

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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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 nonregenerated 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 nonregenerated tail of each studied osteoderm must be known in order to avoid mistakes related to their important interspecies and intraspecies variability. *J. Morphol.* 000:000–000, 2015. © 2015 Wiley Periodicals, Inc.

KEY WORDS: aging; anatomy; galliwasps; histology; osteology

INTRODUCTION

Several squamate taxa 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, some 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 gerrhono-

tines (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, nonbeveled 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

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Received 27 April 2015; Revised 25 May 2015; Accepted 5 July 2015.

Published online 00 Month 2015 in Wiley Online Library (wileyonlinelibrary.com). DOI 10.1002/jmor.20421

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 nonregenerated parts of the tail in several taxa (Woodland, 1920; Bustard, 1970), including *Anguis* (Ali, 1949; Bryant and Bellairs, 1967). In Anguidae, 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 intraindividual and intrageneric 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. Because 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 nonregenerated 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 (nonregenerated) 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 (nonregenerated 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 also observed previously prepared trunk osteoderms from MNHN-RA diploglossine specimens: *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: *A. 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 or 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 μ m 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 Hoffstetter (1962).

The nomenclature used follows the terminologies defined by Buffrénil 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. 1A).

RESULTS

External Morphology

Nonregenerated osteoderms. *Trunk osteoderms.* External morphology of trunk osteoderms is similar to that described in previous studies: they are rounded in superficial view, flat in transversal section (Fig. 1F), thin (thickness/length ratio is between 5 and 9%), and display a crescentic gliding surface with a posterior projection, but lack beveled lateral edges (Hoffstetter, 1962; Strahm and Schwartz, 1977) (Fig. 1; Table 1). As previously reported, *Ophiodes* and *Diploglossus* osteoderms (Fig. 1A,B,E) have a “cloud-like” radix system on their anterior portion or gliding surface which is absent in *Celestus* (Fig. 1C,D) (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. Osteoderms of the proximal third of the tail display slight differences with those from the trunk: they are less flat in transversal section, more

