



General palaeontology, systematics and evolution (Vertebrate palaeontology)

An enamel-like tissue, osteodermine, on the osteoderms of a fossil anguid (Glyptosaurinae) lizard

Présence d'un tissu similaire à l'émail, l'ostéodermine, sur les ostéoderms d'un lézard anguidé (Glyptosaurinae) fossile

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ABSTRACT

The Glyptosaurinae, a fossil clade of anguid lizards, have robust osteoderms, with a granular ornamentation consisting of tubercles. In this study, the structural and histological features of these osteoderms are described in order to reconstruct their developmental pattern and further document the possible homology that could exist between vertebrate integumentary skeletons. Glyptosaurine osteoderms display a diploe architecture and an unusually complex structure that includes four tissue types: an intensely remodeled core of woven-fibered bone, a thick basal layer of lamellar bone, a peripheral ring exhibiting histological features intermediate between these two tissues and containing dense bundles of long Sharpey fibers, and a superficial layer made of a monorefringent, acellular and highly mineralized material, different from bone, and comparable in many respects to hypermineralized tissues such as ganoine, enameloids and enamel. We call this tissue osteodermine. The growth pattern of glyptosaurine osteoderms is likely to have involved first metaplasia, at an early developmental stage, then appositional growth due to osteoblast activity. The superficial layer that is well developed at the tubercle level must have resulted from epidermal and dermal contributions, a conclusion that would support previous hypotheses on the role of epidermal-dermal interactions in the formation of squamate osteoderms.

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R É S U M É

Les Glyptosaurinae, un clade fossile de lézards anguidés, ont des ostéoderms robustes, pourvus d'une ornementation granulaire composée de tubercules. La présente étude décrit les caractères structuraux et histologiques des ostéoderms, dans le but d'interpréter leur mode de développement et de compléter l'information relative au degré d'homologie des éléments du squelette dermique chez les vertébrés. Les ostéoderms des glyptosaures ont une architecture en diploé et montrent une structure complexe, incluant quatre types de tissus : de l'os à fibres enchevêtrées au centre, une épaisse couche basale d'os lamellaire, une formation périphérique aux caractéristiques histologiques intermédiaires entre ces deux types tissulaires et très richement pourvue de longues fibres de Sharpey, et une

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couche superficielle composée d'un tissu totalement monoréfringent, acellulaire et fortement minéralisé. Ce tissu est différent de l'os et comparable, à de nombreux égards, aux tissus hyperminéralisés tels que la ganoïne, les émailloïdes et l'émail. Nous avons nommé ce tissu ostéodermine. La formation et la croissance des ostéodermes des glyptosaures impliquaient tout d'abord un processus métaplasique, aux stades précoces du développement, suivi d'une croissance appositionnelle liée à l'activité d'ostéoblastes. Il est probable que la couche superficielle, bien développée au niveau des tubercules, résultait d'une contribution de l'épiderme et du derme, conclusion qui s'accorde avec les hypothèses émises précédemment à propos du rôle des interactions épidermo-dermiques dans la formation des ostéodermes chez les squamates.

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1. Introduction

Intradermal osteoderms, forming continuous shields or occurring as isolated elements, are frequent in all major tetrapod clades. The increasing set of comparative data suggests that most osteoderms are basically made of bone tissue (whatever its origin or developmental pattern) comprising osteocyte lacunae and a collagenous meshwork (reviewed in Vickaryous and Sire, 2009).

However, in the Squamata, osteoderm structure seems to be more complex and liable to substantial variation, even among closely related taxa (Levrat-Calviac, 1986). As a consequence, the precise natures of the tissues they are composed of, as well as their pattern of development and growth, remain uncertain and controversial topics. This situation is particularly obvious for the tissue covering the superficial side of the osteoderms (close to the epidermis) in various taxa. This tissue indeed displays peculiar histological and histochemical characteristics in contrast with the common features of bone. This is why some authors questioned its relevancy to osseous tissues (Levrat-Calviac, 1986), or considered that it could be a kind of hypermineralized tissue similar to enamel, ganoine, etc. (Moss, 1969, 1972; Vickaryous and Sire, 2009). Its formation would therefore involve a morphogenetic contribution of local epidermal cells. For other authors, the superficial layer of squamate osteoderms is a peculiar form of osseous tissue resulting from the metaplastic transformation of the outer strata of the dermis (*stratum laxum*), as exemplified by detailed histological descriptions in *Tarentola* and *Anguis* (Haines and Mohuiddin, 1968; Levrat-Calviac and Zylberberg, 1986; Zylberberg and Castanet, 1985).

The Glyptosaurinae (Anguinae) are large lizards occurring throughout the Eocene in the holarctic region, and are among the squamates that possess the thickest and most broadly developed osteoderm shield. However, there was no detailed description of the histological structure of glyptosaurine osteoderms up to now (see Keller, 2009, for basic microanatomical features). The present study, necessarily limited to the questions compatible with fossil material, is intended to contribute to further documenting osteoderm structure in squamates by the study of a taxon that offers particularly favourable conditions for histological observations because of the volume and excellent preservation of its osteoderms.

2. Material and methods

2.1. Palaeontological samples

The fossil material comprises 26 well-preserved osteoderms attributed to Glyptosaurinae, registered under the references LAV 1258 to LAV 1273 in the paleontological collections of Montpellier II University (16 osteoderms), and SNB 1022–SNB 1031 (10 osteoderms) in the collections of the Muséum national d'histoire naturelle (Paris, France).

The Glyptosaurinae are considered to be Anguinae by most authors (Augé and Sullivan, 2006; Conrad and Norell, 2008; Hoffstetter, 1955), although Hill (2005) proposed to consider them as the sister group of a clade including the Scincoidea (Scincidae, Cordylidae, Gerrhosauridae) and Anguinae. Among the Anguinae, they occupy a relatively marginal position, being closer to the Anguinae and Gerrhonotinae than to the Diploglossinae, and forming a sister taxon to the clade that includes the Anguinae and Gerrhonotinae. Up to now, some 13 genera and 28 species of glyptosaurine are encountered in Late Cretaceous–Early Oligocene sites in the USA, Europe, and Asia. The size and general aspect of these lizards must have been comparable to that of extant heloderms, i.e., 60 to 80 cm long animals with extensive, robust osteoderm shields covering most of the body, including the skull roof (review in Hoffstetter, 1955).

The specimens used for this study were collected in the 1970s and 1980s in the Phosphorites du Quercy (de Bonis et al., 1973; Crochet et al., 1981; Rage, 2006), a broad fossiliferous area located in southwestern France, dated Early Eocene to Early Miocene (Rage, 2006). The specimens referenced LAV 1258 – 1273 are from the Bartonian (standard level MP 16, Middle Eocene of Lavergne locality), whereas the specimens referenced SNB 1022 – 1031 are from the Priabonian (standard level MP 18 of Sainte Néboule locality; Late Eocene).

