

# Osteoderm morphology in recent and fossil euphractine xenarthrans

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## Abstract

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The presence of osteoderms within the integument, forming a carapace, is one of the most distinctive features of armadillos with the external morphology of these elements forming the basis of most systematic schemes. This is especially true for fossil taxa, where these elements are most frequent in the palaeontological record. A detailed study of osteoderms from the cephalic shield and different regions of the dorsal armour of *Chaetophractus villosus* (Euphractinae, Xenarthra) was made and compared to those of the extant genus *Dasypus* (Dasypodinae, Xenarthra), and the extinct genus †*Eutatus*. Three distinct histological zones were recognized: outer and inner zones are thin, formed by regular compact bone, the middle zone is thicker, with large cavities that contain mainly adipose tissue, hair follicles, and sweat and sebaceous glands. The internal structure of †*Eutatus* (also a member of Euphractinae) osteoderms is close to that of *C. villosus*, consistent with the notion that these taxa are phylogenetically closely related. In contrast, *Dasypus* shows marked differences. *Dasypus* shows hair follicles associated with both gland types (sweat and sebaceous) and connected to foramina on the external surface. Although not observed in adult *C. villosus*, it has been documented during embryonic development, only to atrophy later in ontogeny. Furthermore, the presence of red bone marrow is rare in *C. villosus*, but widespread in *Dasypus novemcinctus* osteoderms. These results suggest an early split of both subfamilies and support the hypothesis that the Euphractinae are more derived than the Dasypodinae.

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## Introduction

Armadillos (Cingulata, Dasypodidae) are the xenarthrans with the greatest temporal and geographical distribution. The oldest fossils date back to the early Tertiary [more than 50 million years ago (Ma)] of Brazil (Oliveira and Bergqvist 1998; Bergqvist *et al.* 2004), and their current distribution ranges approximately between 40°N and 40°S in the American continent. One of their most distinctive anatomical features is the presence of a protective dorsal cover (carapace) formed by osteoderms overlaid by epidermal horny scales. These osteoderms cover the head dorsally (cephalic shield)

and the trunk (dorsal shield), and encase the tail (caudal sheath), except in the genus *Cabassous* ('naked tailed armadillo', Wetzel 1985). In addition, osteoderms may also be present within the integument above the rostrum, in the ventral surface of the trunk and in the limbs. However, these elements do not contribute to continuous shields in these areas and may not be associated with horny scales.

Isolated osteoderms are the elements most frequently preserved in the fossil record of the Cingulata, and therefore their external morphology has traditionally been the basis for most systematic schemes of this group (e.g. Ameghino 1897, 1902a,b; Simpson 1948; Scillato-Yané 1982; Carlini and

Scillato-Yané 1996; Carlini *et al.* 2002a,b,c, 2004, 2005a,b; Krmptic *et al.* 2004, 2007a; Ciancio *et al.* 2005). The living representatives of the family are grouped in eight genera and 21 species (Wetzel 1985; Nowak 1999; Parera 2002). The extinct diversity is much higher, with nearly 40 extinct genera and 200 extinct species (Scillato-Yané 1980; Mones 1986; McKenna and Bell 1997).

According to McKenna and Bell (1997), the Dasypodidae are divided into three subfamilies: Dasypodinae, Euphractinae and Tolypeutinae. Dasypodinae contains the oldest representatives of the family, included in †Astegotheriini (Late Palaeocene–middle Miocene) (Bergqvist *et al.* 2004; Carlini *et al.* 1997, 2005b). This subfamily and the Euphractinae include most of the known species, both living and extinct. The Tolypeutinae are only recorded since the middle Miocene (*c.* 13 Ma) and did not attain significant diversity at any time (Carlini *et al.* 1997).

The species studied in the present work, *Chaetophractus villosus* (Desmarest 1804) Fitzinger 1871, belongs to Euphractinae (Euphractini), and occurs exclusively in the Neotropical region. The current distribution of *C. villosus* includes the Paraguayan Chaco, southern Bolivia, Argentina (extending latitudinally to southern Santa Cruz province) and southern Chile (from Bio Bio Region to Aisen Region) (Wetzel 1985; Redford and Eisenberg 1992); it has also been introduced in Isla Grande of Tierra del Fuego (Poljak *et al.* 2007).

Although the presence of osteoderms in xenarthrans is a unique feature among mammals, the first histological descriptions of osteoderms of *Chaetophractus villosus* were not made before 1931; at the time, the study was limited to pelvic buckler osteoderms and neglected those from the rest of the dorsal shield (Fernández 1931). Here we provide histological description and detailed comparative analysis of the osteoderms from the cephalic shield and the different body regions of the dorsal shield of *C. villosus*, based on serial histological sections. In addition, these descriptions are used as an empirical basis for the study and interpretation of the internal structure of osteoderms from fossil Euphractinae of the genus *Eutatus*, the last representatives of the extinct Eutatini, through application of palaeohistological techniques to serial sections of these materials.

We combined our data with previous work on *Dasypus novemcinctus* (Dasypodidae, Dasypodinae) (Cooper 1930; Hill 2006; Vickaryous and Hall 2006), to compare basic patterns of osteoderm histology in dasypodids, to contrast the morphological differences between the two most diverse subfamilies, which split at least 40 Ma (Delsuc *et al.* 2004).

The histological study of osteoderms of living dasypodids will allow deeper and better supported analyses and interpretations of osteoderm morphology in fossil dasypodids (Krmptic *et al.* 2005). The fact that certain soft tissues are associated with distinct osteological structures provides a solid basis for the reconstruction of soft tissues in osteoderms of extinct dasypodids (Hill 2004, 2006). The orientation of

collagen fibres governs that of hydroxyapatite crystals in bony tissue in many amniote clades (Scheyer and Sánchez-Villagra 2007). Precisely this biomineralization, which is preserved in fossil materials, allows reconstruction of the location of associated soft tissues lost during fossilization.

