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Body location and tail regeneration effects on osteoderms morphology – are they useful tools for systematic, paleontology and skeletochronology in diploglossine lizards (Squamata, Anguinae)?

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Short title: Osteoderm morphology of diploglossines?

Abstract

Although diploglossine osteoderms were mentioned in several systematic and paleontological studies, their morphological variability in single specimens or within species remains paradoxically undescribed. This is mainly the effect of the lack of attention paid hitherto to the morphological and histological characteristics of the tail osteoderms. This study demonstrated

that a previously undescribed morphological variability exists in these osteoderms, especially in those resulting from tail regeneration. Indeed, regenerated osteoderms display a plesiomorphic anguid morphology that was previously considered to be absent in Diploglossinae. We also provide the first histological description of diploglossine osteoderms and new information about the obvious differences in growth dynamic between regenerated and non-regenerated osteoderms. These new data raise questions about the usefulness of diploglossine osteoderms in systematic, paleontological and skeletochronological studies. Our study shows that the exact position on the trunk or on a regenerated or non-regenerated tail of each studied osteoderm must be known in order to avoid mistakes related to their important inter- and intraspecies variability.

Key words: Aging, Anatomy, Galliwasp, Histology, Osteology.

Introduction

Several squamate families are known to present bony plates, osteoderms, that occur within the dermis under epidermal horny scales on the head or the whole body (Moss, 1969; Vickaryous and Sire, 2009). Among these families, members of the clade Anguinae display osteoderms on most of their head and body. These osteoderms differ from those of Scincidae by being composed of a single element while scincid osteoderms are compound (Hoffstetter, 1962). Among the anguines, two distinct types of osteoderms were described (Hoffstetter, 1962). The first is mainly encountered in gerrhonotines (Gerrhonotinae + Anguinae *sensu* Pyron et al., 2013) whose members bear a rigid armor formed of osteoderms strongly linked to each other. In this case, osteoderms are beveled along their lateral edges, which increases contact surfaces between osteoderms and thus, strengthens the shield that they form (Hoffstetter, 1962; Meszoely, 1970). The second type of osteoderms is encountered in Diploglossinae (*sensu* Pyron et al., 2013) and in

Anguis Linnaeus, 1758, the armor of which is formed of rounded, non-beveled osteoderms allowing shield flexibility (Hoffstetter, 1962; Meszoely, 1970). This last condition is supposed to be apomorphic within Anguinae (Meszoely 1970). In addition, osteoderm morphology in the three diploglossine genera (*Celestus* Gray, 1839; *Diploglossus* Wiegmann, 1834 and *Ophiodes* Wagler, 1828) has been especially well described and studied by Strahm and Schwartz (1977) who proposed a character for the distinction of *Diploglossus* and *Celestus*: the occurrence of a cloud-like radix system of nutritive canals under the gliding surface of the osteoderms which is visible without special preparation due to the thinness of the osteoderm. As a consequence osteoderms were commonly used in anguid systematics (Hoffstetter, 1962; Strahm and Schwartz, 1977; Gauthier, 1982; Mead et al., 1999).

However, the taxonomic significance of anguid osteoderms suffers obvious limitations because it refers exclusively to trunk osteoderms and takes no account of osteoderm morphology from other regions of the body. Moreover, it is commonly known that anguids, like other lizards, can regenerate their autotomized tail (Bryant and Bellairs, 1967; Guibé, 1970), and that scale morphology may strongly differ between regenerated and non-regenerated parts of the tail in several taxa (Woodland, 1920; Bustard, 1970), including *Anguis* (Ali, 1949; Bryant and Bellairs, 1967). In Anguinae, osteoderm histology and ultrastructure were described in detail in *Anguis fragilis* Linnaeus, 1758 by Zylberberg and Castanet (1985), the same authors also described osteoderms from other taxa, for instance in the gecko *Tarentola* (Levrat-Calviac and Zylberberg, 1986); however, possible peculiarities of regenerated osteoderms were not considered. As a consequence, potential intraspecific variability in anguid osteoderm morphology and structure remains largely unknown, a situation that can be especially problematic in paleontology.

The aim of this study is to further document intra-individual and intra-generic variability of osteoderm morphology and structure in diploglossine lizards. Our intention is to consider the influence of growth and regeneration on these characteristics, and assess the final relevance of osteoderm description in systematic and paleontological studies.

Materials and Methods

The biological sample comprised 82 anguid osteoderms collected from 17 specimens and including 52 diploglossine osteoderms and 30 osteoderms from other subfamilies (Gerrhonotinae -genera *Barisia* Gray, 1838; *Gerrhonotus* Wiegmann, 1828 and *Elgaria* Gray, 1838- and Anguinae -genera: *Ophisaurus* Daudin, 1803 and *Anguis*-).

Most of the diploglossine osteoderms (36) were directly extracted from six dry or alcohol preserved museum specimens belonging to the Muséum national d'Histoire naturelle (MNHN-RA, Paris, France), the Senckenberg Museum of Frankfurt (SMF, Germany) and the Museum of Comparative Zoology of Harvard (MCZ, USA): *Celestus bivittatus* (Boulenger, 1895) (SMF 79022), *Celestus occiduus* (Shaw, 1802) (MNHN-RA 1227), *Diploglossus monotropis* (Kuhl, 1820) (MNHN-RA 1906.0229 and SMF 93787), *Diploglossus plei* Duméril and Bibron, 1839 (MCZ 131518), and *Ophiodes striatus* (Spix, 1824) (SMF 99342). Osteoderm sampling was made with a scalpel and the position of each osteoderm on the body was recorded. Since the morphology of trunk osteoderms was already well-known (Strahm and Schwartz, 1977) we only sampled two trunk osteoderms on each specimen in order to check previously published observations. These osteoderms were all extracted on the lateral part of the trunk just behind the forelimb. In order to take account of the impact of regeneration processes on osteoderm morphology we first checked the regenerated or non-regenerated condition of the studied tail

parts on each alcohol preserved specimens using the MECADEV (CNRS-MNHN) radiography platform. Our observations showed that these six specimens had regenerated their tail to variable extents. For some of them this condition (regenerated tail) could not be assessed on the sole basis of external tail morphology and scale pattern. Consequently, osteoderms were extracted from the proximal (non-regenerated) and distal (regenerated) thirds of the lateral part of the tail on the basis of their x-ray images. Additional osteoderms from the mid-length of the tail (non-regenerated on *D. monotropis* and regenerated on *C. occiduus*) were collected only on the MNHN-RA specimens. Our *D. plei* specimen (MCZ 131518) was a dry skeleton an only regenerated tail and some trunk osteoderms were observable.

Additionally, we observed previously prepared trunk osteoderms from MNHN-RA diploglossine specimens were also observed: *C. striatus* Gray, 1838 (synonym of *Celestus stenurus* (Cope, 1862)) (MNHN-RA 1897.0010), *D. monotropis* (MNHN-RA 1906.0229), *D. delasagra* (Cocteau, 1838 in Cocteau and Bibron, 1843) (MNHN-RA 2859) and *Ophiodes vertebralis* Bocourt, 1881 (MNHN-RA 7144), as well as osteoderms from other anguid genera: *Anguis fragilis* (MNHN-RA 1922.0089), *Barisia imbricata* (Wiegmann, 1828) (MNHN-RA 6136), *Gerrhonotus liocephalus* Wiegmann, 1828 (MNHN-RA 1637), *Elgaria multicarinata* (Blainville, 1835) (MNHN-RA 2002), *Ophisaurus harti* (Boulenger, 1899) (MNHN-RA 1908.0006), *Ophisaurus koellikeri* (Günther, 1873) (MNHN-RA 1912.0469), and *Ophisaurus ventralis* (Linnaeus, 1766) (MNHN-RA 7046).