2.2. Methods

A subsample of 15 complete and perfectly preserved osteoderms from the cranial (six specimens) and postcranial territories was selected in order to be studied by using four distinct morphological approaches.

2.2.1. Ground sections

In nine osteoderms, 60 to 80 μm -thick sections were made according to the usual procedure for this kind of preparation (de Buffr enil and Mazin, 1989). These sections were used for histological observations by means of a microscope, in natural or polarized light.

2.2.2. Scanning electron microscopy (SEM)

This technique was applied to one cranial and one postcranial osteoderm. They were broken transversely, glued on a copper support with the broken surface upwards, coated with a thin layer of gold/palladium, and observed in a JEOL SEM 35 operating at 25 kV.

2.2.3. Computerized micro tomography

This technique was applied to two osteoderms (cranial and postcranial) in order to reveal local differences in the mineralization rate of the osteoderms, as reflected by regional contrasts in X-ray opacity appearing on virtual sections. The tomograph was a Viscom X8050, operated at 60 kV. Virtual sections were then made with the software Mimics 13.3 (Materialise ed., Belgium) after treatment of the rough files, and local X-ray opacity in the sections, expressed in Hounsfield units, was measured along a vector oriented from the deep to the superficial sides of the osteoderms (Fig. 4C).

2.2.4. Electron microprobe

This technique was used for mapping the local concentration of calcium and phosphorus, the main two components of the mineral phase of vertebrate mineralized tissues. Semiquantitative chemical maps were obtained on an analytical scanning electron microscope (Philips XL30) with an energy dispersive spectrometer (EDS) PGT Imix 10 system with a 10 mm² Ge detector at UMR IDES (Universit e Paris Sud). Operating conditions used were 15 kV accelerating voltage. Samples were embedded in a synthetic resin and polished by using various grades of diamond paste. The polished surfaces were lightly etched in 2% formic acid for 10 sec at room temperature to reveal microstructural details of the tissues. The elements Na, Mg, P, S, Ca, Mn, Fe, Sr and K were selected to illustrate aspects of tissue composition and possible diagenesis.

3. Results

3.1. Morphological features

Morphologically, glyptosaurine osteoderms (Fig. 1A–E) are characterized by a large size (up to 8.2 \times 3.5 mm), an important thickness (up to 2 mm, or more), a hexagonal (cranial osteoderms) or rectangular shape, a variably pronounced dorsal keel, and a typical granular ornamentation (Hoffstetter, 1962). This osteoderm morphology is distinctive of the Glyptosaurinae; the osteoderms of another anguid taxon, the Gerrhonotinae, have a similar aspect, but they are proportionally thinner (Aug e, 2005). The generic identification of our specimens is problematic because none of them was found in association

with taxonomically significant remains (see Sullivan and Aug e, 2006). They may belong to two genera that are relatively common in the Phosphorites du Quercy, *Placosaurus* Gervais, 1848–1852 and *Paraplicosauriops* Aug e and Sullivan, 2006. In the following, they are considered as belonging to the Glyptosaurinae, with no further specification.

Seventeen of them have a roughly rectangular shape, with an area up to 37 mm² and a mean thickness 0.9 to 1.25 mm (Fig. 1A,B). They represent postcranial osteoderms. The other ones are from the cranial region (Fig. 1C,D); they figure irregular hexagons measuring 10–23 mm² for a mean thickness 1.97–2.4 mm.

The superficial ornamentation of all osteoderms consists of numerous tubercles (Fig. 1A,C,E), either blunt or ended by a sharp apex that create a rough surface, typical of the granular ornamentation. Some tubercles are partly superimposed or seem to have fused with each other, especially at the top of osteoderm keel. In a single osteoderm the diameter at the base of the tubercles may vary from 0.3 to 0.5 mm. The tubercles have a whitish, vitreous coloration that clearly contrasts with the brown color acquired by the other regions of the osteoderms during fossilization. The anterior, “gliding surface” of each rectangular osteoderm (Fig. 1A) is smooth (this part is covered by the preceding osteoderm: Hoffstetter, 1962). The superficial side of the osteoderms also displays vascular pits located between the tubercles (Fig. 1A,C). The deep surface bears 6–8 vascular pits the diameters of which range from 60 to 240 μm (Fig. 1B). The peripheral (equatorial) margin of the cranial osteoderms, and the lateral sides of the postcranial ones, display deep folds and indentations indicative of sutural surfaces (Fig. 1D). Vascular pits also perforate these surfaces.

3.2. Microanatomical organization

Simple sections or breaks of the osteoderms show that all of them share the same basic diploe architecture (as already mentioned by Keller, 2009), with a central cancellous core bordered by two compact cortices (Figs. 1F,G and 2A). However, postcranial osteoderms are more compact than cranial ones, and only display small rounded cavities. In all cases, the cortices house vascular canals that are generally more numerous in cranial than in postcranial osteoderms. Vascular canals consist of either primary or secondary osteons that communicate with the large inner cavities, and open at the deep and superficial sides of the osteoderms by pits as mentioned above. Primary, simple vascular canals occur in some specimens, but they are generally sparse. A striking feature of all osteoderms is the conspicuous difference between the tissue composing the superficial, tubercular layer and the subjacent bony layers (Fig. 1F,G). Most of the volume of the osteoderms is occupied by typical bone that colored in brown during the fossilization process. Conversely, the superficial layer is whitish, vitreous, often translucent or even transparent. This layer is entirely compact and no cavity, i.e. small vascular canals or osteocyte lacunae, can be identified, whatever the magnification used.

