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The oldest record of flea/armadillos interaction as example of bioerosion on osteoderms from the late Miocene of the Argentine Pampas

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ABSTRACT

We describe perforations recognized in mobile osteoderms of the extinct armadillos (Cingulata, Dasypodidae) *Chasicotatus ameghinoi* (Euphractinae, Eutatini) and *Vetelia perforata* (Euphractinae, Euphractini), recovered from the late Miocene of Argentine Pampas. We interpret that fleas corresponding to the genus *Tunga* (Siphonaptera) produced these marks. These parasites locate in the carapace of the host, perforating the osteoderms by mechanical and chemical processes as the neosome grows. This finding constitutes the oldest record of flea/armadillos interaction. It also reflects the antiquity of this important specialization and reinforces the proposal indicating an early co-evolution between these parasites and their hosts. These traces, produced in the bone tissue when the host was still alive, provide new information on the trophic relationships in past ecosystems.

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1. Introduction

Paleopathology is the study of past diseases affecting human and animals (Ruffer, 1913; Dutour, 2013). Ectoparasites are present in most living vertebrates, and usually produce lesions in their hosts. The structure and composition of the ectoparasite communities is derived from the co-evolution of hosts with ectoparasites in a specific environment (Hugot, 2006; Poulin, 2007; Gandon et al., 2008; Dittmar, 2010).

Dasypodidae (Xenarthra, Cingulata), a group characteristic of the Neotropical region, are the most diversified xenarthrans with 21 living species included in 8 genera (Fernicola et al., 2008; Loughry et al., 2015). Numerous parasitological analyzes were performed in living armadillos (Mauri and Navone, 1993; Ezquiaga, 2013; and references therein), but this discipline has been scarcely considered in the study of fossil cingulates (Hammond et al., 2014; Pirrone and Luna, 2015).

Bioerosion is defined as every form of biologic penetration into hard substrates. Pirrone et al. (2014: 195) defined bioerosion trace fossil in bones as "biogenic structure that cut or destroy hard osseous tissues as result of mechanical and/or chemical processes". The classification of trace fossils is mainly based on their morphology, which should represent an expression of animal behavior (Bromley, 1996). In most cases, the morphology of the traces can be evaluated; however, the type of behavior is only inferred (Pirrone et al., 2014). In this context, traces are a very useful tool to recognize evidences of parasitism in the fossil record (Dittmar, 2010).

The main goal of this paper is to describe trace fossils identified in osteoderms of the Dasypodidae *Chasicotatus ameghinoi* (Euphractinae, Eutatini) and *Vetelia perforata* (Euphractinae, Euphractini) from the late Miocene of Argentine Pampas. Taking into account the recorded characteristics and the comparisons with similar marks found in osteoderms of extant armadillos, we propose that these traces correspond to lesions produced by the activity of fleas. These findings constitute the oldest evidence of the interaction between these ectoparasites and armadillos and represent an example of bioerosion structures in bone tissues of living organisms.

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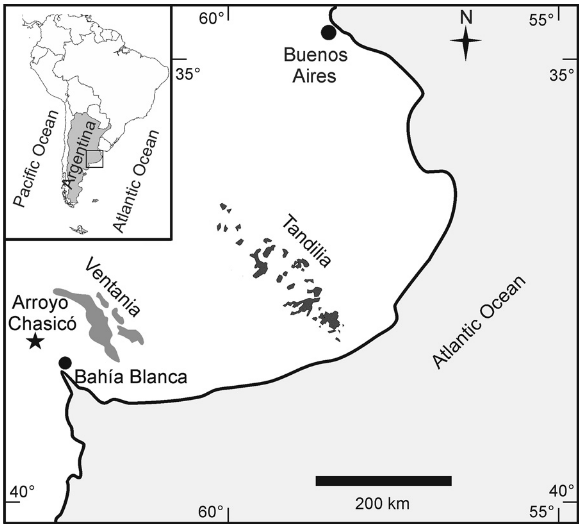

Fig. 1. Location map showing Arroyo Chasicó locality, Buenos Aires Province, Argentina.