In the alcohol preserved specimens two osteoderms were removed in each of the three of four sampled body parts in order to cross-section one of them for histological observations. Consequently, among the sampled osteoderms, 16 were photographed before being embedded in a polyester resin, sectioned along their transversal axes, mounted on a glass slide and then

polished to obtain ground sections some 100 μm thick. The slides were observed with a compound microscope in natural and polarized light. Unfortunately, we were unable to cross-section all the other sampled osteoderms that were already mounted on microscope slides during the studies of R. Hoffstetter (1962).

The nomenclature used follows the terminologies defined by Buffr enil et al. (2010) and Strahm and Schwartz (1977). Following these terms, the osteoderms present a superficial (=external *sensu* Strahm and Schwartz, 1977) surface and a basal (=internal *sensu* Strahm and Schwartz, 1977) surface. This superficial surface is divided into an anterior portion called the gliding surface that lie below the osteoderm of the previous row and a posterior portion that is connected to the apex of the epidermal scale (Fig. 1: A).

Results

External morphology

Non-regenerated osteoderms

Trunk osteoderms (Fig. 1; Table. 1)

External morphology of trunk osteoderms is similar to that described in previous studies: they are rounded in superficial view, flat in transversal section (Fig. 1: F), thin (thickness/length ratio is between 5 to 9%), and display a crescentic gliding surface with a posterior projection, but lack beveled lateral edges (Hoffstetter, 1962; Strahm and Schwartz, 1977). As previously reported, *Ophiodes* and *Diploglossus* osteoderms have a “cloud-like” radix system on their anterior portion or gliding surface which is absent in *Celestus* (Strahm and Schwartz, 1977). This character has been considered as ontogenetically variable (Wilson et al., 1986), which is in agreement with our

observations because radix system on our smallest specimen of *Diploglossus* is less developed (*D. delasagra* MNHN-RA 2859).

Osteoderms of the proximal part of the tail (Fig. 2; Table. 1)

Osteoderms of the proximal third of the tail display slight differences with those from the trunk: they are less flat in transversal section, more transversally arched (Fig. 2: F) and thicker (thickness/length ratio between 9 to 13%). Additional differences occur in *Celestus* where the proximal third of tail osteoderms bear a cloud-like radix system that does not occur on trunk osteoderms. Whereas in *Ophiodes* these osteoderms are slender than trunk osteoderms, and also present a reduction of their gliding surface, a smaller posterior projection and a less developed radix system.

Osteoderms of the mid-length of the tail (Fig. 3; Table. 1)

Because most of our specimens have a regenerated tail forming more than two-thirds of the full length of the tail we could only observe osteoderms from the mid-length of a non-regenerated tail on two specimens (*D. monotropis* -MNHN-RA 1906.0229- and *C. bivittatus*). These osteoderms present different morphologies. The *D. monotropis* osteoderm (Fig. 3: A) has a subcircular shape in superficial view, very similar to the more proximal ones, but is divided into two smaller osteoderms of rectangular shape only partially fused together in superficial view that are nested together by two small hooks on the basal side of the osteoderm. This division may represent the transition from an ovoid shape for the proximal ones to a rectangular shape for the distal ones but unfortunately, morphology of the non-regenerated distal osteoderms remains unknown to us. The *C. bivittatus* osteoderm (Fig. 3: B) is even more distinct. Its general shape is strongly arched in transversal section and it presents an important reduction of its gliding surface that is void of

radix system and posterior projection. Both these osteoderms are thin (thickness/length ratio of 7 and 9%, respectively).

Regenerated osteoderms

Osteoderms of the mid-length of the tail (Fig. 4; Table. 1)

In three of our specimens (*D. monotropis* - SMF 93787-, *D. plei* and *C. occiduus*) the second third of the tail was regenerated. The morphology of these osteoderms is very different from that previously described since they bear a pit-like ornamentation on their posterior portion and are all beveled along their lateral edges. This last character shows that these osteoderms are more tightly imbricated and so form a less flexible shield than the osteoderms of the trunk and non-regenerated part of the tail. This condition is considered as plesiomorphic within Anguillidae (Meszoely, 1970) and was absent in the non-regenerated osteoderms. However, their morphology is still highly variable. In MCZ 131518 (*D. plei*) and MNHN-RA 1227 (*C. occiduus*) (Fig. 4: A & B) the osteoderms are oval in superficial view, and thicker than non-regenerated ones (thickness/length ratio between 17 to 25%). In SMF 93787 (*D. monotropis*) (Fig. 4: C), the osteoderms seem to result from the fusion of two rectangular osteoderms (as shown by the presence of a superficial furrow on their longitudinal axis) to form simple osteoderms beveled along their lateral edges. However, histological observations reveal that this osteoderm formed as a whole, in one piece and that it was probably newly formed (see below: Table. 1); its morphology may thus reflect an early developmental stage.

Osteoderms of the distal part of the tail (Fig. 5; Table 1)

Two clearly different osteoderm morphotypes were observed in the distal third of regenerated tails of our specimens. The first one, in *D. plei* and *C. occiduus*, is strictly similar to the

osteoderms previously described in the mid-length of the tail of the same specimens (Fig. 5: A & B).

The second type was observed in *D. monotropis*, *C. bivittatus* and *O. striatus* (Fig. 5: C-F). These osteoderms are rectangular instead of ovoid in superficial view, beveled on their medial or lateral sides and their posterior portion is ornamented with ridges or small pits. They are thin (thickness/length ratio between 7 to 13%) and lack “cloud-like” radix system on their gliding surface. This gliding surface is broad in the largest *D. monotropis*, but proportionally much smaller in all the other specimens, which suggest that this character could be ontogenetically variable.

The two morphotypes do not reflect ontogenetic variability because both are present in “adult” osteoderms (see below). They could possibly be linked to osteoderm position on the tail portion that was regenerated after autotomy, but this remains hypothetical.

Osteoderm histology

Non-regenerated osteoderms

All non-regenerated osteoderms from the trunk and tail display a similar histological structure. They are made of three distinct tissue types, located, respectively, in superficial and basal regions of the osteoderms, and in the core the osteoderms (Fig. 6):

- 1) The superficial cortex is composed of a tissue type intermediate between parallel-fibered and lamellar bone (Fig. 7 cortex a) and is the thinnest cortex of the osteoderm (less than one fifth of the osteoderms’ maximal thickness) (Fig. 7: A). In polarized and natural light this cortex shows an alternation of very thin dark and slightly thicker light strata decreasing in thickness from

cortical depth to the periphery of the osteoderms. A similar structure but with far thicker strata is also observed in the deep cortex of the osteoderms and will further described below. Some flat osteocyte lacunae occur in this tissue. The outer surface of this cortex displays evidence of extensive superficial resorption and, in four of our five specimens, this cortex was either partly or entirely eroded (Fig. 7: D). This resorptive process is not followed by secondary reconstruction deposits.