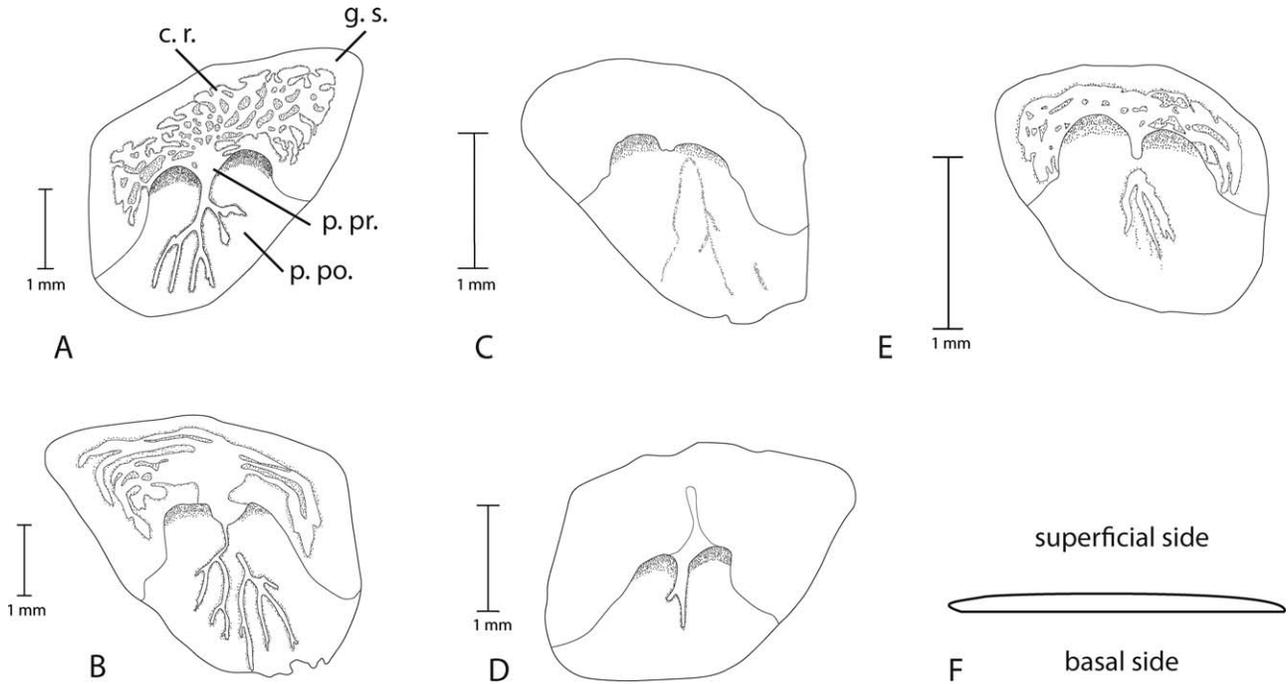


Fig. 1. Trunk osteoderms in superficial view. (A) *D. monotropis* (MNHN-RA 1906.0229) c. r.: cloud-like radix system of the gliding surface, g. s.: gliding surface or anterior portion of the osteoderm, p. po.: posterior portion of the osteoderm, p. pr.: posterior projection of the gliding surface, (B)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

transversally arched (Fig. 2F), and thicker (thickness/length ratio between 9 and 13%) (Fig. 2; Table 1). 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 (Fig. 2C,D). 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 “cloud-like” radix system (Fig. 2E).

Osteoderms of the mid-length of the tail. 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 nonregenerated tail on two specimens (*D. monotropis* -MNHN-RA 1906.0229- and *C. bivittatus*) (Fig. 3; Table 1). These osteoderms present different morphologies. The *D. monotropis* osteoderm (Fig. 3A) 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 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 osteoderms to a rectangular shape for the distal ones but unfortunately, morphology of the nonregenerated distal osteoderms remains unknown to us. The *C. bivittatus* osteoderm (Fig. 3B) 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 “cloud-like” 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.* In three of our specimens (*D. monotropis* -SMF 93787-, *D. plei* and *C. occiduus*), the second third of the tail was regenerated (Fig. 4; Table 1). The morphology of these osteoderms is very different from those previously described because 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 plesiomorphic within Anguidae (Meszoely, 1970) and was absent in the nonregenerated osteoderms. However, their morphology is variable. In MCZ 131518 (*D. plei*) and MNHN-RA 1227 (*C. occiduus*; Fig. 4A,B), the osteoderms are oval in superficial view, and thicker than nonregenerated ones (thickness/length ratio between 17 and 25%). In SMF 93787 (*D. monotropis*) (Fig. 4C), the osteoderms seem to result from the fusion of two sub-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

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	Transversally arched	9-13%	Yes	Yes	No	Ridges
<i>Celestus</i>	Proximal third of the tail	No	Ovoid	Transversally arched	10-12%	Yes	Yes	No	Ridges
<i>Ophiodes</i>	Proximal third of the tail	No	Ovoid	Transversally arched	9%	Yes	Yes	No	Ridges
<i>Diploglossus</i>	Mid-length of the tail	No	Subcircular	Transversally arched	7%	Yes	Yes	No	Ridges
<i>Celestus</i>	Mid-length of the tail	No	Ovoid	Transversally arched	9%	No	No	No	Ridges
<i>Diploglossus</i>	Mid-length of the tail	Yes	Ovoid	Transversally arched	17%	No	No	Yes	Pit-like
<i>Celestus</i>	Mid-length of the tail	Yes	Ovoid	Transversally arched	25%	No	No	Yes	Pit-like
<i>Diploglossus</i>	Distal third of the tail	Yes	Ovoid to subrectangular	Transversally arched	12-17%	No	No	Yes	Pit-like or ridges
<i>Celestus</i>	Distal third of the tail	Yes	Ovoid to subrectangular	Transversally arched	7-22%	No	No	Yes	Pit-like or ridges
<i>Ophiodes</i>	Distal third of the tail	Yes	Subrectangular	Transversally arched	9%	No	No	Yes	Ridges

that this osteoderm formed as a whole, in one piece and that it was probably newly formed (see below: Table 2); its morphology may, thus, reflect an early developmental stage.

Osteoderms of the distal part of the tail. Two clearly different osteoderm morphotypes were observed in the distal third of regenerated tails of our specimens (Fig. 5; Table 1). 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. 5A,B).