## Materials and Methods

### Histology

We dissected carapaces from three specimens of *Chaetophractus villosus* (adult individuals) that had been fixed in 10% formaldehyde. The carapaces studied were obtained from road-killed specimens of Buenos Aires Province (Bahía Samborombón), Argentina. These materials were deposited in the reference collection of the Vertebrate Palaeontology Department at Museo de La Plata. For each specimen, small portions of different carapace regions were decalcified (approximately 10 osteoderms from each region). To evaluate different techniques, two decalcification processes were used: one set of osteoderms was treated with Bouin solution, and another was submerged in 8% nitric acid. Once decalcified, tissues were dehydrated using a 70% to 100% graduated ethanol series and embedded in paraffin. Osteoderms were cut into 5- $\mu$ m thick longitudinal and cross serial sections. Sections were stained using haematoxylin and eosin, Masson trichrome and Periodic Acid–Schiff, to ensure visualization of most tissues. The histological slides are housed in the reference collection of the Vertebrate Palaeontology Department at Museo de La Plata.

### Palaeohistology

For the analysis of fossil materials we used osteoderms from individuals of the extinct genus †*Eutatus* Gervais 1867 deposited in the collection of the Vertebrate Palaeontology Department at Museo de La Plata (MLP 69-IX-5-3). Osteoderms were mechanically prepared (for coarse fraction sediments) and by means of 100 volume hydrogen peroxide (1 : 10) (for fine fraction), to remove sediment from pores, foramina and cavities. Next they were embedded in coloured polyester resin and placed in a vacuum chamber to remove air bubbles. Once the resin solidified, preparations were sliced using a metallographic cutter and then polished with a metallographic grinding disc to obtain thin sections. Digital photographs were obtained every 150  $\mu$ m and incorporated on a database for each osteoderm. The digital database is housed in the collection of the Vertebrate Palaeontology Department at Museo de La Plata added to the specimen number (MLP 69-IX-5-3).

### Terminology

In describing the general morphology of osteoderms, researchers have used different terms to indicate the specific

structures. These terms are an improvement, but can become confusing, here we summarize the terminology used here and the equivalent used by other authors:

External and internal surfaces of the osteoderm = superficial and deep surfaces of the osteoderm (Hill 2006), external surface foramina = central perforations (Simpson 1948) or pits for hair follicle (Hill 2006), cranial portion = base (Holmes and Simpson 1931) and caudal portion = tongue (Holmes and Simpson 1931).

## Results

### *External morphology of the carapace of *Chaetophractus villosus**

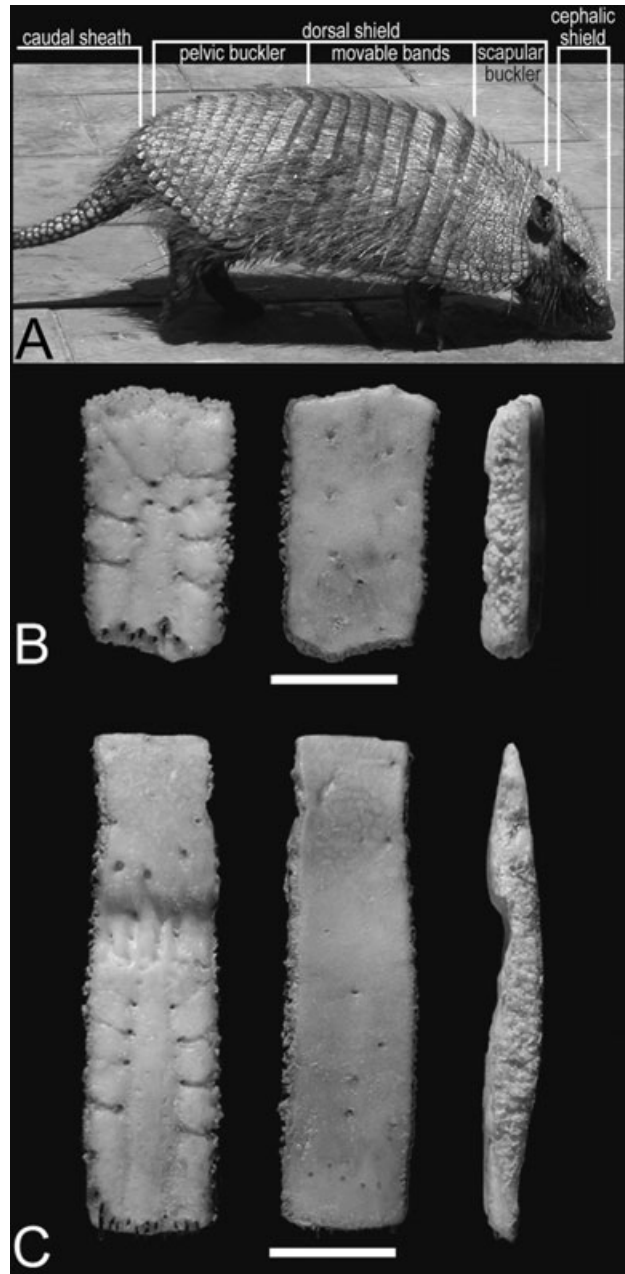
The carapace of *Chaetophractus villosus* (Fig. 1A) includes, from head to tail: cephalic or head shield, dorsal shield and caudal sheath. In turn, the dorsal shield comprises three movable nuchal bands, a scapular buckler, a region of movable bands (or rings) and a pelvic buckler. The dorsal shield comprises two major types of osteoderms: fixed osteoderms forming both bucklers (scapular and pelvic) and movable osteoderms arranged in rows forming the movable bands.

The external surface of fixed osteoderms is ornamented consisting of a central figure and several smaller peripheral figures (Figs 1B,C and 2A,B). All figures are convex and delimited by well-defined sulci. The central figure is elongated and occupies the posterior two-thirds of the osteoderm. The peripheral figures are located anterior and lateral to the central figure.

Surrounding the central figure there are foramina, which we name as external surface foramina. These are arranged at the intersections between the sulci between peripheral figures and the one surrounding the central figure. Some osteoderms also present foramina in the sulci between adjacent peripheral figures. The posterior margin of each osteoderm bears piliferous follicle foramina, from which emerge the hairs that partially cover the carapace.