2) The core of each osteoderm is composed of a thin cortex of woven-fibered bone (Fig. 7 cortex b) the thickness of which represents less than one fourth of osteoderms' maximal thickness (Fig. 7: A). Remnants of primary bone tissue in this cortex are monorefringent in polarized light and show high density of rounded osteocyte lacunae, distributed unevenly within the bone matrix. This tissue also displays evidence of strong remodeling, in the form of numerous large resorption bays, the walls of which are partly reconstructed by secondary endosteal deposits of lamellar bone tissue, separated from primary deposits by resorption lines (Fig. 7: B (arrows)). This central bone cortex is bordered in upper (superficial) and lower (basal) positions by two quite distinct bone formations.

3) The deep (basal) cortex (basal plate *sensu* Buffrénil et al., 2010) (Fig. 7 cortex c) is composed of parallel-fibered bone extending on one third to one half of osteoderms' maximal thickness (Fig. 7: A). As in the superficial cortex, this cortex shows in polarized and natural light an alternation of thin dark and thicker light strata. Decrease in thickness of these strata from cortical depth (where they are some 20 μm thick) to periphery (where stratum thickness is less than 10 μm) indicates that they represent cyclic growth marks (later designated as CGMs) rather than bone lamellae (Fig. 7: C). These strata also display an irregular birefringence in polarized light, also supporting this conclusion. The more important thickness of these CGMs, as compare to

these observed in the superficial cortex, indicate that bone accretion over the basal cortex was more active than on the superficial cortex (Fig. 7: B). In both cortexes (superficial and basal) osteocyte lacunae are flattened and less densely distributed than in the core region. Their morphology and their occurrence in both light and dark strata, with no change in their orientation, are further evidence in favor of the parallel-fibered bone tissue type. Many obliquely oriented Sharpey's fibers (collagen fibers that anchor the osteoderm to outer adjacent structures - Francillon-Vieillot et al., 1989-) of variable length cross this cortex (Fig. 7: C).

The precise number of CGMs in each osteoderm was uneasy to assess because the deep cortical cortexes was often resorbed. Despite these limitations, the number of counted growth lines was relatively stable in the non-regenerated osteoderms of a single specimen (Table 2). The maximum number of CGMs observed in the basal plate of non-regenerated osteoderms, 13 CGMs, was consistent with a yearly deposit of these marks since available data attribute a maximal longevity more than 12.3 years for *Diploglossus* and *Celestus* (de Magalhaes and Costa, 2009; Henderson and Powell, 2009). In addition, the specimen presenting the highest number of CGMs, *D. monotropis* (SMF 93787), was kept in captivity until its death and so may have reached an advanced age. Therefore the number of CGMs in the basal cortex was likely to correspond to the age, in years, of the animal (one CGM by year).

In the osteoderms of SMF 93787 (*D. monotropis*) (Fig. 7: A-E) the deep cortex is superficially bordered by an additional cortex of similar structure but with blurred cyclic growth marks and lesser birefringence (Fig. 7 cortex d). Some broadly spaced CGMs can nevertheless be observed, together with densely-packed bundles of Sharpey's fibers obliquely orientated. The density of Sharpey's fibers could indicate a preferential anchoring area, and suggests the local occurrence of a particular mechanical context. Moreover, the broad spacing of the CGMs is likely to reflect a

local resumption of growth (possibly due to traction of Sharpey's fibers?) after a previous stage of growth slackening.

In the lateral parts of the osteoderms the CGMs of the basal plate are in continuity with those of the superficial cortex, a situation suggesting that a unique, continuous osteogenetic process, creating the superficial cortex, occurs all around the osteoderm.

With reference to the histological observations developed above, the growth of the osteoderms can be reconstructed as follows: Their oldest part (core of osteoderms), composed of woven-fibered tissue, could be produced by a metaplastic process that directly transforms the dermis into bone, as it occurs in lepidosaurian (Zylberberg and Castanet, 1985; Buffr enil et al., 2011) and crocodylian (Vickaryous and Hall, 2008) osteoderms. After this initial stage, classical osteoblastic osteogenesis takes place, and produces the deep and superficial cortices. Cortical growth is clearly more active on the basal osteoderm surface, as shown by the spacing of the CGMs.

Regenerated osteoderms

Regenerated osteoderms display a histological organization very similar to the non-regenerated osteoderms yet with some differences including the occurrence of a fourth type of tissue in the largest osteoderms (Fig. 8). Their histological structure is also more variable because they may represent different growth stages, from those bearing no basal plate and only a woven-fibered core (Fig. 9: A) to some presenting a thick basal plate with numerous (most likely non-annual; see below) CGMs (Fig. 9: B). The four tissues encountered are the following:

- 1) The superficial cortex (Fig. 9 cortex a) of regenerated osteoderms present some differences with the superficial cortex of non-regenerated osteoderms. In the regenerated osteoderms, this

cortex is thicker, the CGMs are wider and their number more variable, and osteocytes lacunae are far more numerous (Fig. 9: C) than in the non-regenerated osteoderms. In addition, unlike in the non-regenerated osteoderms, no strong resorption of this cortex was observed.

2) All regenerated osteoderms present a core of woven-fibered bone (Fig. 9 cortex b) strictly similar to that of non-regenerated osteoderms (Fig.9: B-E). The difference is that this cortex is thicker since it can occupy more than half of the total thickness on the smaller osteoderms to one third on the large osteoderms.

3) The basal plate (Fig. 9 cortex b) is also similar to that of non-regenerated osteoderms. Its thickness is highly variable: thin in the small osteoderms with no clearly distinct CGMs (Fig. 9: A) and thick in larger osteoderms displaying many CGMs (Fig. 9: B). The main difference with non-regenerated osteoderms is about the number of growth lines. In the largest osteoderms the number of CGMs is significantly higher (up to a maximum of 25) than in the non-regenerated osteoderms of the same specimen (Fig. 9: B, Table 2); conversely, the number of growth marks is smaller in the smallest regenerated osteoderms (Fig. 9: A, C, Table 2). Unlike the situation of non-regenerated osteoderms, no cortex possibly related to growth resumption was observed.

4) On the biggest regenerated osteoderms (MNHN-RA 1906.0229, *D. monotropis*; and MNHN-RA 1227, *C. occiduus*) a fourth type of tissue occurs in the lateral margins of the sections (Fig. 9: D). This tissue displays irregular birefringence, a high density of rounded osteocytes lacunae and numerous, long Sharpey's fibers parallel to each other and nearly perpendicular to the orientation of the basal plate Sharpey's fibers. It represents woven-fibered tissue with extremely dense bundles of anchorage (Sharpey's) fiber bundles (Sharpey's fiber bone). It was not observed in the non-regenerated osteoderms but is similar to that described by Buffr nil et al. (2010) in the same

area of glyptosaurine osteoderms. The occurrence of this tissue may be linked to the fact that all these osteoderms are beveled along their lateral edges and strongly linked to each other by Sharpey's fiber. Thus its occurrence could also be an anguoid plesiomorphic character.

Discussion

Our results clearly show that intra-individual and intra-generic morphological variability of diploglossine osteoderms is much greater than previously supposed. We reported morphology of regenerated and non-regenerated osteoderms of the tail and pointed out a probable reappearance of plesiomorphic anguoid characters in regenerated osteoderms.