The second type was observed in *D. monotropis*, *C. bivittatus*, and *O. striatus* (Fig. 5C,D,E,F). These osteoderms are subrectangular 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 and 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

Nonregenerated osteoderms. All nonregenerated osteoderms from the trunk and tail display a similar histological structure. They are made of three distinct tissue types, located, in superficial and basal regions of the osteoderms, respectively, 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. 7A,F). 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 be 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 (Fig. 7A) or entirely eroded (Fig. 7D). 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

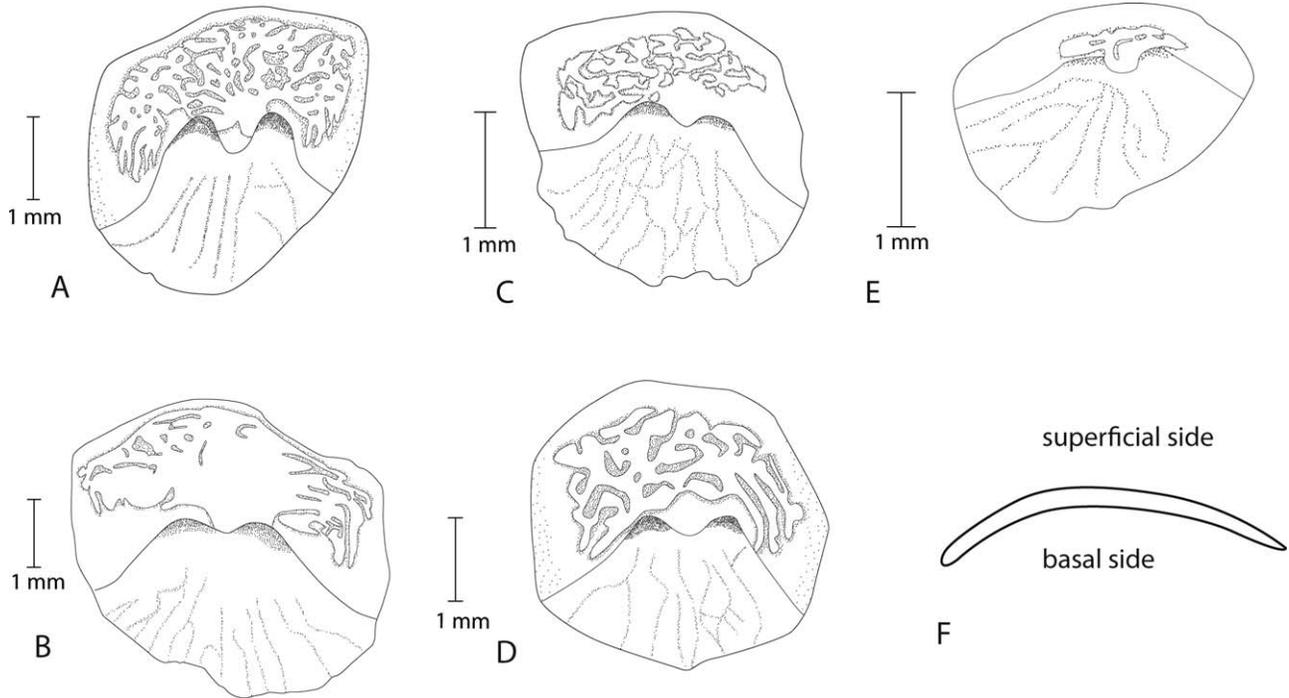


Fig. 2. Osteoderms of the proximal third of the tail in superficial view. (A) *D. monotropis* (MNHN-RA 1906.0229), (B) *D. monotropis* (SMF 93787), (C) *C. bivittatus* (SMF 79022), (D) *C. occiduus* (MNHN-RA 1227), (E) *O. striatus* (SMF 99342), (F) shape of the transversal section of a transversally arched osteoderm.