At first glance the surface of the osteoderms is smooth, but under magnification (25×) it is seen to be rough by the presence of various small orifices and short meandering sulci. These structures correspond to the openings and courses of blood vessels and nerves situated below the epithelium forming the horny scales.

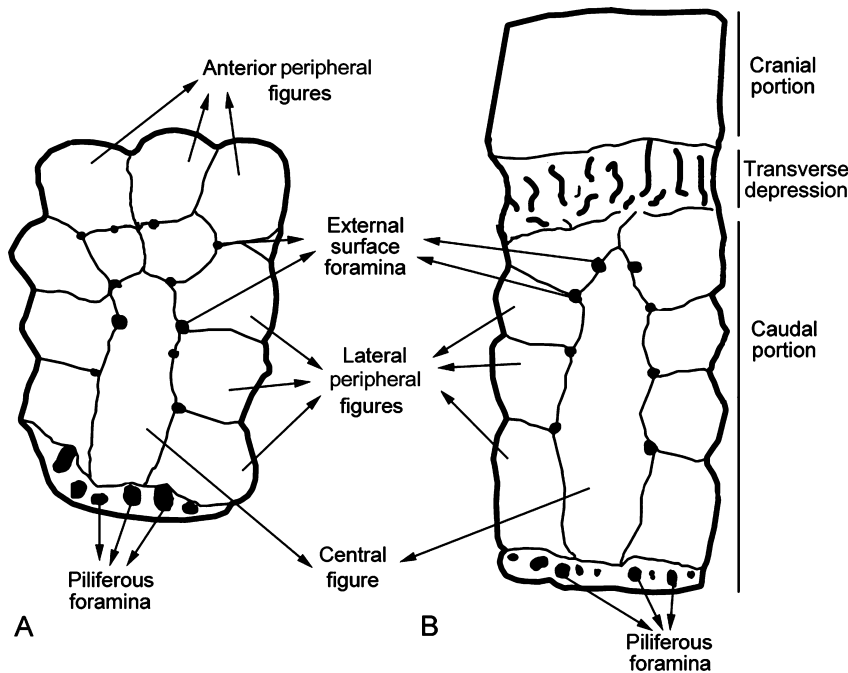
Movable osteoderms have two distinct regions (Figs 1C, 2B). The cranial portion is elevated, not exposed, lacking ornamentation, and occupies about the proximal one-third of the osteoderm. This area always lies under the caudal portion (the external surface) of osteoderms from the preceding row. The caudal portion overlaps the posteriorly adjacent osteoderm, comprising the remaining two-thirds of the osteoderm, and with the external surface ornamented similarly to the previous description for fixed osteoderms, i.e. one elongated central figure and smaller peripheral figures; however, in contrast with fixed osteoderms, normally only lateral peripherals are present.



**Fig. 1**—*Chaetophractus villosus*. —A. Adult individual; —B. Fixed osteoderm from the dorsal shield; —C. Movable osteoderm from the dorsal shield; the osteoderms are viewed in external, internal and side views. Scale bar 5 mm.

A transverse depression with numerous longitudinal grooves that give it a 'striated' appearance is present between the cranial and caudal portions of the osteoderm.

The internal surface of each osteoderm (both fixed and movable) is relatively unmarked, with some irregularly distributed foramina that correspond to apertures for neurovascular bundles into the osteoderm.



**Fig. 2**—*Chaetophractus villosus*. —**A**. Line drawing of fixed osteoderm from dorsal shield showing external structures mentioned in the text; —**B**. Line drawing of movable osteoderm from dorsal shield showing external structures mentioned in the text.

The previous description corresponds to typical osteoderms situated over the midline area of the dorsal shield. Towards the lateral margins of the shield osteoderms become modified and progressively asymmetrical. Marginal osteoderms are smooth, flattened and with a protruding posterolateral apex directed backwards; combined, these osteoderms are arranged in a series forming a serrated carapace margin.

Externally, each osteoderm from the cephalic and dorsal shields and from the caudal sheath is covered by an individual horny scale. This scale is thicker over the most prominent areas of the figures (both central and peripheral) and thinner at the sulci. In movable osteoderms the horny scale covers only the caudal portion, while the anterior region never has this type of cover and is only covered, in areas of greater mobility, by a smooth soft integument, not significantly cornified.

#### General histology of *Chaetophractus villosus* osteoderms

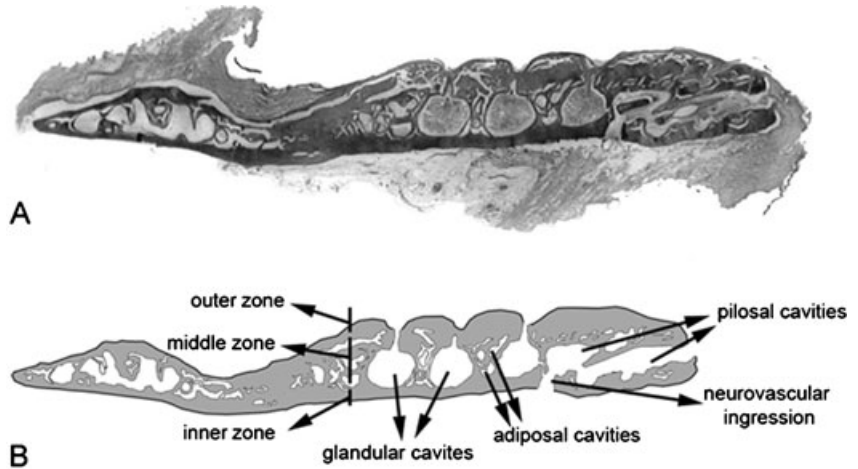
As commented above, we used two different techniques for decalcification of osteoderms. Bouin solution was slower (15–20 days), but better for maintaining structures and eliminating all calcium deposits; this technique was adequate for detailed description of tissues. The nitric acid solution was faster (4–5 days) but it required accurate time control, because excessive exposure can easily destroy the osteoderm; moreover, decalcification was not complete and the osteoderms often retained some small calcium deposits. This latter technique was useful for general descriptions.