Morphological variability of diploglossine osteoderms

We reported a previously unknown intra-individual morphological variability in diploglossine osteoderms. Non-regenerated osteoderms from the trunk display all the characteristic features already described in literature; conversely tail osteoderms deviate from this morphology depending of their position on the tail. Osteoderms of the proximal third of the tail are similar to those from the trunk but less flat, more transversally arched and slightly thicker. This character should thus be re-defined and restricted as only applicable to trunk osteoderms. As previously reported by Wilson et al. (1986), the expansion of the radix system seem partly subject to ontogenetic variability and was discarded by Savage et al. (2008) for this reason. However, no radix at all occurs on trunk osteoderms of *Celestus* specimens we observed (Fig. 1). Thus, we disagree with Savage et al. (2008) and believe that this character can still be useful if carefully used.

Osteoderms from the mid part of the tail present two different morphologies that strongly differ from the morphology of the trunk osteoderms. On *D. monotropis* (MNHN-RA 1906.229), the

shape of these mid tail osteoderms is similar to the others osteoderms from trunk and proximal third of the tail but it's formed of two fused rectangular osteoderms linked by a nip on their basal side (Fig. 3: A). This could indicate the occurrence of osteoderm of different morphology in the more distal part of the non-regenerated tail but since all were regenerated in our material we were unable to observe it. The second morphology observed on *C. bivittatus* (SMF 79022) was even more different, the general shape was less rounded, the gliding surface reduced, the osteoderm is transversally arched rather than flat, and lack the cloud-like radix system and the posterior projection characteristic of Diploglossinae that occurred on all the others non-regenerated osteoderms (Fig. 3: B). This *Celestus* osteoderm did not bear any of the diploglossine characters except that it is not beveled along their lateral edges.

Histologically, all non-regenerated osteoderms present the same basic organization with three different types of tissues: a core of woven fibered bone, a basal plate of parallel-fibered bone presenting CGMs, and an superficial cortex composed of an intermediate tissue type between parallel-fibered and lamellar bone (Fig. 6 and 7). A similar organization was observed in glyptosaurine osteoderms (Buffrénil et al. 2011) but their basal plate is made of lamellar bone and their superficial cortex includes a hyper-mineralized tissue that does not occur in diploglossines. Both these organizations differ from that of another anguid osteoderms: *Anguis fragilis* (Zylberberg and Castanet, 1985) that is simpler with only two types of tissue: a superficial cortex of woven-fibered bone and a basal plate of parallel fibered bone. These facts suggest that this simple organization could be an *Anguis* apomorphic character. However, diploglossine non-regenerated osteoderms share the absence of the Sharpey's fiber bone and a parallel-fibered basal plate with *Anguis fragilis* osteoderm, two characters that do not occur in glyptosaurine osteoderms.

Regenerated osteoderms are morphologically highly variable because they reflect different growth stages and positions on the tail (Fig. 4 and 5). However, clear general differences with the non-regenerated osteoderms can be noted. They tend to be thicker and bear ornamentation formed of small punctures on their posterior portion. But the most striking difference is that the regenerated osteoderms are beveled along one or both of their lateral edges. Such morphology, indicative of a strong link between adjacent osteoderms, was not known to occur in diploglossine anguids. This strong link is histologically represented in our diploglossine osteoderms by a tissue very rich in Sharpey's fiber (Sharpey's fiber bone) occurring in the lateral borders of the osteoderms, that is absent in the non-regenerated osteoderms (Fig. 8 and 9). This type of tissue was previously reported in other squamates (Levrat-Calviac and Zylberberg, 1986; Buffrénil et al., 2010, 2011) but also in the xenarthan mammal *Dasyurus novemcinctus* Linnaeus, 1758 by Vickaryous and Hall (2006). The diploglossine body is entirely covered with osteoderms that usually lack such links that allow sliding between them and the movement of the animal (Hoffseteter, 1962). This condition is considered as apomorphic in regard of the plesiomorphic condition encountered in Gerrhonotinae (Meszoely, 1970). The occurrence of osteoderms beveled along their lateral edges in the regenerated diploglossine tail is consequently very surprising and seems to be a reversion to a more ancestral condition (atavism) after regeneration.

Barbour and Stetson (1929) hypothesized a similar phenomenon when comparing the scaling pattern of the regenerated tail of modern *Sphenodon* Gray, 1831 to scaling pattern of the fossil sphenodontid *Homeosaurus maximiliani* Wagner, 1853. In *Ophisaurus gracilis* (Gray, 1845), Boulenger (1888) observed the occurrence of quadrangular scales on the trunk but cycloid scales on the regenerated tail. No observations were made about the morphology of the regenerated osteoderms from this last species but the shape of the regenerated scales could match with the

regenerated osteoderms we described in Diploglossinae but could also be similar to the non-regenerated osteoderms of this family. Further investigations are needed to clearly assess the phylogenetic significance of these differences between regenerated and non-regenerated osteoderms.

The use of osteoderms in systematic and paleontology studies

Many characters are traditionally used to separate anguid genera by their osteoderms (Hofftetter, 1962; Meszoely, 1970) including thickness, length/width ratio, expansion and shape of the gliding surface and, the most important, the occurrence or absence of a lateral bevel. However, this study points out that these characters are prone to intra-individual and intra-generic variability depending of the position, age and regenerated or non-regenerated condition of the osteoderms. These biases can be easily avoided by using trunk osteoderms of adult specimens. Observation of the osteoderms used in some classical systematic studies (Hofftetter, 1962; Meszoely, 1970; Strahm and Schwatz, 1977), reveals that only trunk osteoderms of adult specimens were taken into account. That is why we do not question the reliability of the pre-existing characters established by these studies. However, this question becomes far more problematic in paleontological studies when isolated osteoderms of unknown body localization are considered. Fortunately, our result does not demonstrate that such osteoderms cannot be used at all, and we found that combination of characters still allow a reliable identification of diploglossine osteoderms.

-diploglossine osteoderms from trunk and proximal part of the tail: rounded shape, flat, not beveled along their lateral edges, present a crescent shape gliding surface with a posterior peak. *Celestus*: absence of a cloud-like radix system on the gliding surface. We believe that the

distinction between osteoderms from the trunk and proximal part of the tail is difficult and thus we only consider here characters applicable to both of them. The other diploglossine genera (*Diploglossus* and *Ophiodes*) cannot be distinguished on the basis of morphology of isolated osteoderms. These osteoderms are similar to *Anguis* osteoderms but *Anguis* lack posterior projection of the gliding surface.

-diploglossine osteoderms from regenerated tail: ovoid shape never clearly rectangular, gliding surface broadly extended on one of both of the lateral margins and never of band appearance, beveled on one of both of the lateral edges, posterior portion ornamented with small ridges or pits, possible occurrence of a posterior projection and lack of medial keel. However, some *Ophisaurus* osteoderms presents a morphology that cannot be distinguished from diploglossine regenerated osteoderm.

Consequently, although some combinations of characters seem still typical of diploglossines, the important morphological variability of regenerated osteoderms makes their identification complicated because they can provide ambiguous taxonomic conclusions. Therefore, as previously suggested for other taxa (Hill and Lucas, 2006; Burns, 2008), we recommend avoiding the use of isolated osteoderms (not located on a particular body part) in systematic studies and especially in paleontological studies.