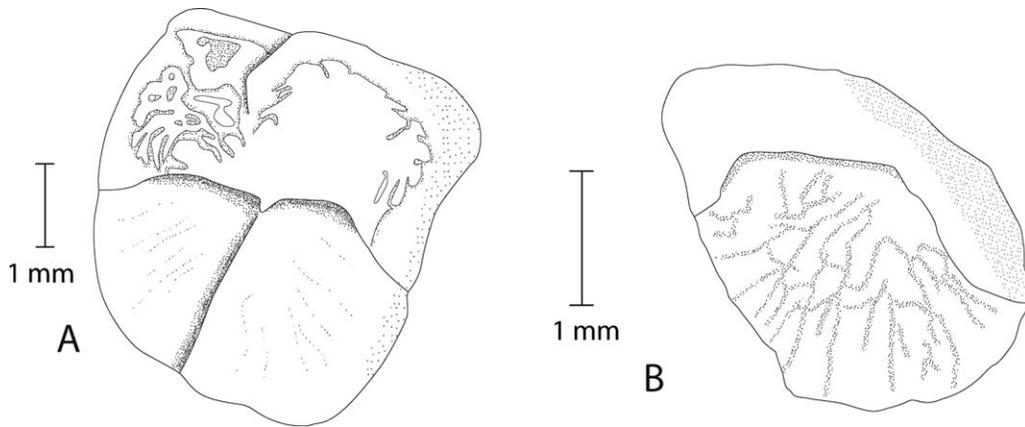


Fig. 3. Osteoderms of the mid-length of the nonregenerated tail in superficial view. (A) *D. monotropis* (MNHN-RA 1906.0229) and (B) *C. bivittatus* (SMF 79022).

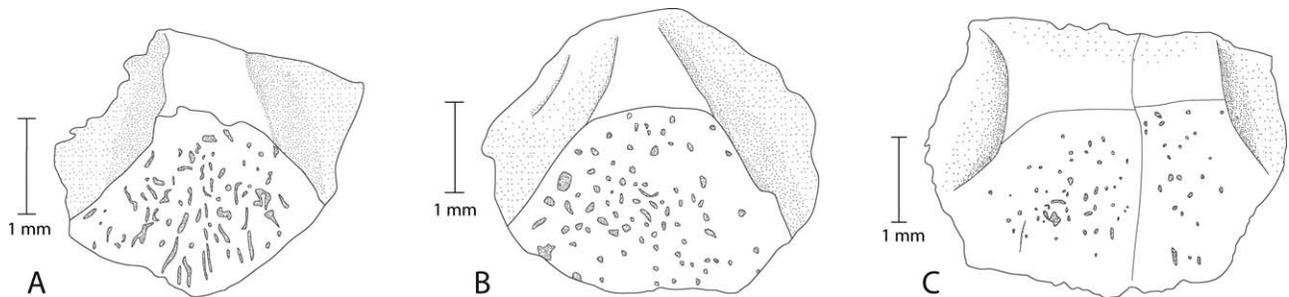


Fig. 4. Osteoderm of the mid-length of the regenerated tail in superficial view. (A) *C. occiduus* (MNHN-RA 1227), (B) *D. plei* (MCZ 131518), (C) *D. monotropis* (SMF 93787).