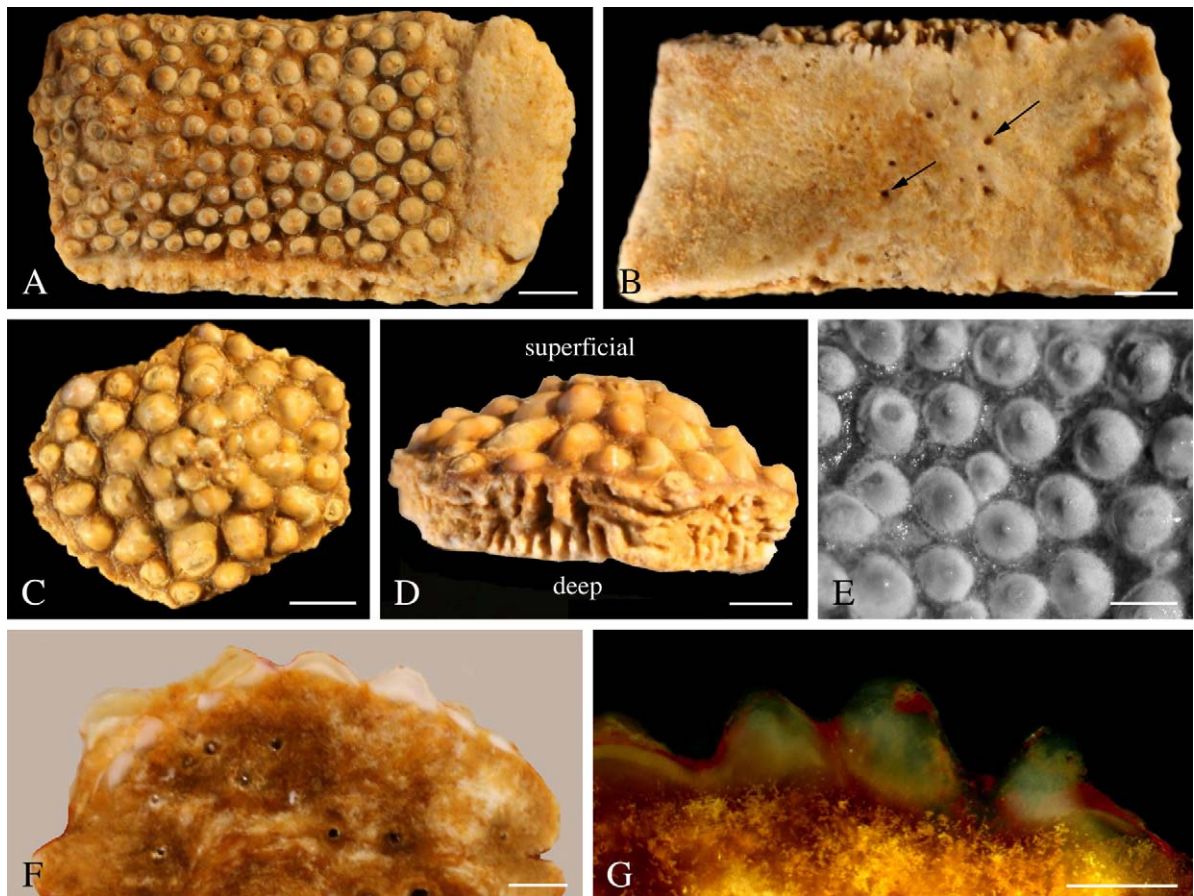


Fig. 1. General aspect of glyptosaurine osteoderms from the Phosphorites du Quercy (Lavergne and Sainte Néboule sites). A. Ornamented surface of a postcranial osteoderm. B. Deep side of a postcranial osteoderm showing the vascular pits (arrows). C. Surface of a cranial osteoderm. D. Lateral view of a cranial osteoderm showing a sutural surface. E. Close view of several tubercles forming the granular ornamentation of the osteoderm. F–G. Transverse sections of cranial osteoderms showing the different structures of the tissues composing the superficial and deep regions. Note the translucent aspect of the tubercle tissue. Scale bars: A–D = 1 mm; E–G = 0.5 mm.

Fig. 1. Aspect général des ostéodermes de Glyptosaurinae des Phosphorites du Quercy (sites de Lavergne et Sainte Néboule). A. Surface bien ornementée d'un ostéoderme postcrânien. B. Face profonde d'un ostéoderme postcrânien montrant les pertuis vasculaires (flèches). C. Surface d'un ostéoderme crânien. D. Vue latérale d'un ostéoderme crânien montrant la surface suturale. E. Détail de plusieurs tubercles formant l'ornementation granulaire à la surface d'un ostéoderme. F–G. Coupes transversales d'ostéodermes crâniens montrant les différences structurales des régions superficielle et profonde. Noter l'aspect translucide du tissu composant les tubercles de la région superficielle. Barres d'échelle : A–D = 1 mm ; E–G = 0,5 mm.

3.3. Histological structure

Cranial and postcranial osteoderms display four, clearly distinct tissues (Fig. 2). The first one consists in woven-fibered bone located in the core of the osteoderms (Fig. 2B). This tissue extends laterally into a broad, but relatively thin layer, the thickness of which is less than one third of the total osteoderm thickness. Vascular canals are mainly located in this region that displays evidence of intense remodeling. This process resulted in extensive Haversian substitution and broad resorption bays bordered by secondary lamellar bone.

In the basal region of the osteoderms the second tissue type consists in a thick layer of typical lamellar bone (Fig. 2C). This tissue contains numerous Sharpey's fibers that create two fiber systems: short (length 30–40 μm), robust (diameter up to 2.5 μm) and vertically-oriented fibers located deep into the lamellar cortex (Fig. 2C,D);

and long bundles of fibers oriented obliquely and located at the extremities of the lamellar bone layer. Periodic growth lines occur throughout the basal lamellar layer. The deepest half of the basal region has been subjected to an active resorption process, mainly progressing downward, that created extremely broad lacunae partly reconstructed by secondary deposits (imbalanced remodeling; Fig. 2C).

The basal layer laterally merges with a third type of tissue, a bony tissue particularly rich in Sharpey's fibers. These fibers are long, parallel to each other, but their direction makes an angle of some 30–40° with the fibers of the basal region (Fig. 2D). The bone tissue that houses such fibers is intermediate between woven-fibered and parallel-fibered bone, and displays an irregular birefringence in polarized light. It is located near sutural surfaces, where it forms an equatorial ring. Toward the center of the osteoderm, this tissue merges with the core of woven-fibered bone, while