Use of osteoderms in skeletochronology

We observed that, in polarized light, the basal plate shows an alternation of light and dark strata progressively decreasing in thickness towards bone periphery. Such deposits are undoubtedly cyclic growth marks reflecting cyclical decreases in individual growth rate but the histological variability of the osteoderms raises question about the possibility of their use for estimating the

age of the specimen using skeletochronology (Castanet et al., 1977; Castanet, 1978). Our observations show that the number of growth lines is roughly stable in the non-regenerated osteoderms of a single specimen (Table 2) and that they could possibly be yearly deposits. Non-regenerated osteoderms seem therefore potentially useful for skeletochronology. However, the number of CGMs observed in regenerated osteoderms is every time very different depending of the “maturity” of the regenerated osteoderm (Table. 2). In addition, our observations show a clear difference in growth cyclicity between non-regenerated and regenerated osteoderms. Taking account of this bias, it is clear that regenerated osteoderms cannot in any manner be used to estimate the age of a specimen.

To conclude, our results show that diploglossine osteoderms display an important morphological variability at both intra-individual and intra-specific levels according to their location on the body (trunk, non-regenerated tail or regenerated tail) and age. Tail regeneration strongly modifies the morphology of diploglossine osteoderms that present a plesiomorphic anguid condition after regeneration that is absent in non-regenerated ones. These new data make the use of osteoderms in systematic and paleontological studies subject to caution when a doubt exists about their exact localization on the body and about their regenerated or non-regenerated condition. We have also shown that regenerated and non-regenerated tail osteoderms probably do not follow the same growth rates and that the application of skeletochronology should be limited to non-regenerated osteoderms.

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Table 1: Summarize of the main characters observed on diploglossine osteoderms.

Taxon	Anatomical location	Regenerated	Shape in superficial view	Shape of transversal section	Length/Thickness ratio	Posterior projection of the gliding surface	Occurrence of a cloud like radix system on the gliding surface	Beveling of the lateral edges	Ornamentation of the posterior portion
<i>Diploglossus</i>	Trunk	No	Ovoid	flat	5 - 6%	Yes	Yes	No	Ridges
<i>Celestus</i>	Trunk	No	Ovoid	flat	6 - 9%	Yes	No	No	Ridges
<i>Ophiodes</i>	Trunk	No	Ovoid	flat	6%	Yes	Yes	No	Ridges
<i>Diploglossus</i>	proximal third of the tail	No	Ovoid	Transversaly arched	9 - 13%	Yes	Yes	No	Ridges
<i>Celestus</i>	proximal third of the tail	No	Ovoid	Transversaly arched	10 - 12%	Yes	Yes	No	Ridges
<i>Ophiodes</i>	proximal third of the tail	No	Ovoid	Transversaly arched	9%	Yes	Yes	No	Ridges
<i>Diploglossus</i>	mid-length of the tail	No	Subcircular	Transversaly arched	7%	Yes	Yes	No	Ridges
<i>Celestus</i>	mid-length of the tail	No	Ovoid	Transversaly arched	9%	No	No	No	Ridges
<i>Diploglossus</i>	mid-length of the tail	Yes	Ovoid	Transversaly arched	17%	No	No	Yes	Pit-like
<i>Celestus</i>	mid-length of the tail	Yes	Ovoid	Transversaly arched	25%	No	No	Yes	Pit-like
<i>Diploglossus</i>	Distal third of the tail	Yes	Ovoid to rectangular	Transversaly arched	12 - 17%	No	No	Yes	Pit-like or ridges
<i>Celestus</i>	Distal third of the tail	Yes	Ovoid to rectangular	Transversaly arched	7 - 22%	No	No	Yes	Pit-like or ridges
<i>Ophiodes</i>	Distal third of the tail	Yes	Rectangular	Transversaly arched	9%	No	No	Yes	Ridges

Table 2: Number of Cyclic Growth Marks (CGMs) observed in osteoderm sections.

Specimen	Anatomical location	Regenerated	Number of strata in the basal part
<i>D. monotropis</i> (SMF 93787)	Body	No	13
<i>D. monotropis</i> (SMF 93787)	Tail anterior third	No	10
<i>D. monotropis</i> (SMF 93787)	Taile posterior third	Yes	4
<i>D. monotropis</i> (MNHN 1906.229)	Body	No	7
<i>D. monotropis</i> (MNHN 1906.229)	Tail anterior third	No	7
<i>D. monotropis</i> (MNHN 1906.229)	Tail median third	No	7
<i>D. monotropis</i> (MNHN 1906.229)	Taile posterior third	Yes	12
<i>C. bivittatus</i> (SMF 79022)	Body	No	3
<i>C. bivittatus</i> (SMF 79022)	Tail anterior third	No	6
<i>C. bivittatus</i> (SMF 79022)	Taile posterior third	Yes	1
<i>C. occiduus</i> (MNHN 0.1227)	Body	No	4
<i>C. occiduus</i> (MNHN 0.1227)	Tail anterior third	No	8
<i>C. occiduus</i> (MNHN 0.1227)	Tail median third	Yes	28
<i>C. occiduus</i> (MNHN 0.1227)	Taile posterior third	Yes	14
<i>O. striatus</i> (SMF 99342)	Body	No	3
<i>O. striatus</i> (SMF 99342)	Tail anterior third	No	5
<i>O. striatus</i> (SMF 99342)	Taile posterior third	Yes	0

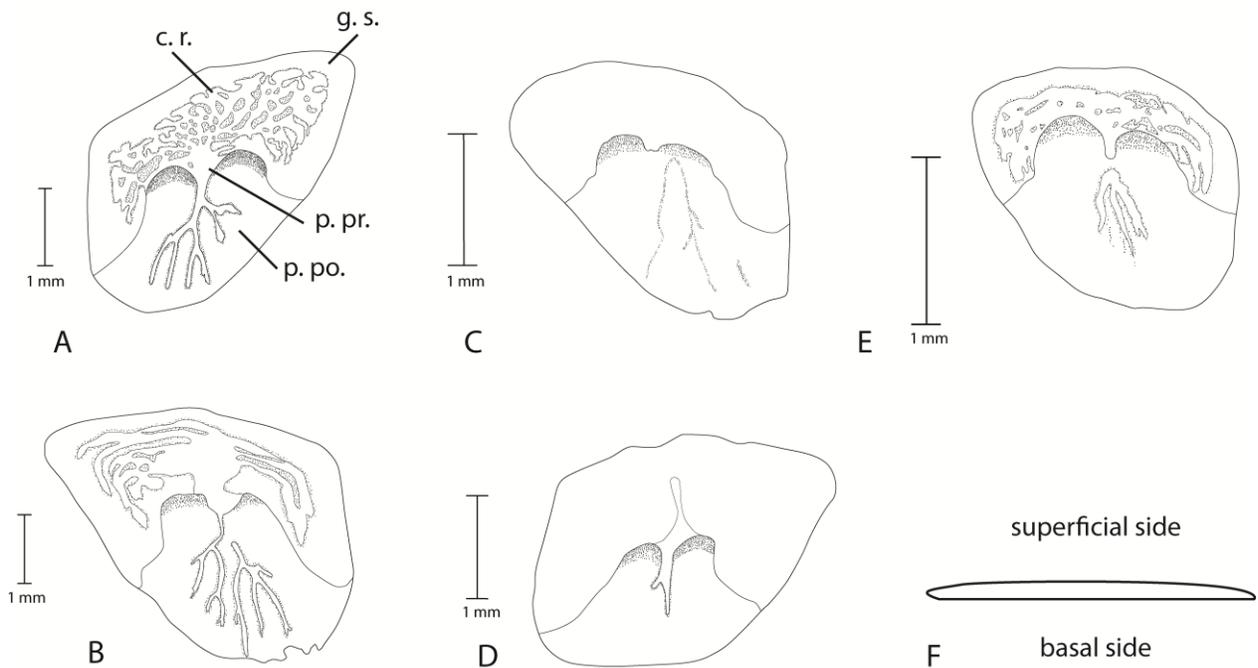


Figure 1: Trunk osteoderms in superficial view: A: *Diploglossus monotropis* (MNHN-RA 1906.0229) c. r.: cloud-like radix system of the gliding surface, g. s.: gliding surface or anterior portion of the osteoderm, p. po.: posterior portion of the osteoderm, p. pr.: posterior projection of the gliding surface; B: *Diploglossus monotropis* (SMF 93787); C: *Celestus bivittatus* (SMF 79022); D: *Celestus occiduus* (MNHN-RA 0.1227); E: *Ophiodes striatus* (SMF 99342). F: Shape of the transversal section of a flat osteoderm.