Osteoderms of *C. villosus* are formed by compact bone. Longitudinal sections show three zones arranged in

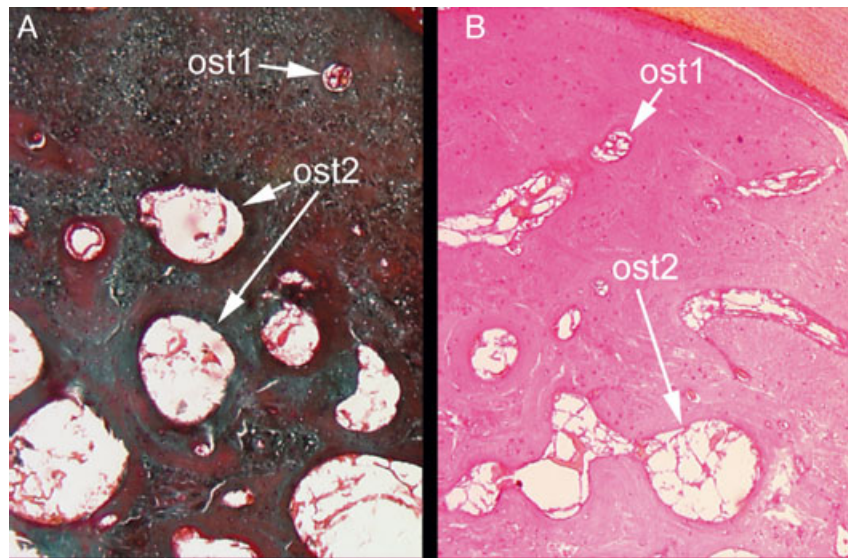
dorsoventral succession. The outer and inner zones are comparatively thin and formed by regular compact bone with primary and secondary osteons (Figs 3A,B and 4A,B). In contrast, the middle zone is thicker, representing about 50% of osteoderm thickness, and consists of bony lamellae arranged concentrically around large cavities. These cavities are filled mainly with adipose tissue, hair follicles, and sweat and sebaceous glands (Fig. 5). In a single osteoderm, from the cephalic shield, we identified elements corresponding to red bone marrow (Fig. 6).

Sebaceous and sweat glands are frequently encountered together, the former dorsally and the latter more ventrally, within a single cavity. Both open into the external surface of the osteoderm by means of separate ducts that open at surface foramina (Fig. 7).

Distally, the osteoderm has several larger elongated and obliquely arranged cavities that hold the hair follicles (posterodorsal–proximoventral direction) (Fig. 3A). The proximal portion of these follicle cavities penetrates to the boundary inner zone, and the posteriormost portion opens dorsally on the posterior margin of the osteoderm, where hairs emerge through each of the hair foramina. Each hair follicle is occasionally associated with a sebaceous gland, or more rarely, a sweat gland. Cavities holding glands are generally located anterior to those that correspond to hair follicles, whereas those that do not contain glands or hair follicles are filled with adipose tissue. All cavities are connected by means of transverse tubes that correspond to Volkman's canals with blood vessels and nerves (Fig. 8). The outer zone has gland canals that open onto the external surface of the osteoderm, as well as small orifices for passage



**Fig. 3**—*Chaetophractus villosus*. Movable osteoderm, longitudinal section (general view). —**A**. Histological section; —**B**. Representation of **A**, bone is in grey, and the general cavities occupied by soft tissues appear white. Arrows show the main tissues that occupy the cavities, and the three zones recognized in osteoderm section are marked.



**Fig. 4**—*Chaetophractus villosus*. Histological details of pelvic shield osteoderms demonstrating the primary and secondary osteon. —**A**. Trichromic stain 4 ×; —**B**. Haematoxylin & eosin 4 ×. ost1, primary osteon; ost2, secondary osteon.

of vessels and nerves toward the surface. Thin bundles of Sharpey's fibres, arranged perpendicularly to the long axis of the osteoderm, occur in this zone.

The inner zone also shows small canals for entrance of vessels and nerves into the osteoderm (Fig. 9A,B). This zone is much more collagen-rich than the external one; collagen forms thick bundles of Sharpey's fibres arranged obliquely to the greater axis of the osteoderm.

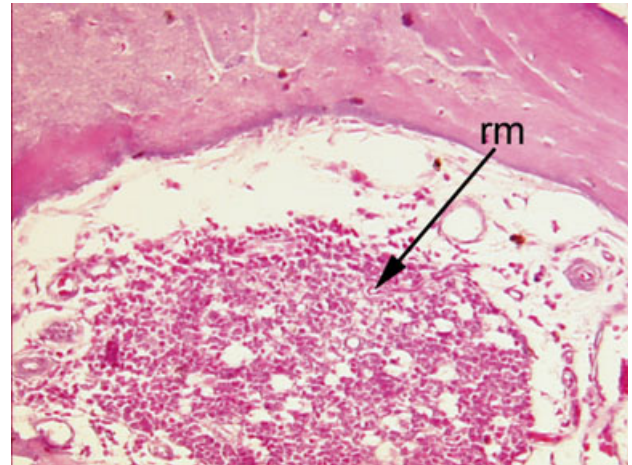
The surface of the lateral areas of contact between adjacent osteoderms is formed by numerous denticle-like projections interdigitating with those from neighbouring osteoderms. Adjacent osteoderm surfaces are joined by means of Sharpey's fibres between them that are thicker ventrally. In addition, at this level these fibres show a more regular arrangement that resembles that of the internal fibres of the osteoderm (Sharpey's fibres) (Fig. 10).