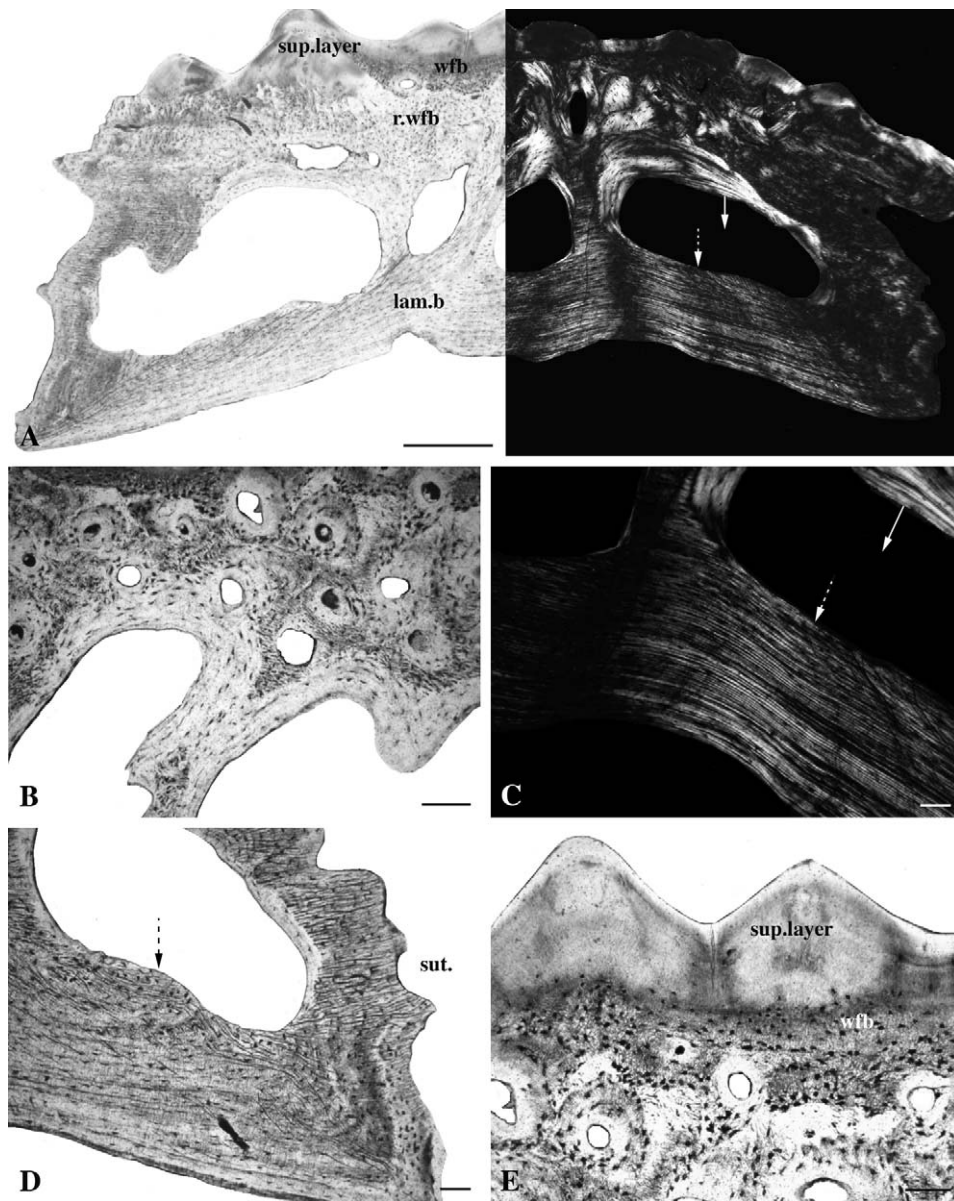


Fig. 2. Basic histological structure of the glyptosaurine osteoderms. A. Transverse section of a cranial osteoderm. Left: ordinary transmitted light; right: polarized light. B. Closer view at the woven-fibered tissue in the core of the osteoderm. C. Lamellar bone in polarized light. D. Osseous tissue rich in long anchoring fibers, subjacent to sutural surfaces. E. Tubercles forming the superficial layer. Dashed arrows indicate the direction of inner resorption and solid arrow secondary reconstruction. lam. b: lamellar bone; r.wfb: remodelled woven-fibered bone; sup. layer: superficial layer; sut.: suture; wfb: woven fibered bone. Scale bars: A, B = 0.5 mm; C–F = 100 μ m.

Fig. 2. Structure générale des ostéodermes. A. Coupe transversale d'un ostéoderme crânien observée, à gauche, en lumière transmise et, à droite, en lumière polarisée. B. Détail du tissu osseux à fibres enchevêtrées, situé au centre des ostéodermes. C. Os lamellaire observé en lumière polarisée. D.: Os riche en longues fibres d'ancrage, localisé sous les sutures. E.: Tubercules formant la couche superficielle. Les flèches en pointillés indiquent la direction de la résorption et les flèches pleines la reconstruction secondaire. Barres d'échelle: A, B = 0,5 mm ; C–F = 100 μ m.

its deep part shows evidence for extensive, imbalanced remodeling.

The fourth tissue type forms the superficial layer and is particularly thick, up to 400 μ m, at the tubercle level. Its histological characteristics completely differ from those of the three bone tissues described above (Figs. 2E and 3). It is indeed composed of a vitreous, avascular and acellular tissue that is totally monorefringent and does not

contain differentiated structures, with the exception of poorly-defined periodical growth marks (Fig. 3A,B). The limit between this tissue and the underlying woven-fibered bone is sharp. It is noteworthy that the superficial layer has the same histological aspect in all osteoderms studied, and that it does not display any sign of diagenetic alteration, even when the underlying bone tissue is heavily altered (Fig. 3C–E).