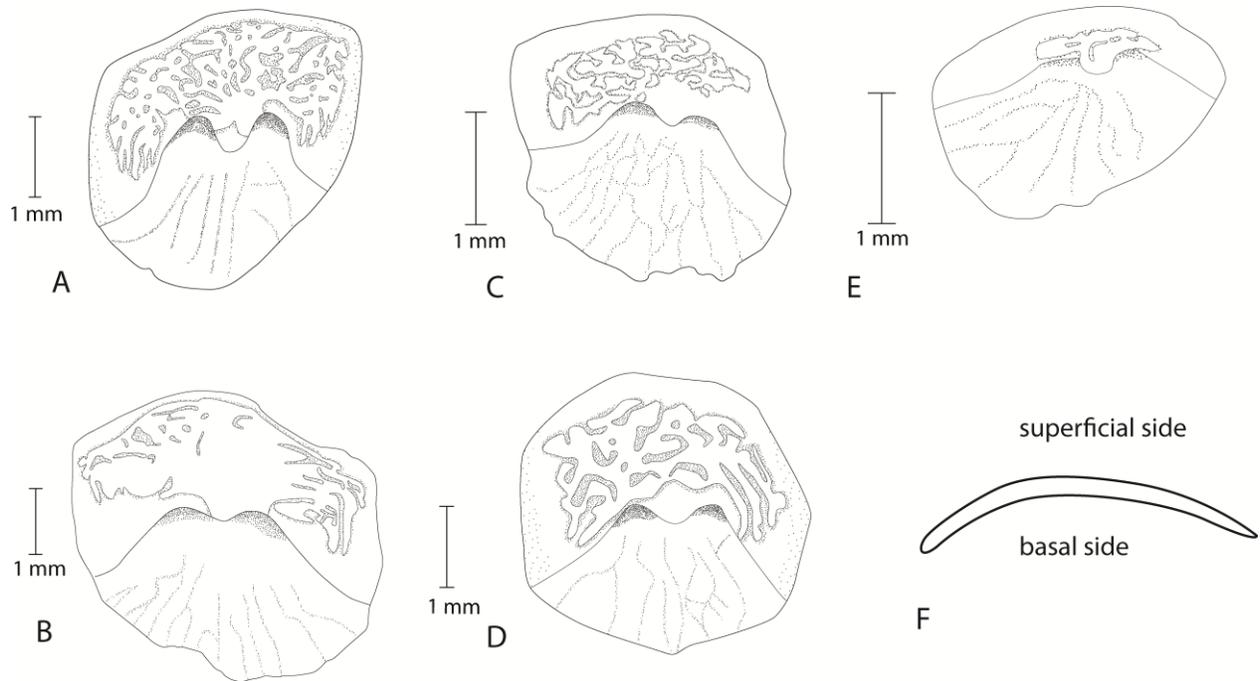


Figure 2: Osteoderms of the proximal third of the tail in superficial view: A: *Diploglossus monotropis* (MNHN-RA 1906.0229); B: *Diploglossus monotropis* (SMF 93787); C: *Celestus bivittatus* (SMF 79022); D: *Celestus occiduus* (MNHN-RA 1227); E: *Ophiodes striatus* (SMF 99342). F: Shape of the transversal section of a transversally arched osteoderm.

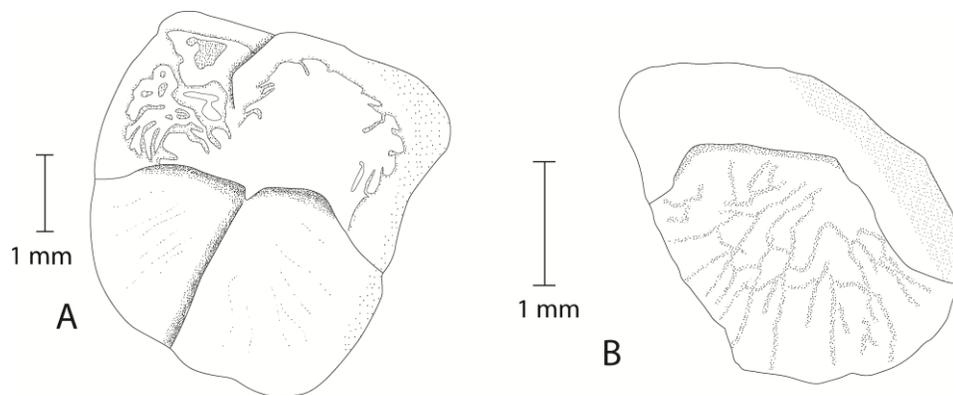


Figure 3: Osteoderms of the mid-length of the non-regenerated tail: A: *Diploglossus monotropis* (MNHN-RA 1906.0229) and B: *Celestus bivittatus* (SMF 79022).

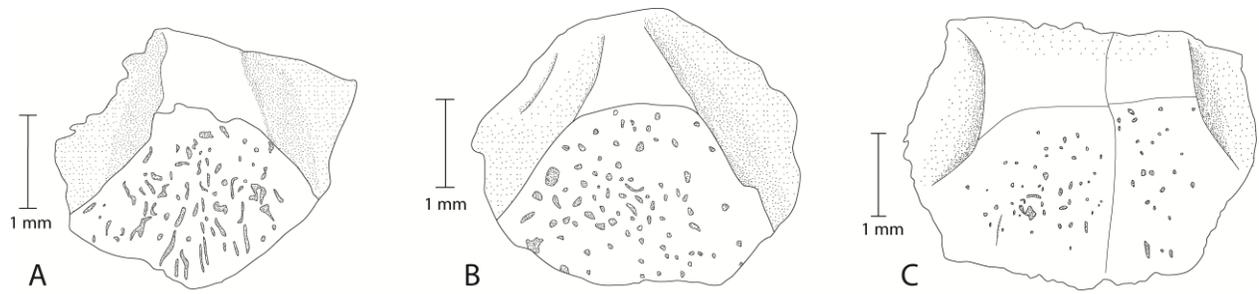


Figure 4: Osteoderm of the mid-length of the regenerated tail in superficial view: A: *Celestus occiduus* (MNHN-RA 1227); B: *Diploglossus plei* (MCZ 131518); C: *Diploglossus monotropis* (SMF 93787).