#### *Histology and morphology of osteoderms from different regions*

**Cephalic shield.** This element has a pear-shaped to subtriangular outline, covers most of the skull dorsally excluding the anterior half of the nasal region and has paired lateral notches at the level of the orbital region. Osteoderms of the middle and posterior regions of the shield are the largest, with ornamentation similar to that of the dorsal shield described above. The anterior marginals are smaller, lack ornamentation and although they vary in shape, are generally polygonal. Osteoderms of the cephalic shield are not arranged in distinct rows. Osteoderm cross-sections exhibit a poorly developed middle region, with up to three sweat glands and no sebaceous glands (Figs 11, 12). The posterior half of the osteoderm shows scarce hair follicles arranged as previously described. Occasionally, small, paired sebaceous



**Fig. 5**—*Chaetophractus villosus*. Histological sections of movable osteoderms. —**A**. Detail of adiposal cavity full of adipose tissue, haematoxylin & eosin (H&E) stain, 10 ×; —**B**. Detail of the arrangement of sebaceous and sweat glands in a glandular cavity, H&E stain 4 ×; —**C**. Detail of hair follicle, trichromic stain, 1.25 ×. at, adipose tissue; hf, hair follicle; sg, sebaceous gland; swg, sweat gland.



**Fig. 6**—*Chaetophractus villosus*. Histological section of cephalic shield osteoderm, details of red bone marrow, haematoxylin & eosin stain, 10 ×. rm, red bone marrow.



**Fig. 7**—*Chaetophractus villosus*. Histological details of a glandular cavity of movable osteoderm, containing sebaceous gland (dorsally) and sweat gland (ventrally). Haematoxylin & eosin stain, 4 ×. gc, gland cavity; gd, gland ducts; sg, dorsal sebaceous gland; swg, ventral sweat gland.

glands can be observed in association with one of the hair follicles.

*Scapular buckler*. This is the cranialmost section of the dorsal shield and covers the scapular region of the trunk. Five longitudinal rows of osteoderms are present medially, while laterally up to seven rows may be present. These osteoderms are mainly quadrangular and rectangular, although some are pentagonal in outline, especially in areas with multiple rows. The caudalmost row has the longest osteoderms; these are



**Fig. 8**—*Chaetophractus villosus*. Section of outer zone of pelvic shield osteoderm shows details of a Volkman's canal, haematoxylin & eosin stain, 4 ×. cb, compact bone; hs, horny scale; vd, Volkman's canal.

rectangular and their ornamentation is similar to that of the osteoderm of the first movable band.

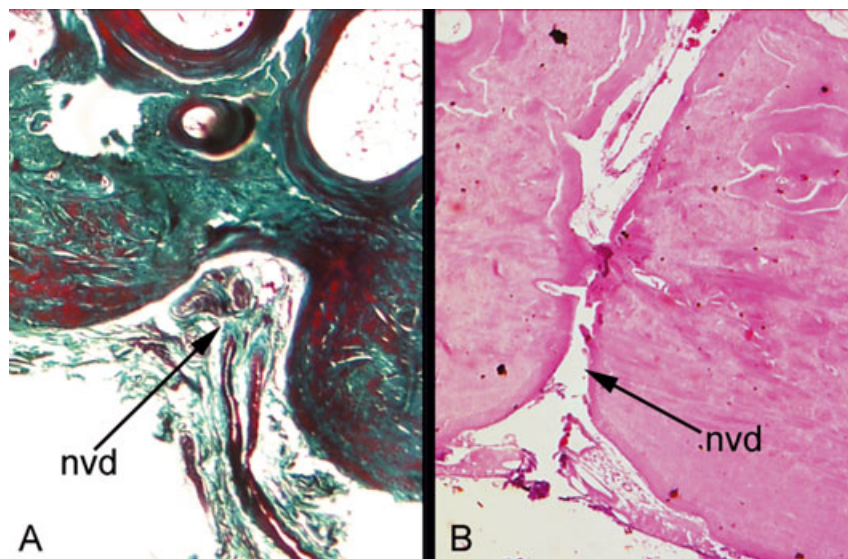
Cross-sections of osteoderms from this buckler show a middle zone comparable with that described above (Fig. 13). There are relatively more glands compared with the cephalic shield, and both sweat and sebaceous glands occur in each glandular cavity. Hair follicles are more numerous than in cephalic shield osteoderms.

**Movable bands.** This region comprises six to eight rows of movable osteoderms. The cranial portion of osteoderms from the first movable band lies below the last row of the scapular buckler, and the last movable band overlies the cranial portion of the first row of (semifixed) osteoderms of

the pelvic buckler. The number of elements in each band ranges between 32 and 39.

Histological sections of these osteoderms show structural differences between the cranial and caudal portions. The cavities of the middle zone of the cranial portion contain only adipose tissue and neurovascular bundles, whereas the caudal portion of the osteoderm contains spherical cavities with glands (one sweat gland and two sebaceous glands per cavity). Cavities with hair follicles are located posteriorly to these glandular cavities. The general structure of the rest of the osteoderm is similar to the previous description (Fig. 3A).

**Pelvic buckler.** The pelvic buckler consists of about 10 rows over the sagittal plane. The first row is called semifixed because the osteoderms have a proximal cranial portion that is covered by the caudal portion of the last movable band, but in contrast to movable band osteoderms, the contact of the semifixed row with the next row is serrated. The remaining osteoderms of this region are fixed and rectangular, although becoming more isodiametric toward the margins. Marginal osteoderms are subtriangular. The number of peripheral figures around each central figure ranges mainly between 10 and 13. The number of osteoderms per row varies between 35 and 10 (in the latest row that demarcates the caudal notch). Hair follicles range between 2 and 12, more frequently four to eight (Table 1). The number of non-hair foramina on the external surface varies between 2 and 11, more frequently five to seven (Table 1). Frequently the third and fourth rows of the medial region of this shield include one to three (often two) osteoderms with large surface foramina (sometimes merged in a single foramen) at the level of the sagittal plane; these represent the openings of the pelvic glands. These structures are typical of many Euphractinae species (Fernández 1922; Estecondo *et al.* 1997, 1998, 1999).