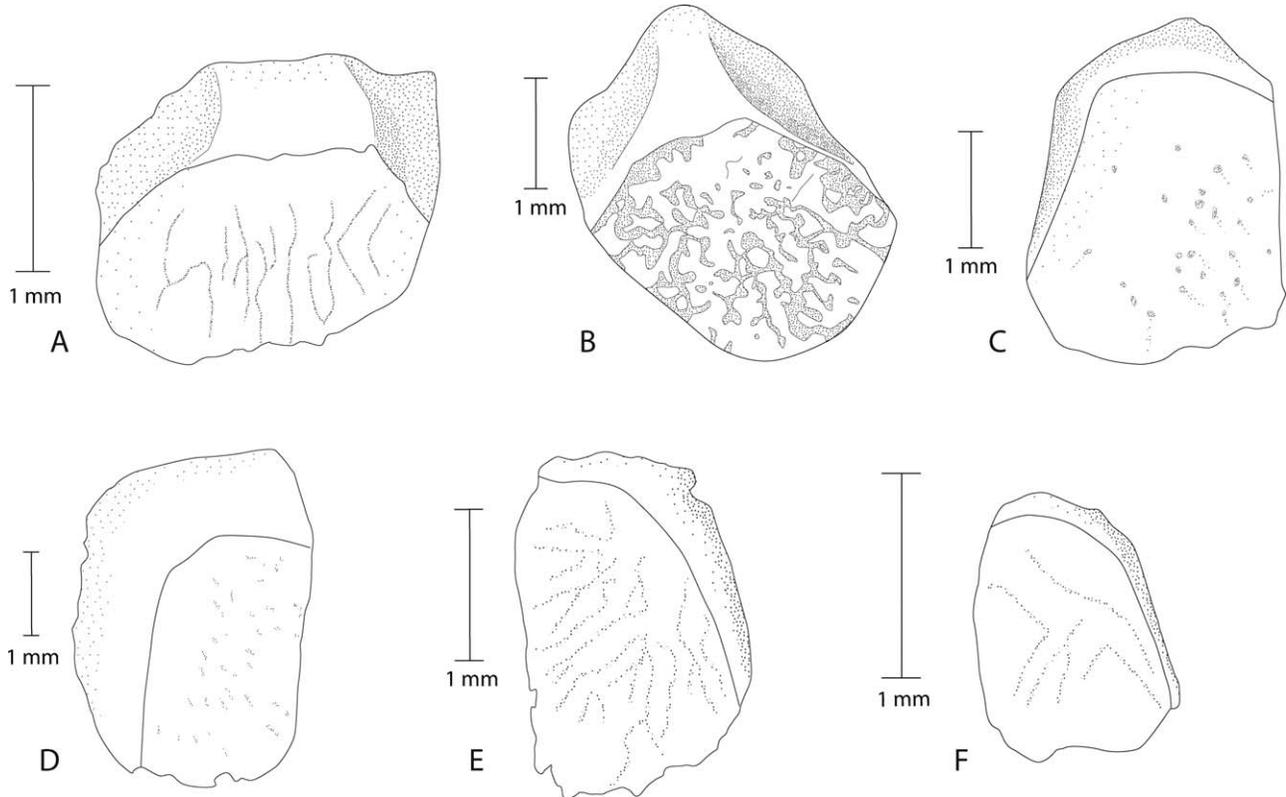


Fig. 5. Osteoderm of the distal third of the regenerated tail in superficial view. (A) *C. occiduus* (MNHN-RA 1227), (B) *D. plei* (MCZ 131518), (C) *D. monotropis* (SMF 93787), (D) *D. monotropis* (MNHN-RA 1906.0229), (E) *C. bivittatus* (SMF 79022), (F) *O. striatus* (SMF 99342).

b) the thickness of which represents less than one fourth of osteoderms' maximal thickness (Fig. 7A,F). 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. 7B 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* Buffr n l 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. 7A,F). 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. 7C,F). 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. 7B,F). In both cortices (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. 7C,F).

The precise number of CGMs in each osteoderm was difficult to assess because the deep cortical

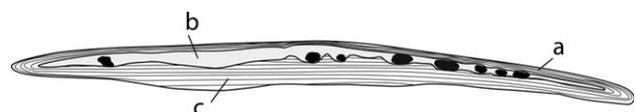


Fig. 6. Schematic representation of a section of a nonregenerated osteoderm. (a) Superficial cortex, (b) woven-fibered core cortex, (c) basal plate.