2. Materials and methods

Dasypodidae osteoderms were recovered in the fossiliferous locality Arroyo Chasicó, southwestern Buenos Aires Province, Argentina (Fig. 1). The bearing levels correspond to the Arroyo Chasicó Formation, assigned to the late Miocene based on a radiometric dating that yielded an age of 9.23 ± 0.09 Ma (Zárate et al., 2007). The materials are housed in the Museo Municipal de Ciencias Naturales "Vicente Di Martino" (Monte Hermoso, Buenos Aires, Argentina) under the acronym MMH-CH, and Museo Municipal de Ciencias Naturales "Carlos Darwin" (Punta Alta, Buenos Aires, Argentina) under the acronym MD-CH.

Both MD-CH-06-391 and MMH-CH-88-6-92 are complete mobile osteoderms of *Chasicotatus ameghinoi*, while MMH-CH-82-4-6 is a broken mobile osteoderm of *Vetelia perforata*, all of them corresponding to the dorsal carapace. The three specimens were disarticulated and isolated, belonging to different individuals.

The characteristics of the traces, including shape, size, depth, location and texture of the internal surface, were analyzed in all specimens. Materials were observed with a Leica MS 5 binocular light microscope, and some of them were also photographed using a JEOL 35 CF SEM scanning electron microscope belonging to the Unidad de Administración Territorial del Centro Científico y Tecnológico CONICET Bahía Blanca (CCT-CONICET-BB). Fossil materials were compared with specimens of living armadillos housed in the mammal collection of the Facultad de Ciencias Exactas y Naturales, Universidad Nacional de La Pampa (Santa Rosa, La Pampa, Argentina).

3. Results

Each osteoderm presents a single perforation, with subcircular shape (Fig. 2a-f). Taking into account the classification of traces according to their morphology proposed by Pirrone et al. (2014), these perforations can be considered as isolated chambers. Size varies between the specimens; larger and smaller diameters of each perforation are respectively 1.25 mm and 1.1 mm in MD-CH-06-391, 1.15 mm and 1.05 mm in MMH-CH-88-6-92, and 3.55 mm and 3.35 mm in MMH-CH-82-4-6.

The perforations in MD-CH-06-391 (Fig. 2a,b) and MMH-CH-88-6-92 (Fig. 2c,d) are limited to the external zone, corresponding to the cortical bone, while in MMH-CH-82-4-6 (Fig. 2e,f) reaches to the internal zone, corresponding to the spongy bone. They are located in the dorsal surface of the osteoderm. In MD-CH-06-391 osteoderm, the perforation is situated in the middle region that separates the cranial portion from the caudal portion (Fig. 2a,b),