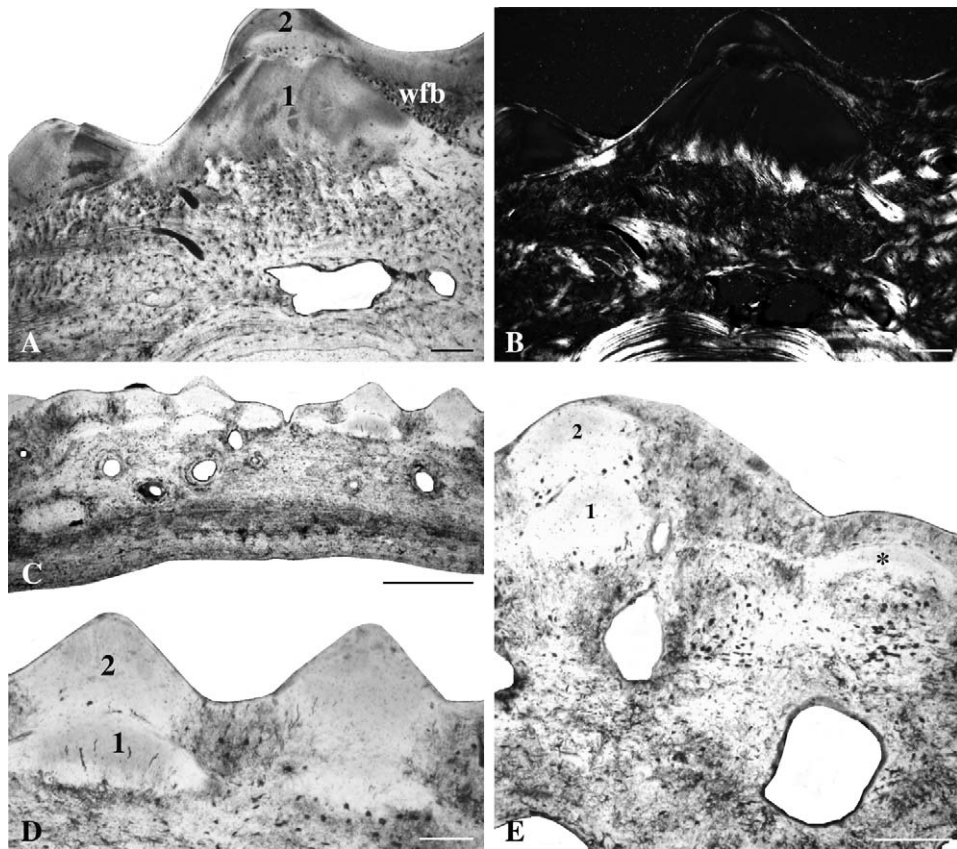


Fig. 3. Structure of the superficial layer. A. Detail of tubercles that were formed in two (1, 2) successive generations. Tubercle 1 has been subjected to resorption and woven-fibered bone has been deposited before tubercle 2 was formed. B. Same section as in A, viewed in polarized light. C. Superimposed generations of tubercles in a postcranial osteoderm. D. Detail of two (1, 2) superimposed, successive tubercles. E. Two superimposed tubercles (1, 2) on the left and tubercle covered by bone (asterisk). Scale bars: A–E = 100 μ m; C = 0.5 mm.

Fig. 3. Histologie de la couche superficielle. A. Détail de tubercules formés en deux (1, 2) générations successives. Le tubercule 1 a été partiellement résorbé et de l'os à fibres enchevêtrées a été déposé avant la formation du tubercule 2. B. Même coupe que A observée en lumière polarisée. C. Générations superposées de tubercules dans un ostéoderme postcrânien. D. Détail d'ostéodermes superposés. E. A gauche, deux tubercules (1, 2) sont superposés et, à droite, un tubercule a été recouvert d'os (astérisque). Barres d'échelle A–E: 100 μ m; C = 0,5 mm.

In several osteoderms, the superficial acellular layer comprises two superimposed strata of tubercles, often separated by a thin sheet of woven-fibered bone. This organization means that two successive generations of tubercles occurred at the same location, spaced with a short period, during which bone tissue was deposited (Fig. 3C–E). The superficial layer lacks Haversian systems, but in some osteoderms there is evidence for an erosion process restricted to the apex of the tubercles belonging to the first generation (Fig. 3A,B). Either a second tubercle was formed directly onto the resorbed surface of the previous one, or woven-fibered bone was deposited prior to the formation of a new tubercle. Conversely, the tissue located between the tubercles never displays any sign of resorption. Also, by place some tubercles are covered by woven-fibered bone and they do not protrude at the osteoderm surface (Fig. 3E).

3.4. Scanning electron microscopy (SEM)

Our histological observations were confirmed by SEM pictures showing a superficial layer devoid of osteocyte

lacunae, in contrast to the subjacent woven-fibered bone tissue that displays abundant cell lacunae (Fig. 4A,B). The boundary between the two tissues is not as clear-cut as suggested by histological observations. In particular, tissue indentations create a strong anchorage of the superficial layer into the woven-fibered bone.

3.5. Assessment of local mineralization rates

Transverse virtual sections obtained at every level of the osteoderms using computerized microtomography demonstrate unambiguously that the tissue composing the tubercles of the superficial layer is much more mineralized than the underlying bone tissue. The typical graph of X-ray opacity in the osteoderms, as measured along a depth-to-surface vector, is presented on Fig. 4C. The region of the graph that topographically corresponds to bone tissue (be it lamellar or remodeled woven-fibered bone) features a plateau at some 50 to 100 Hounsfield units (HU), whereas the region corresponding to the tubercles of the superficial layer displays a steep pick culminating at some 950 HU or more (the range of Hounsfield scale