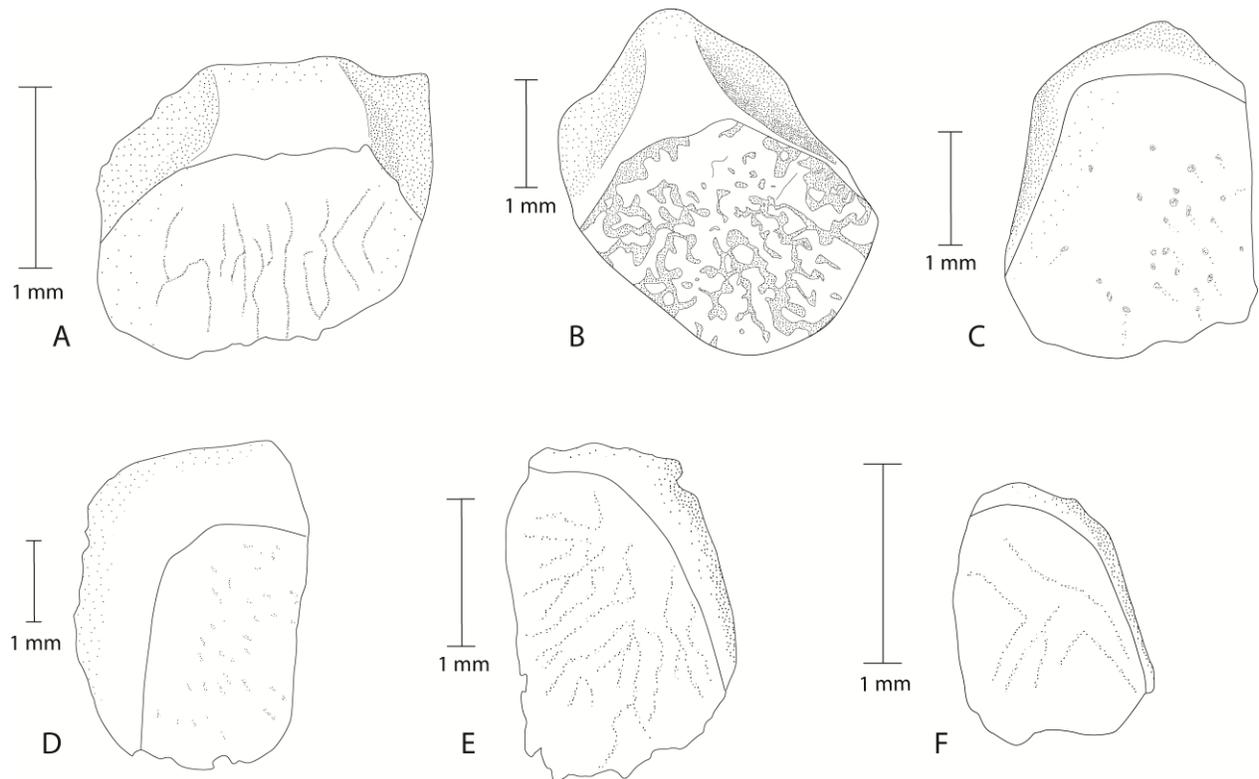


Figure 5: Superficial view of osteoderm of the distal third of the regenerated tail: A: *Celestus occiduus* (MNHN-RA 1227); B: *Diploglossus plei* (MCZ 131518); C: *Diploglossus monotropis* (SMF 93787); D: *Diploglossus monotropis* (MNHN-RA 1906.0229); E: *Celestus bivittatus* (SMF 79022); F: *Ophiodes striatus* (SMF 99342).

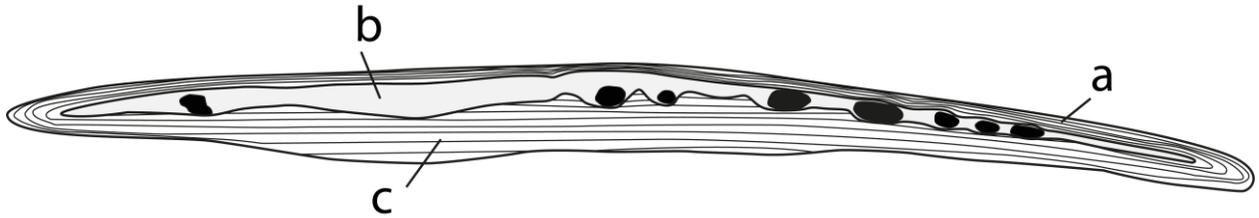


Figure 6: Schematic representation of a section of a non-regenerated osteoderm. a: Superficial cortex; b: Woven-fibered core cortex; c: Basal plate.

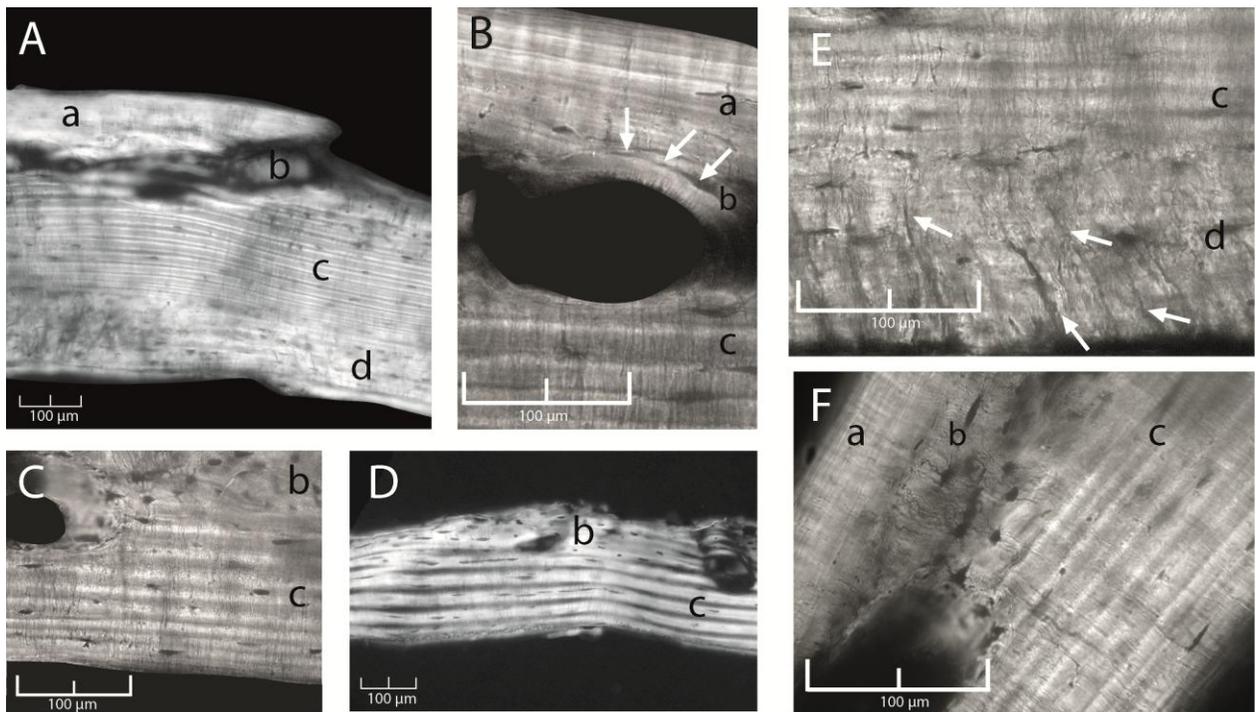


Figure 7: A-B-C-E-F: Non-regenerated proximal third of the tail osteoderm (*Diploglossus monotropis* SMF 93787) – Transverse section in polarized light. D: Non-regenerated mid-length of the tail osteoderm (*Diploglossus monotropis* MNHN-RA 1906.0229) – Transverse section in polarized light. a: Superficial cortex sometimes partially (A) or totally (D) resorbed with poorly-defined and densely packed alternation of light and dark lamellae. b: Woven-fibered core with large resorption bays presenting reversion lines and a redeposit of lamellar bone (B: arrows - F) .

c: Basal plate with alternation of light and dark strata (CGMs) decreasing in size (C-F). d: Secondary deposit under the basal plate with less marked and more spaced strata, and high density of horizontally orientated Sharpey's fibers (E: arrows).