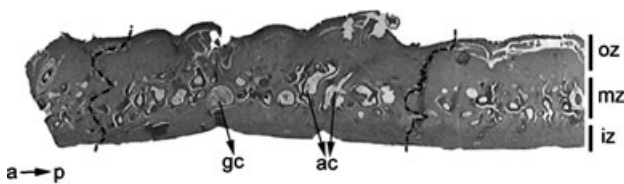
**Fig. 9**—*Chaetophractus villosus*. Histological details of an inner zone of movable osteoderms show neurovascular bundle entrance. —**A.** Trichromic stain 4 ×; —**B.** Haematoxylin & eosin stain, 4 ×. nvd, neurovascular bundle entrance.



**Fig. 10**—*Chaetophractus villosus*. Histological details of an arrangement of Sharpey's fibres between two pelvic shield osteoderms, Periodic Acid-Schiff stain, 20 ×. ost, osteoderm; sf, Sharpey's fibres.

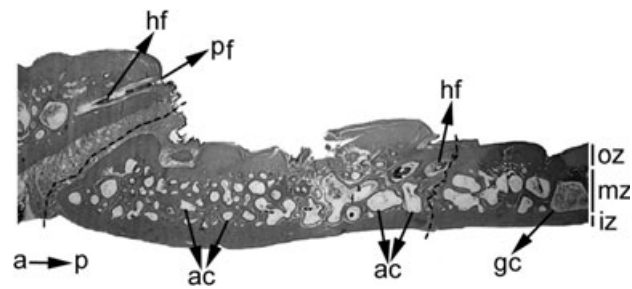


**Fig. 12**—*Chaetophractus villosus*. Histological section of cephalic shield osteoderm shows a glandular cavity occupied only by a sweat gland, haematoxylin & eosin stain, 4 ×. gd, gland duct; swg, sweat gland.



**Fig. 11**—*Chaetophractus villosus*. Histological section of cephalic shield osteoderms (general view) showing the poor development of the middle zone compared to osteoderms from other body regions. Dotted line pointed out the limits of a single osteoderm. a, anterior; ac, adiposal cavity; gc, glandular cavity; iz, inner zone; mz, middle zone; oz, outer zone; p, posterior.

Histological sections show the typical arrangement with three bone zones and some hair follicles posteriorly, associated with sweat glands. Histology of osteoderms of this region agrees with the general description (Fig. 14). Some sections show epidermal and dermal papillae, often accompanied by invaginations of bony tissue.



**Fig. 13**—*Chaetophractus villosus*. Histological section (general view) of scapular shield, showing the general internal arrangement of osteoderms and the well-developed middle zone compared to osteoderms from other body regions. Dotted line pointed out the limits of a single osteoderm. a, anterior; ac, adiposal cavity; gc, glandular cavity; hf, hair follicle; iz, inner zone; mz, middle zone; oz, outer zone; p, posterior; pf, piliferous foramen.

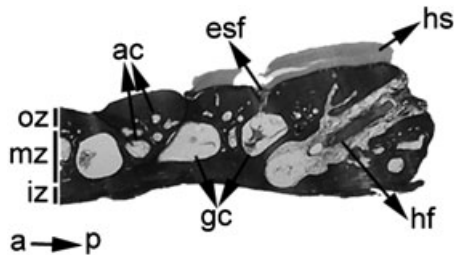
**Table 1** Statistical data for number of external surface foramina and piliferous foramina for osteoderms of the pelvic buckler of *C. villosus*.

	External surface foramina		Piliferous foramina		Osteoderms (n)
	Mean	Rank	Mean	Rank	
Specimen 1	6.04	2–9	6.08	2–12	146
Specimen 2	6.30	2–11	6.56	4–10	164
Specimen 3	6.49	3–11	7.23	4–11	165

*External and internal morphology of osteoderms of †Eutatus sp.*

The osteoderms of *Eutatus* sp. have a rugose surface with numerous orifices (larger foramina and smaller punctuations)





**Fig. 14**—*Chaetophractus villosus*. Histological section (general view) of pelvic shield osteoderm, showing the general internal arrangement of osteoderm. a, anterior; ac, adiposal cavity; esf, external surface foramina; gc, glandular cavity; hf, hair follicle; hs, horny scale; iz, inner zone; mz, middle zone; oz, outer zone; p, posterior.

and small furrows. Ornamentation of the external surface consists of a central figure delimited by peripheral figures (Figs 15, 16). The sulci delimiting the central figure do not reach the posterior margin of the osteoderm, so that the outline of the central figure becomes less distinct. The anterior half of the osteoderm demonstrated external surface foramina, located at the intersections of sulci between peripheral figures with the sulcus surrounding the central figure. A deep transverse furrow at the posterior margin of each osteoderm is divided by transverse septa that delimit a few large compartments. Since the first studies of eutatines (e.g. from classical works such as Burmeister 1883; Ameghino 1889, and later works), these have been attributed to hair follicle foramina (i.e. piliferous pits). The large size of these hair foramina has been cited as one of the most distinctive Eutatini characters. On the internal surface, the osteoderm surface is smooth, with various irregularly distributed perforations.

Palaeohistological sections of osteoderms from the midline of the pelvic buckler of *Eutatus* sp. specimens show the same three well-defined dorsoventral zones found in *C. villosus*: an outer zone formed by regular compact bone, a middle zone with bony lamellae arranged mainly around large cavities, and an inner zone also formed by regular compact bone (Fig. 17) that is diplôe-like (sensu Hill 2006). The middle zone forms up to 70% of the osteoderm thickness.