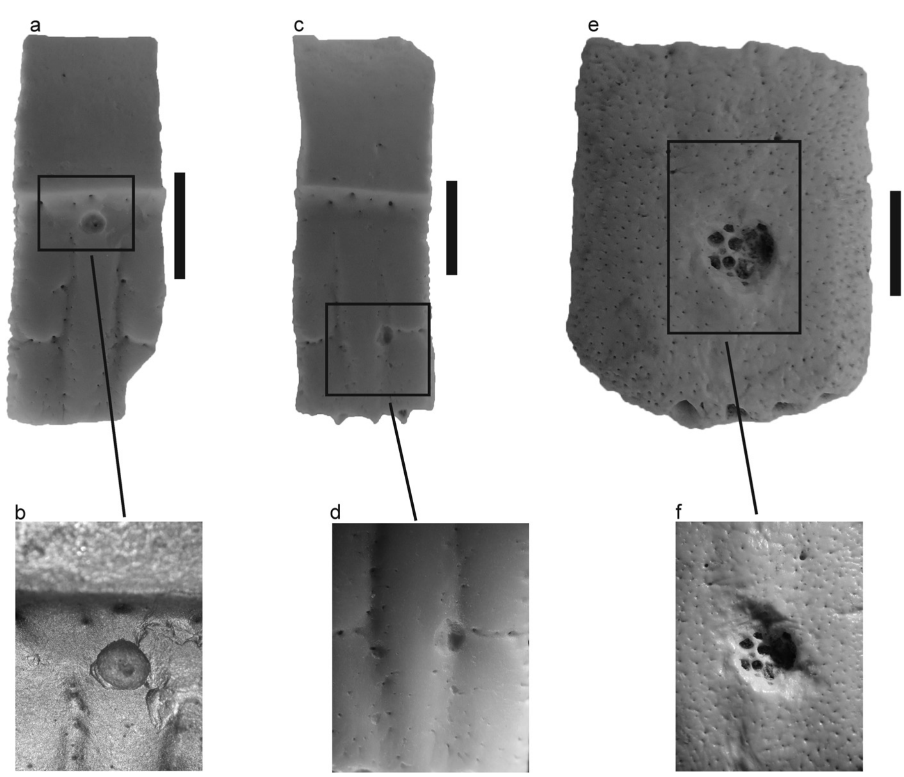

Fig. 2. Mobile osteoderms with perforations: (a) specimen MD-CH-06-391, (b) detail of the trace, (c) specimen MMH-CH-88-6-92, (d) detail of the trace, (e) specimen MMH-CH-82-4-6, (f) detail of the trace. Scale bar = 0.5 cm.

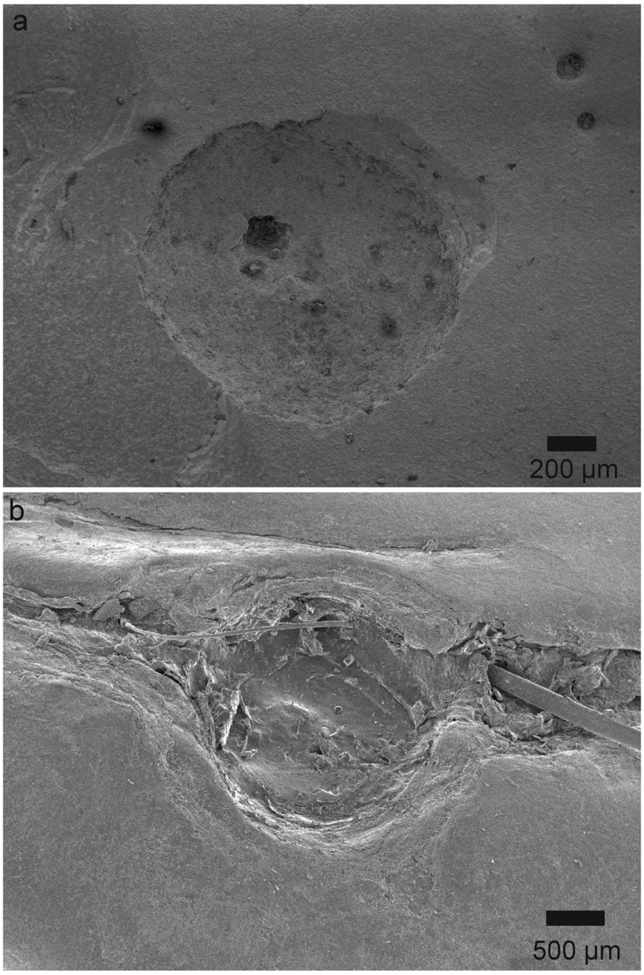

Fig. 3. SEM images of perforations: (a) specimen MD-CH-06-391, (b) living specimen of *Chaetophratus vellerosus*.

in MMH-CH-88-6-92 osteoderm, the perforation is situated in the caudal portion, particularly in one of the grooves that separate the central figure from the peripheral figures (Fig. 2c,d), and in MMH-CH-88-6-92 osteoderm, the perforation is situated in the caudal portion, specifically in the central figure (Fig. 2e,f).

The internal surface of the perforations presents irregular texture with corroded appearance. Moreover, in some sectors, stepped edges with evidences of torn bone tissue were recognized. The MD-CH-06-391 osteoderm shows a small subcircular hole, located in the central zone of the perforation (Fig. 3a).

4. Discussion

Armadillos are one of the best-represented fossil groups of mammals in the Cenozoic of South America. They present a flexible carapace, composed by numerous osteoderms (~800) covering their body (Wetzel et al., 2007; Superina et al., 2014). *Chasicotatus ameghinoi* and *Vetelia perforata* are two extinct taxa very abundant in the late Miocene of Argentina (Urrutia et al., 2008; Scillato-Yané et al., 2010).