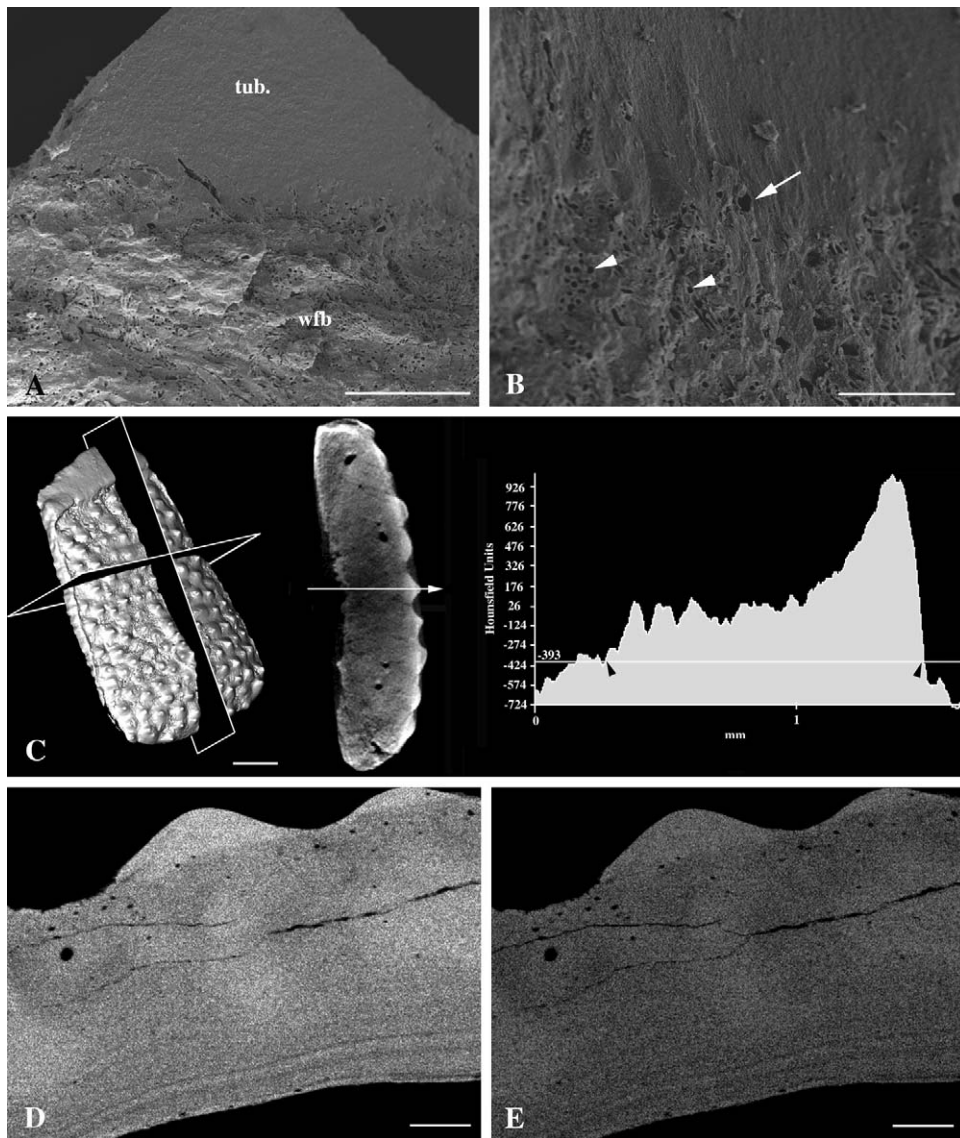


Fig. 4. Scanning electronic microscopy of the osteoderms, and assessment of their mineral content. A. General view of a broken osteoderm showing the tubercle structure (tub.) and the sub-jacent woven-fibered tissue (wfb). B. Closer view at the boundary between the superficial layer and the woven-fibered bone tissue; note the presence of numerous osteocyte lacunae (arrowheads) and several openings of small vascular canals (arrows) in the woven-fibered bone. C. Computed tomography of a postcranial osteoderm. Left side: 3-D reconstruction of the osteoderm with location of the transverse virtual section shown in the center of the figure. Center: virtual transverse section. The arrow on the virtual section shows the vector along which radio-opacity was measured. Right side: quantification of X-ray opacity. The arrowheads indicate the limit of the measurements, i.e., the area between the deep side and the outer surface of the osteoderm. D–E: Electron microprobe maps of calcium (D) and phosphorus (E) concentrations in the various regions of a postcranial osteoderm. Scale bars: A: 10 μm ; B: 50 μm ; C: 1 mm; D, E: 200 μm .

Fig. 4. Microscopie à balayage et estimation du taux de minéralisation des ostéodermes. A. Vue générale d'un tubercule fracturé (tub.) et de l'os à fibres enchevêtrées sous-jacent (wfb). B. Détail de la région frontière entre le tissu du tubercule et l'os; noter les nombreuses logettes ostéocytaires (têtes de flèches) et la présence de petits canaux vasculaires (flèches) dans l'os à fibres enchevêtrées. C. Microtomographie informatisée d'un ostéoderme postcrânien. À gauche: reconstitution tridimensionnelle de l'ostéoderme avec localisation de la coupe virtuelle montrée au centre de la figure. Au centre: coupe transversale virtuelle. La flèche montre le vecteur selon lequel la mesure de radio-opacité a été effectuée. À droite: quantification de l'opacité aux rayons X. Les têtes de flèches indiquent la limite des mesures, depuis la face profonde jusqu'à la surface de l'ostéoderme. D–E. Cartographie par microsonde électronique de la concentration du calcium (D) et du phosphore (E) dans les différentes régions d'un ostéoderme postcrânien. Barres d'échelle: A: 10 μm ; B: 50 μm ; C: 1 mm; D, E: 200 μm .

is -1000 to $+1000$ HU). There is thus a considerable difference in the mineral content of these two regions. The normal X-ray opacity of bone is in the range $400 - 900$ HU (Shetty et al., 2010); a mean value of 650 HU can thus be considered representative of bone *sensu lato*. It therefore

seems likely that the osteoderms underwent pronounced demineralization during fossilization. This demineralization process might have been uneven (depending on e.g. the local histological features of the osteoderm), a situation that could interfere with X-ray density measurements

and incites to carefulness in considering the absolute values of these measurements. It nevertheless remains that X-ray tomography reveals a considerable difference in mineral content between the superficial layer that displays values reaching the upper limit of Hounsfield scale in spite of possible demineralization, and the underlying osseous regions.

The mapping of calcium (Fig. 4D) and phosphorus (Fig. 4E) densities on osteoderm sectional surfaces using an electronic probe also gave unambiguous results: the two ions are clearly in higher concentration in the superficial, ornamented layer than in the underlying bone strata. For ion concentration, as well as for X-ray opacity, the highest values are strictly restricted to the tubercles themselves, while the bone regions located below and in some places between the tubercles display the same relative values as the deep bony parts of the osteoderms.

4. Discussion

4.1. Remark on the granular ornamentation

A granular ornamentation comparable to that described here on the surface of glyptosaurine osteoderms is not frequent in tetrapods. Among squamates it seems to be restricted to anguid osteoderms, mainly represented by Glyptosaurinae and Gerrhonotinae (Hoffstetter, 1962). In other taxa the osteoderms are either devoid of superficial ornamentation (e.g., the varanid *Varanus [Megalania] priscus*: Erickson et al., 2003; the gekkonid *Tarentola mauritanica*: Levrat-Calviac, 1986), or they display a “vermiculate” ornamentation consisting of branching grooves (e.g., the anguine *Ophisaurus apodus*: Romer, 1997), or pits separated by crests (e.g., the gekkonid *Tarentola neglecta*: Levrat-Calviac, 1986). Among the Anguinae, the glyptosaurine osteoderms strikingly differ from the slow worm (*Anguis fragilis*: Anguinae) osteoderms, which display a typically vermiculate ornamentation resulting from a perivascular resorption of the superficial layer (Zylberberg and Castanet, 1985). Therefore, from the broadest morphological level, there is a conspicuous variability between the osteoderms of closely related taxa (see on this topic: Hoffstetter, 1962; Levrat-Calviac, 1986). A comparison involving the dermal skeleton of the sarcopterygians as a whole confirms the relative sparseness of the granular ornamentation, but also reveals that the scales of some actinistians (Cœlacanthidae) bear an ornamentation pattern closely resembling that of glyptosaurine osteoderms (Ørving, 1977: pl. 2A,B, 3C). In both cases, the ornamentation of the dermal skeleton consists of individual tubercles 0.4 to 0.7 mm in basal diameter, with an ogive shape ended by a sharp apex, and separated by areas of bare bone.