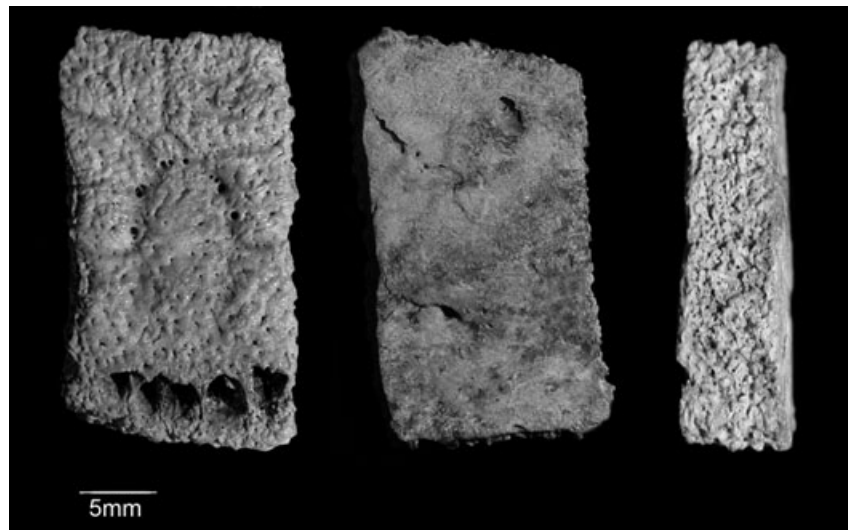
In addition to being traversed by larger canals coming from cavities in the middle zone, the outer zone is also pierced by small canals that branch and open as canals on the surface.

The middle zone contains spherical cavities that connect with foramina on the external surface by means of one or two canals.

There are also elongated cavities in oblique orientation (posterodorsal–anteroventral) with their anteriormost portion at the half of the osteoderm (generally surpassing the halfway line) or located even deeper in the inner zone, and with their posterior portion communicated with hair foramina. Lastly, irregular interconnected cavities appear in areas of the middle layer not occupied by these structures or glandular cavities. The inner zone is traversed by numerous canals extending toward the middle zone.

## Discussion

The results of this work allowed clear identification of internal morphological differences between osteoderms of members of Dasypodinae and Euphractinae (see also Burmeister 1883 and Scillato-Yané 1982; for external morphological differences). As previously mentioned, these two are the major Dasypodidae clades and, according to all available evidence, split in the middle Eocene (more than 42 Ma) (see Carlini *et al.* 2002b, 2005b;



**Fig. 15**—*Eutatus* sp. (MLP 69-IX-5-3). Fixed osteoderm in external, ventral and lateral views (left to right).

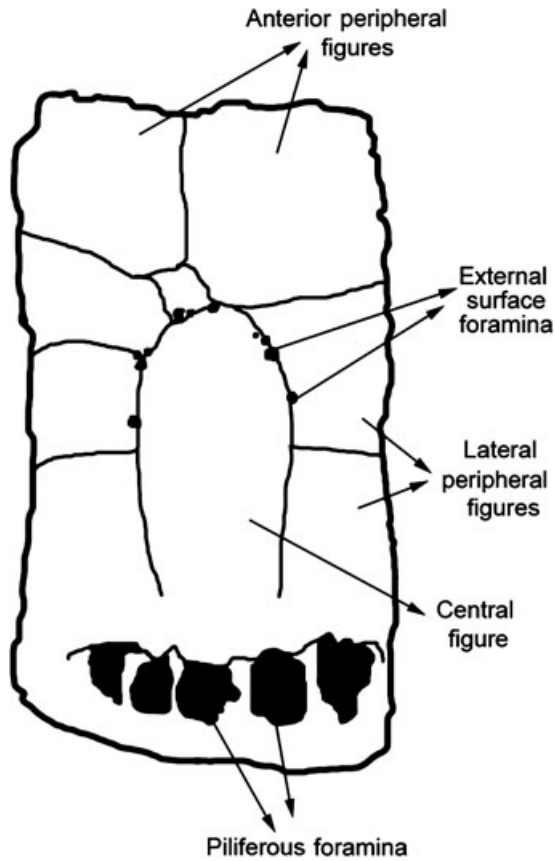


Fig. 16—*Eutatus* sp. (MLP 69-IX-5-3). Line drawing of fixed osteoderm showing external structures mentioned in the text.

Delsuc *et al.* 2002, 2003, 2004). According to the recent and exhaustive works of Vickaryous and Hall (2006) and Hill (2006), and to our own observations (Ciancio *et al.* 2007), in Dasypodinae the middle layer is poorly developed and does not have cavities with adipose tissue. In *Dasypus novemcinctus*, the foramina of the external surface are not only associated with both types of glands, but also with hair follicles (Cooper 1930; Hill 2006; Vickaryous and Hall 2006), a condition not observed in *C. villosus* adults. However, Fernández (1931) noted that hair follicles develop in these cavities in *C. villosus* embryos, only to become atrophied during development (progenesis?). This condition supports the hypothesis that among dasypodids, the Euphractinae are more derived than the Dasypodinae (Carlini *et al.* 1997, 2005a), because they exhibit plesiomorphic features during their ontogeny that are not manifested in postnatal stages. Consequently, the presence of hairs associated with foramina on the dorsal surface of the osteoderm would be a plesiomorphic condition for dasypodids.

A middle zone with multiple cavities filled with adipose tissue does not occur in *D. novemcinctus* (we have not observed this condition in *Dasypus hybridus* either; see Ciancio *et al.*

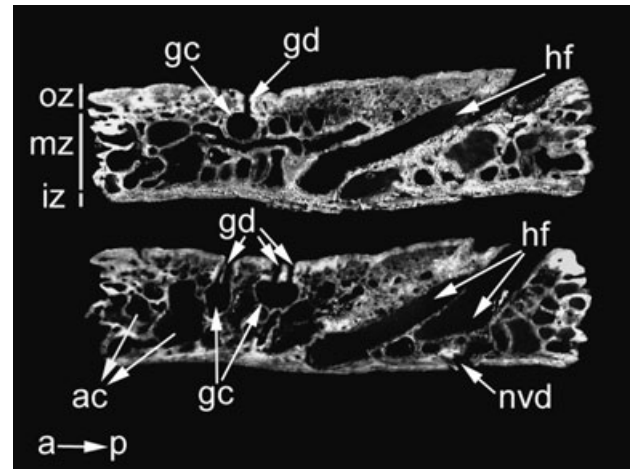


Fig. 17—*Eutatus* sp. (MLP 69-IX-5-3). Palaeohistological sections of fixed osteoderm, showing the general internal arrangement of osteoderms and the development of different zones recognized. a, anterior; ad, adipose cavity; gc, gland cavity; gd, gland duct; hf, hair follicle cavity; iz, inner zone; mz, middle zone; nvd, neurovascular bundle entrance; oz, outer zone; p, posterior.

