotic et al., 2009, 2015; Wolf, 2007; Wolf et al., 2011). The histological structures found vary from compact tissue almost avascular in some sloth osteoderms to strongly remodeled tissues in osteoderms of large glyptodonts (Hill, 2006).

The main characteristics of osteoderms of glyptodonts include a generally “diplo-like” structure, consisting of well-developed trabecular bone interposed between compact bone layers (Hill, 2006; Wolf, 2007), also found in pampatheres (Wolf et al., 2011). Despite the high interspecific variability in osteoderms, several features are common to all glyptodonts (Hill, 2006). These features, which include a high level of mineralized fiber bundles, growth lines, poorly vascularized and dense layers, both superficial and deep, are also common to other vertebrate osteoderms (Scheyer and Sander, 2004). The patterns described here, both to *Neuryurus* sp. and for *Panochthus* sp., are consistent with the “diploe-like” structure as described for glyptodonts.

The histologic pattern for the genus *Panochthus* is still not well defined. Da Costa Pereira et al. (2014) use an osteoderm from *P.* cf. *greslebini* and one from *P. jaguaribensis* that may be a fragment from the cephalic shield. Wolf et al. (2011) describe *P. tuberculatus*, but the description is a combination of other glyptodont features. Therefore, it is not possible to define the unique characteristics of this species. The only well-described species is *P. frenzelianus*, of which the identification of the specimen is not ambiguous (Hill, 2006). These examples demonstrate the necessity of using taxonomically well-identified specimens, so that the pattern of each species can be defined and thus be used in systematic comparisons and analyses.

The description of Da Costa Pereira et al. (2014) for osteoderms of *Panochthus* cf. *greslebini* is consistent with that of the osteoderms described here, corroborating the general pattern observed for the genus. According to these authors, the osteoderm shows collagen fibers oriented parallel to surface in the superficial and deep zone with a smaller organization in the central region and presence of an intermediate zone between the superficial layer of compact bone and trabecular bone, which cannot be characterized as trabecular or compact. Both features can be observed in the osteoderms described herein.

Also according to Da Costa Pereira et al. (2014), *Panochtus jaguaribensis* has thinner trabeculae with larger resorption areas, more rounded and more evenly distributed, and a thicker deep layer of compact bone; in addition, the intermediate layer between the superficial zone and the middle zone is absent. However, the differences may not be related to different species, but could reflect they are not homologous parts.

Hill (2006) describes *Panochtus frenzelianus* as having a relatively compact microstructure with a moderate development of trabecular bone, similar to that found in *P.* cf. *greslebini*. The trabecular area of the two species is greatly remodeled as described for *Doedicurus giganteus*, of which the level of remodeling in trabecular region is the largest among glyptodonts (Hill, 2006). In both taxa, *P. frenzelianus* and *P.* cf. *greslebini*, there is an intermediate layer between the superficial zone of compact bone and the middle zone of trabecular bone, this layer being better developed in *P.* cf. *greslebini* (Da Costa Pereira et al., 2014).

The osteoderms from derived glyptodonts show a poorly defined deep zone. It is formed by moderate to well-vascularized primary compact bone, with several vessels and irregular arrangements of collagen fiber bundles (Wolf et al., 2011). *Propalaehoplophorus*, a basal Miocene glyptodont (Croft et al., 2007), has generally similar osteoderms to pampatheres in relation to its diploe-like structure, displaying similar proportions of the three histological layers (Wolf et al., 2011). This characteristic of proportionality between the three layers is not found in any of the osteoderms described here.

The histologic pattern described for *Neuryurus* is not compatible with the profiles already described for the many species of the genus *Panochthus*, indicating once again how histology can help in taxonomic identification, strengthening the differences in external ornamentation levels. Three main features differentiate the two genera:

- degree of compactness;
- organization of the collagen fibers;
- bone tissue layer classified as an intermediate type between compact and trabecular in the transition from superficial and middle zone.

The osteoderms from *Neuryurus* sp. exhibit a much lower degree of compactness than the ones from *Panochthus*: they do not have a defined organization of the collagen fibers, nor an intermediate layer. Among the glyptodonts with histological patterns described above, *Neuryurus* is the one with a larger trabecular tissue area in the middle zone; this being a remarkable difference for this genus.

The morphology of the Xenarthran osteoderms has long been recognized as taxonomically important, and several taxa were identified based on the characteristics of this morphology (Ameghino, 1889; Hoffstetter, 1958). The great variability in interspecific microstructural pattern of osteoderms reported by several authors (Hill, 2006; Wolf, 2007; Wolf et al., 2011) confirms the great potential of osteoderms in systematic studies of cingulates.

5. Conclusions

Regarding *Neuryurus* sp., the osteoderms have a large trabecular bone area in the middle zone enclosed by small compact bone layers in the superficial and deep zone that are characterized by poorly organized mineralized fiber bundles and few osteons. The middle zone is extremely trabecular with large elongated resorption areas and without an organized pattern; also in this area the concentration of secondary osteons is greater. The histological pattern found in specimens of *Neuryurus* sp. from the State of Pernambuco (northeastern Brazil) coincides with the pattern described for specimens of the State of Rio Grande do Sul (southern Brazil).

The poor spatial organization of the fibers and the trabecular area in the middle zone of osteoderms of *Neuryurus* sp. show a different pattern of that found in *Panochthus* sp. which is characterized by a less spongy middle zone, corroborated by quantitative analysis by the Bone Profiler program. The patterns found for the two genera show that the similarity in external morphology of osteoderms of *Neuryurus* sp. and *Panochthus* sp. is not reflected in their microstructural pattern, where significant differences were found.

The compactness profiles were interpreted as resulting from a good approximation of the degree of global compactness of the specimens. The compactness degree of *Neuryurus* sp. was around 70%, while for *Panochthus* sp. this value was around 90%. These values corroborate the visible difference in histological patterns for both genera, where its middle zone has trabecular areas of different sizes, reflecting on the degree of compactness of osteoderms. Use of the Bone Profiler program proved to be a promising tool in characterizes histological patterns.

Differentiation of *Panochthus* and *Neuryurus* by paleohistology adds a new tool to identify species with similar external morphological patterns. In addition, the Bone Profiler program proved to be a promising tool in helping to characterize the histological patterns. However, for a broader application of histologic features, it is important to use specimens with unambiguous identification as well as a standard histological classification, avoiding confusion when comparing species patterns.

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