4.2. Histological peculiarities of glyptosaurine osteoderms

The histological structure of lizard osteoderms has been described, with variable precision, in 15 species representing 12 genera and six families: Gekkonidae (Levrat-Calviac and Zylberberg, 1986; Levrat-Calviac, 1986), Anguinae

(Moss, 1969; Zylberberg and Castanet, 1985), Scincidae (Moss, 1969), Gerrhosauridae (Moss, 1969), Helodermatidae (Moss, 1969; Vickaryous and Sire, 2009), and Varanidae (Auffenberg, 1981; Erickson et al., 2003). In the most precisely studied taxa (*Tarentola*, *Anguis* and, to a lesser extent, *Heloderma*), the osteoderms display a simple structure, although the description of the composing tissues is sometimes confused due to the use of various terminologies. The osteoderms possess a deep (or basal) layer consisting of well characterized lamellar bone, and a superficial layer that is made of either a tissue poor in collagen fibers (so-called microfibrillar matrix), but containing osteocyte lacunae (e.g., *Tarentola*: Levrat-Calviac and Zylberberg, 1986), or of woven-fibered bone (e.g., *Anguis fragilis*: Zylberberg and Castanet, 1985). Moreover, if the mineralization of the basal layer is always of the inotropic type (i.e., apatite crystals located within and between the collagen fibrils), that of the superficial layer was described as being either inotropic as in *Anguis* or spheritic (i.e., formation of calcified spherules within a microfibrillar matrix), as in *Tarentola* (Levrat-Calviac et al., 1986). Up to now, no inner remodeling has been observed in lizard osteoderms (e.g., Moss, 1969). However, the ornamentation of the superficial layer was supposed to result from perivascular resorption (e.g., *Anguis fragilis* and *Tarentola neglecta*: Levrat-Calviac, 1986; Zylberberg and Castanet, 1985).

Glyptosaurine osteoderms differ from those of modern lizards by the greater complexity of their structure, the presence of a rich vascular supply, and an intense and extensive perivascular remodeling. In addition to its histological peculiarities, the superficial layer displays a remarkable feature: in numerous osteoderms, it resulted from two successive “waves” of formation separated by a period of woven-fibered bone deposition. To date, such a feature was not observed in other squamate osteoderms, but was described in the scales of some actinopterygians (Meunier, 1980; Ørving, 1978) and actinistians (Ørving, 1977). The possible meaning of this characteristic is further considered below (Section 4.4).

4.3. Development of glyptosaurine osteoderms

In squamate taxa for which detailed histological studies are available (Gekkonidae, Scincidae, Anguinae, and Helodermatidae), the osteoderms are considered as resulting from metaplasia (sensu Haines and Mohuiddin, 1968), a process known as a local mineralization of the dermis that is transformed into bone tissue although no osteoblast contributed to its formation (Moss, 1969, 1972; Levrat-Calviac, 1986). In this interpretation, the deep part of the dermis, rich in collagen fibers (*stratum compactum*), becomes the lamellar bone tissue that constitutes the basal part of the osteoderms. Conversely, the superficial part of the osteoderms originates from the upper dermal strata that are relatively poor in collagen fibers (*stratum laxum*). This dual structure is frequently observed in modern squamate osteoderms but the nature and formation of the superficial layer are variable from one taxon to another, and were diversely interpreted in the literature (see synthetic table in Moss, 1969; Vickaryous and Sire, 2009, p.

443). Moreover, the exclusive contribution of a metaplastic process can be questioned because active osteoblasts have been identified on osteoderm surface in *A. fragilis* (Zylberberg and Castanet, 1985) and *T. mauritanica* (Levrat-Calviac and Zylberberg, 1986). In other sauropsid groups, metaplasia would play a prominent role either as the only mechanism of osteoderm formation as, for example, in some sauropod dinosaurs (D'Emic et al., 2009), or as a temporary mechanism, involved only in the earliest stages of osteoderm formation, as in crocodylians (de Buffrénil, 1982; Vickaryous and Hall, 2008) and stegosaur plates (de Buffrénil et al., 1986; Cerda and Powell, 2010; de Ricqlès et al., 2001; Hayashi et al., 2009). The most notable exception to this trend is represented by placodont osteoderms that derive from cartilage anlagen (Scheyer, 2007).

The complex structure of glyptosaur osteoderms is poorly compatible with a growth pattern based exclusively on a metaplastic transformation of the dense and loose dermis. This conclusion relies on four observations made in this study, and listed below.

4.3.1. Tissue diversity

There are four tissue types, all clearly distinct by their locations and histological characteristics. If we refer to the osteoderm structure of *A. fragilis* (cf. Zylberberg and Castanet, 1985), taken here as an example because it is phylogenetically close to the glyptosaurus, we could consider that the woven-fibered tissue in the core of the osteoderms, and the thick lamellar layer in their basal part, are metaplastic transformations of the loose and dense dermal strata, respectively. However, in this interpretation, the origin of the tissue located against the sutures, and that is histologically well distinct from lamellar bone, would be unexplainable because it was situated at the same depth, within the dermis, as the lamellar tissue (Fig. 2A,D). Similarly, if the layer of woven-fibered bone corresponds to the loose dermis, then the superficial layer that is composed of a very different tissue must have another origin.

4.3.2. Bone remodeling

Glyptosaurine osteoderms, unlike those of other squamates, were submitted to intense inner remodeling that undoubtedly resulted from both osteoclast and osteoblast activities. Endosteal osteoblasts are known to be recruited from the osteoblast population lining the outer surface of growing bones, and penetrating the cortices through perivascular spaces (Karaplis, 2008; Krstic, 1988). The obvious occurrence of bone formation during osteoderm remodeling thus implies the presence of peripheral osteoblasts that were in contact with the osteoderm surfaces and likely constituted the equivalent of a periosteal cambium. This hypothesis would agree with the above-mentioned observation of peripheral osteoblasts in *Anguis* and *Tarentola* osteoderms (Levrat-Calviac and Zylberberg, 1986; Zylberberg and Castanet, 1985). Therefore, the growth of the lamellar basal layer, and that of the fiber-rich bone underlying the sutures in glyptosaurine osteoderms could possibly result, at least in great part, from osteoblast activity. Conversely, the superficial layer that is histologi-

cally different from bone tissue would result from another process (see below).

4.3.3. Sutures

Glyptosaurine osteoderms were tightly imbricated with each other, as evidenced by their complex sutural surfaces and the structure of the sub-complete osteoderm shields preserved on some fossils (Hoffstetter, 1955, 1962; Sullivan and Augé, 2006). Therefore, there was no dermal tissue reserve between them for their lateral expansion by metaplasia during growth.

4.3.4. Structure of the superficial layer

In all squamates studied so far, the tissue composing the superficial layer covering the osteoderms displays a roughly homogeneous structure; it houses few cell lacunae, if any, and sometimes reveals some cyclic growth marks (Levrat-Calviac, 1986; Moss, 1969; Zylberberg and Castanet, 1985). Such a simple structure can be interpreted as resulting from metaplasia, when considering for instance the expansion of a continuously growing mineralization front within a dermal region devoid of, or very poor in, collagen fibers. In contrast, the complex structure of the superficial layer in glyptosaurine osteoderms, with tubercles often organized in superimposed successive generations submitted to local resorption and separated from each other by layers of woven-fibered bone, cannot be explained as the result of such a mere metaplastic process.