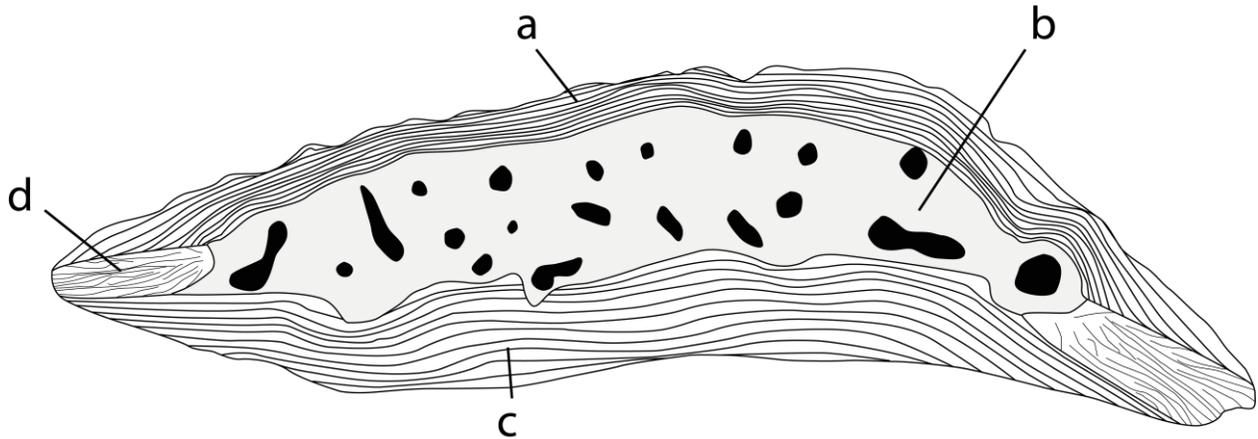


Figure 8: Schematic representation of a section of a large regenerated tail osteoderm. a: Superficial cortex; b: Woven-fibered core cortex; c: Basal plate ; d: Sharpey's fiber bone.

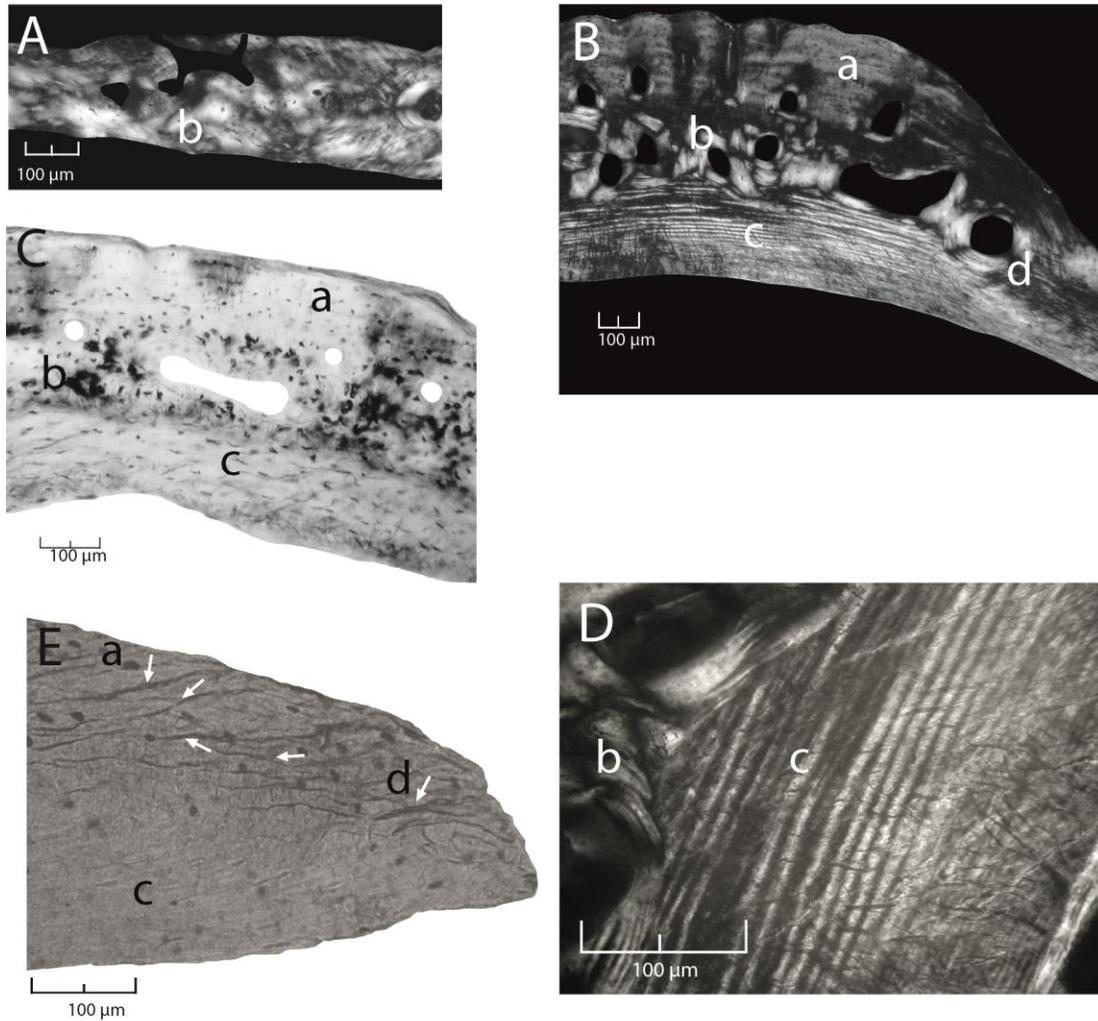


Figure 9: A: Osteoderm of the distal third of a regenerated tail (*Celestus bivittatus* SMF 79022) – Transverse section in polarized light. B-D: Osteoderm of the mid-length of a regenerated tail (*Celestus occiduus* MNHN-RA 0.1227) – Transverse section in polarized light. C: Osteoderm of the distal third of a regenerated tail (*Diploglossus monotropis* SMF 93787) – Transverse section in transmitted light. E: Osteoderm of the distal third of a regenerated tail (*Diploglossus monotropis* MNHN-RA 1906.0229) – Transverse section in transmitted light. a: Superficial cortex similar to the previously described on the non-regenerated osteoderms but with a high density of small osteocyte lacunae (C). b: Woven-fibered core occurring in all osteoderms but

only cortex present in the smallest osteoderms (A) and presenting reversion lines and a redeposit of lamellar bone (E: arrows - D). c: Basal plate presenting a high number of light and dark strata on the biggest specimens (B-D), d: Sharpey's fiber bone presenting a high density of Sharpey's fiber oriented in parallel to the basal plate (E: arrows).