Bioerosion traces in bone tissues are generally produced at the time of death of the animal (associated with predation) or after their death, when the bone is exposed without tissues that protect the outer surface (Pirrone et al., 2014). However, they are not common in bones of living organisms (e.g. Pirrone and Luna, 2015). Armadillos are mainly consumed by mammals (e.g. puma, Andean fox) and raptorial birds (e.g. crowned solitary eagle, black vulture), which usually leave evidences of their activity (e.g. punctures, scoring) in the skeletal elements (Zapata et al., 2005; Ballejo et al., 2012; Montalvo et al., 2007, 2016). The traces studied herein show clear differences with respect to the marks produced by predators and scavengers, which suggests that their origin was not related to the pressure generated by teeth or beaks.

These traces also display differences with respect to the pilliferous foramina and glandular foramina present in the specimens analyzed. Although one trace shows a small hole in the central zone, this characteristic is absent in the others traces, so we discard the possibility of foramina enlargement due to destructive fossil-diagenetic processes. In other osteoderms of dasypodids recovered from the same levels, it was noted that post-burial corrosion generates alterations with very different patterns (Tomassini and Montalvo, personal observation).

There are a great variety of arthropods that parasitize extant armadillos (e.g. fleas, ticks, mites), however *Tunga perforans* (Siphonaptera, Tungidae) is the only known species in which the individual bury themselves into the carapace of their host. The hosts recognized include *Zaedyus pichiy*, *Chaetophractus vellerosus* and *Chaetophractus villosus* (Ezquiaga, 2013; Ezquiaga et al., 2015; Montalvo et al., 2016). The trace fossils described herein show similar characteristics to the lesions produced by these fleas in the osteoderms of extant armadillos (Fig. 3a,b).

Ezquiaga et al. (2015) mentioned that adult gravid females of *T. perforans* penetrate the skin of their host, perforating the osteoderm. After mating and fertilization, they undergo hypertrophy, forming an enlarged structure known as neosome. By mechanical, and probably also chemical processes the neosome develops in the perforation. After oviposition, the neosome involutes with the death of the ectoparasite (Linardi and De Avelar, 2014).

Perforations assigned to *Tunga* were also mentioned in osteoderms belonging to *Zaedyus pichiy*, recovered from the late Holocene deposits of Argentine Patagonia (Hammond et al., 2014). These marks also show similarities with the traces studied in the present work.

The available evidence allows us to consider that the osteoderms of *Chasicotatus ameghinoi* and *Vetelia perforata*, recovered from the late Miocene of the Arroyo Chasicó Formation, were perforated by fleas, probably of the genus *Tunga*. Thus, these trace fossils correspond to isolated chambers used before the oviposition. Since *T. perforans* develops the neosome in the osteoderms of armadillos, it is possible to propose that Miocene fleas, with a similar behavior, were the producers of the traces studied. The size and depth of the traces suggest that in the specimens MD-CH-06-391 and MMH-CH-88-6-92 (smaller and shallower perforations) had already started the process of cicatrization of lesions, while in the specimen MMH-CH-82-4-6 (larger and deeper perforation) this process would have not occurred, so it is possible to infer that the neosome was still inside the osteoderm at the time of death of the host.

5. Conclusions

The specimens studied herein constitute the oldest record of fleas/armadillos interaction and reflects the antiquity of this great specialization (perforation of osteoderms). Based on molecular phylogenetic analyzes, Zhu et al. (2015) indicated that Tungidae likely evolved only shortly after the K-Pg boundary. Gibb et al. (2016), also based on molecular phylogenetic analyzes, considered that the origin of the armadillos corresponds to the middle Eocene (ca. 45 Ma). Taking into account these data, this new finding reinforces the previous proposal that suggests an early co-evolution process between these ectoparasites and armadillos (see Delsuc et al., 2004; Whiting et al., 2008; De Avelar, 2010; Linardi and De Avelar, 2014).

These trace fossils, produced in the bone tissue when the host was still alive, brings novel information on the trophic relationships in the Argentine Pampas during the Neogene. Additionally, this work increases the diversity of hosts for these ectoparasites.

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