Our observations, combined with the comparative data presently available in the literature, suggest the following interpretation. The early stage of development of glyptosaurine osteoderms started by means of a metaplastic process, as it occurs in all sauropsids, including the taxa in which another osteogenic mechanism is involved in later ontogenetic stages (e.g., crocodylians). The core of woven-fibered tissue was initiated at this stage, close to capillary blood vessels. Then osteoblasts differentiated around the osteoderm anlagen, and organized into a periosteal cambium. The osteogenic activity of these cells contributed to extend the bony layers of the osteoderm through the deposition of: bone tissue around the capillary blood vessels, thus creating primary osteons; lamellar bone on the deep surface; and the bone sub-jacent to the sutures. The histological difference between the latter two osseous formations (Sharpey's fibers being let apart) could merely reflect unequal growth rates (see, on this topic, de Margerie et al., 2004; de Ricqlès, 1976), bone deposition being faster in the lateral regions of the osteoderms in relation with their flat (polygonal or rectangular) morphology. In the meantime, remodeling of the bone matrix at the level of vascular cavities involved, on the one hand, osteoclasts, the precursors of which (monocytes) were brought in situ by the rich vascular supply of the osteoderms and, on the other hand, endosteal osteoblasts that were recruited from the outer cell layer and reached the appropriate loci through migration along the walls of the vascular canals opening at the osteoderm surface. The formation of the superficial layer was, to a great extent, independent from the processes considered for the

other parts of the osteoderms, and is commented upon below.

4.4. Nature and formation of the superficial layer

The vitreous, acellular tissue composing the superficial layer and the tubercles of glyptosaurine osteoderms neither exists in *A. fragilis* nor in *Ophisaurus* sp, two anguid lizards. The nature of this tissue cannot be settled with certainty in a fossil taxon, because developmental, histochemical and ultrastructural data are unavailable. Histologically, this tissue displays none of the typical characteristics of bone: it lacks altogether vascular canals, osteocyte lacunae, canaliculi or any kind of intra-osseous tubules that could have housed cytoplasmic extensions of osteoblasts. Moreover, its vitreous, transparent aspect on sections, and its completely monorefringent reaction to polarized light, suggest the absence (or very poor development) of a fibrillar, collagenous network. In addition, this tissue is clearly hypermineralized as compared to bone. Consequently, the histology of this tissue is inconsistent with any kind of bone tissue, including acellular bone (a tissue known, among living vertebrates, in acanthomorph actinopterygians only) that has a normal collagen network (Meunier, 1987). Also, this tissue cannot be considered as some kind of atypical osseous formation resulting from metaplasia for the reasons exposed above. A convergent conclusion, based on histochemical and ultrastructural data, had tentatively been approached by Levrat-Calviac (1986) about the superficial layer of *Tarentola* osteoderms.

At a broad comparative level, this tissue looks similar, at both the histological and microanatomic levels, to various hypermineralized tissues covering the integumentary skeleton of non-tetrapod vertebrates (review in Sire et al., 2009). These tissues include enamel and enameloids (odontodes and dermal denticles), ganoine (scales of the Polypteridae and Lepisosteidae), hyaloine (scutes of armored Siluriformes) and limiting layer (elasmoid scales). In addition, both the organization of the tubercles and the structure of the superficial layer of glyptosaurine osteoderms are strikingly comparable to various published pictures showing either the superposition and superficial resorption of odontodes on the scales of Polypteridae (Meunier, 1980; Meunier and Gayet, 1992), or the growth pattern of actinistian odontodes, with bone layers wedged between successive odontode generations (Ørving, 1977). Such a similarity is further substantiated by the mineralization rate of the superficial layer of glyptosaurine osteoderms that is much more compatible with enamel than with bone, as shown by the quantitative analysis of X-ray opacity on CTscan sections. However, it is noteworthy that the superficial, hypermineralized tissues occurring on the scales of non-tetrapod sarcopterygians are generally associated with dentine, which is obviously not the case in glyptosaurine osteoderms.

Therefore, considering these similarities, the most parcimonious conclusion is to consider that the tissue composing the superficial layer and the tubercles of glyptosaurine osteoderms actually belongs to the broad category of hypermineralized, enamel-like tissues. We call this particular tissue osteodermine.

The structural data presented here for osteodermine strongly suggest a similar mineralization process as previously described for the hypermineralized, enamel-like tissues: deposition of a loose organic matrix involving either dermal and epidermal or only epidermal contributions, mineralization and maturation, an important step during which most of the organic matrix is removed through proteolytic activity.

4.5. Remark on the distribution of hypermineralized tissues in sarcopterygians

In non-glyptosaurine squamates, the existence of a similar, non-osseous layer has been considered by Moss (1969, 1972) in *Heloderma* osteoderms. Vickaryous and Sire (2009) also concluded to the existence of such a tissue on the osteoderms of *Tarentola annularis* and *T. mauritanica*, as yet suggested by Levrat-Calviac (1986). Apart from lizards, such a tissue is unknown in other tetrapod osteoderms, whatever the clade to which they belong.

In the sarcopterygian clade, the occurrence of hypermineralized tissues on postcranial elements of the dermal skeleton has been hitherto clearly settled for basal taxa only: actinistians, some fossil dipnomorphs (i.e., dipnoans and porolepiforms: see review in Sire et al., 2009) and piscine stem tetrapodomorphs (the paraphyletic “osteolepiforms”: Vickaryous and Sire, 2009). Such a feature was not observed in any known tetrapod taxon, with a possible exception for some squamates as mentioned above (Witzmann, 2009; Witzmann and Soler-Giron, 2010 for basal tetrapods).

In teeth and various dermal skeletal elements, the formation of enamel-like tissues results from epidermal-dermal interactions, with an ultimate contribution of the epidermal basal layer cells in the deposition of organic matrix at the surface (Levrat-Calviac and Zylberberg, 1986; Moss, 1969, 1972; Sire et al., 2009). According to present documentation, such epidermal-dermal interactions, unexpressed but latent in deeply nested tetrapod taxa, would be expressed during osteoderm formation in certain squamates. The discontinuous distribution of this character raises the question whether the osteodermine of squamate osteoderms is a neof ormation, representing a developmental convergence with the scales of basal sarcopterygians, or a true homology (Vickaryous and Sire, 2009). The latter hypothesis would, of course, involve the persistence, during sarcopterygian evolution, of a potential capacity of epidermal cells to interact with the dermis to produce hypermineralized tissues in other regions than in the oral cavity. Up to now, there is no definite answer to this question, although the most parsimonious interpretation seems to be the second one. A significant contribution to solve this question would be to bring direct evidence for an epidermal participation to osteodermine formation in squamate osteoderms; an objective that could be achieved for instance by looking for remnants of enamel proteins in fossil and modern material or by demonstrating the expression of enamel proteins in epidermal cells located close to the osteoderm surface. Studies on these topics are currently in progress.

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