2007), and such an association would only be present in the cranial portion of mobile osteoderms (see Cooper 1930; Vickaryous and Hall 2006; Hill 2006). Finally, Vickaryous and Hall (2006) identified red and yellow bone marrow in the osteoderms of *D. novemcinctus*, but we observed adipose tissue, possibly yellow bone marrow in the osteoderms and only found remains of red bone marrow in one cephalic shield osteoderm in *C. villosus*. The retention or loss of red marrow in osteoderms could also be of phylogenetic value.

Fernández (1931) hypothesized that the large amount of adipose tissue occurring in the middle zone of *C. villosus* could function as thermal insulation, or as a strategy to decrease shield weight. According to our analysis of *Eutatus*, the presence of a relatively larger middle zone would indicate greater amount of adipose tissue; this, together with the inferred development of hair, could be interpreted as an adaptation to colder climates like those that would have progressively developed during the Pleistocene (Zachos *et al.* 2001). Furthermore, we note that the osteoderms of both euphractines show great development of cavities that house adipose tissue, in contrast to the condition in Dasypodinae. Taking into account the known distribution of living representatives of both clades, we observe that the Dasypodinae are distributed in tropical to subtropical regions, whereas the Euphractinae occur from subtropical areas to cold temperate zones in the extreme south of the Southern Cone (Wetzel 1985; Redford and Eisenberg 1992; Parera 2002). Similar conclusions have been drawn about environmental requirements of extinct dasypodids, and the main cladogenetic events of the family have been compared with past marine temperatures, permitting a significant correlation between

temperature and relative diversity of the different groups (Carlini *et al.* 2005b and references therein). Lastly, the lack of significant differences between the middle zones of *D. novemcinctus* and †*Dasypus bellus*, given that the osteoderms of the latter are twice (or more) the size of those of the former (see Hill 2006) and three times that of small species (e.g. *D. hybridus*), lends additional support to the hypothesis that cross-sectional osteoderm structure is not necessarily correlated with body size. These data seem to reinforce the hypothesis that a plausible role of adipose tissue distributed within osteoderms is to serve as thermal insulation.

The study of osteoderms of living dasypodids allows us to propose homology hypotheses as well as make comparisons and interpret the structures preserved in osteoderms of extinct species, including the interpretation about the types of soft tissues that could be present in these elements. Thus, *Eutatus* sp. (Eutatini, Euphractinae) has cavities that continue as canals opening into foramina on the external surface that, according to the histological study of *C. villosus*, would correspond to glandular cavities and their respective canals. Similarly, it is probable that these cavities were occupied by more than one type of gland, because it is frequent to observe two canals issuing from a single cavity (Fig. 17). This arrangement is very similar to the condition observed in *C. villosus*, in which sweat and sebaceous glands share a single cavity and open via separate canals, in contrast to the description of Fernández (1931) who stated that both glands opened via a single duct.

The oblique cavities connecting with foramina on the posterior margin probably correspond to hair follicles. Contrasting with the condition in *C. villosus*, in *Eutatus* sp. more than one hair would exit through each hair foramen, given that up to three of these cavities can be observed opening onto the same foramen.

The remaining blind cavities (i.e. those that do not exit to the exterior) are inferred to have contained mostly adipose tissue. Lastly, the perforations observed both on the external surface and on the internal surface of the osteoderm would correspond to entrance and exit of neurovascular bundles, as observed in *C. villosus*.

General osteoderm structure in *Eutatus* sp. is very similar to that of *C. villosus*. This agrees with the hypothesis that these taxa are more closely related to each other than to representatives of Dasypodinae (Gaudin and Wible 2006). However, it may also be noted that the Eutatini and Euphractini show some differences within the basic Euphractinae pattern of internal structure of osteoderms (i.e. outer and inner zones, thin and formed by regular compact bone; and a middle zone, thicker and with large cavities surround for bony lamellae concentrically arranged). In fact, the development of the middle zone is variable, and it has equal or greater relative development in *Eutatus* sp. than in *C. villosus*. The zone presumed to be the insertion area of hair follicles is more extended anteriorly in the osteoderms of *Eutatus* sp. On the other hand, each of the hair foramina of

*C. villosus* communicates with a single cavity in which only one follicle is implanted, whereas the homologous foramina of *Eutatus* are much larger and communicate with several cavities that would probably have held more than one hair follicle; this would indicate greater pilosity than in *C. villosus*.

### Final considerations

Osteoderms have always been the most frequent and abundant elements in the fossil record of Dasypodidae, and most extinct species are based on osteoderm morphology. However, it is noteworthy that relatively few characters from shield or osteoderm morphology have been included in most phylogenetic analyses of the group. Diverse phylogenetic analyses of Dasypodidae have included molecular data (Delsuc *et al.* 2002, 2003, 2004), and craniodental characters (see, e.g. Engelmann 1985; Gaudin and Wible 2006). In contrast, very few have included characters from the dorsal shield (Abrantes 2006), or integument (Hill 2005, 2006). Carlini *et al.* (1997) have partially proposed a phylogenetic scheme based on characters of osteoderms and dorsal carapace for both Glyptodontidae and Dasypodidae. The present study of osteoderm histology and morphology, together with those made by Hill (2006) and Vickaryous and Hall (2006), shows that the anatomical characteristics of these structures are also relevant for the study of relationships. Furthermore, it is consistent with previous hypotheses about the main cladogenetic event within the Dasypodidae. Lastly, there is remarkable agreement between the palaeoecological requirements of different groups of dasypodids inferred from non-skeletal data sources and osteoderm histology (Krmptotic *et al.* 2007a,b).

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