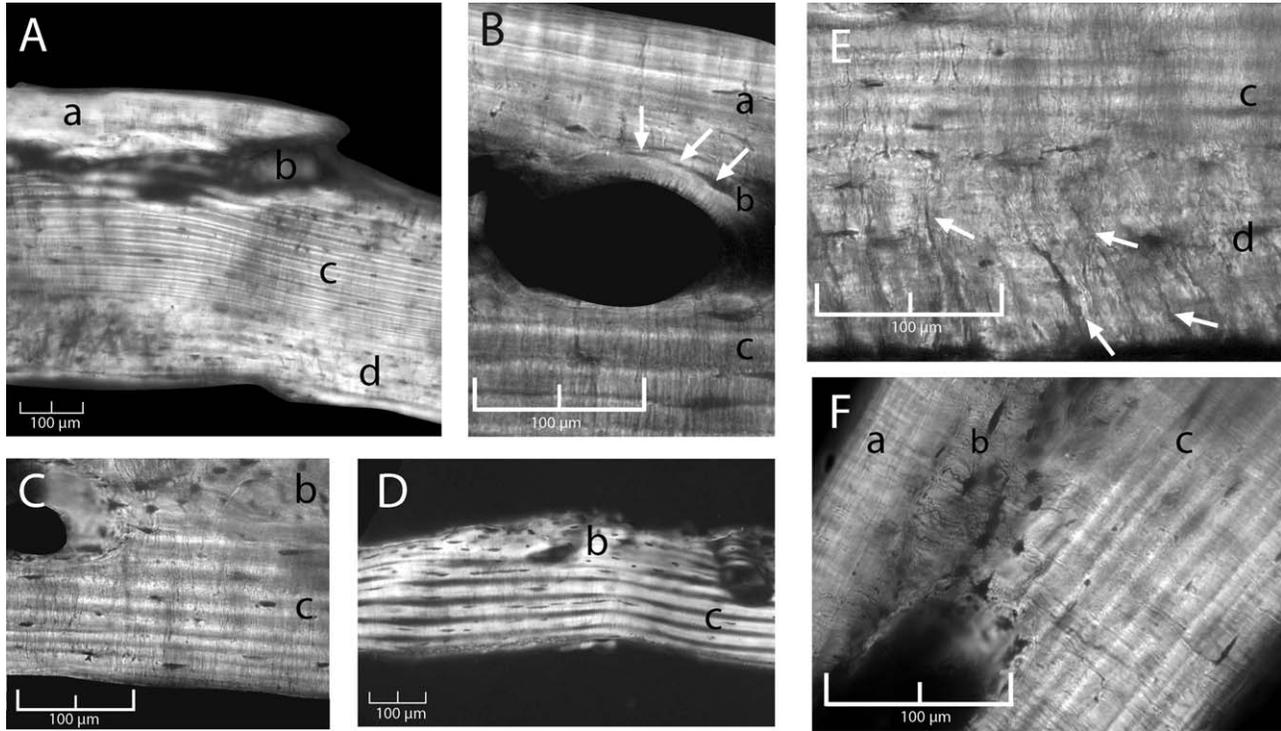


Fig. 7. (A, B, C, E, F) Nonregenerated proximal third of the tail osteoderm (*D. monotropis* SMF 93787)—transverse section in polarized light. (D) Nonregenerated mid-length of the tail osteoderm (*D. monotropis* MNHN-RA 1906.0229)—transverse section in polarized light. (a) Superficial cortex sometimes 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).

cortexes was often resorbed. Despite these limitations, the number of counted growth lines was relatively stable in the nonregenerated osteoderms of a single specimen (Table 2). The maximum number of CGMs observed in the basal plate of nonregenerated 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. 7A–C,E,F), the deep cortex is basally bordered by an additional cortex of similar structure but with blurred CGMs and lesser birefringence (Fig. 7; cortex d). Some broadly spaced CGMs can nevertheless be observed, together with densely-packed bundles of Sharpey's fiber obliquely orientated (Fig. 7E). 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; Buffrénil 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 nonregenerated osteoderms yet with some differences including the occurrence of a fourth type of tissue in the largest

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)	Tail 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)	Tail 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)	Tail 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)	Tail 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)	Tail posterior third	Yes	0

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. 9A) to some presenting a thick basal plate with numerous (most likely nonannual; see below) CGMs (Fig. 9B). 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 nonregenerated 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. 9C) than in the nonregenerated osteoderms. In addition, unlike in the nonregenerated 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 nonregenerated osteoderms (Fig. 9B–E). The difference is that this cortex is thicker since it can occupy more than half of the total thickness on the smaller osteoderms (Fig. 9A) to one third on the large osteoderms (Fig. 9B).
3. The basal plate (Fig. 9; cortex b) is also similar to that of nonregenerated osteoderms. Its thickness is highly variable: thin in the small osteoderms with no clearly distinct CGMs (Fig. 9A) and thick in larger osteoderms displaying many CGMs (Fig. 9B,D). The main difference with nonregenerated 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 nonregenerated osteoderms of the same specimen (Fig. 9B,D; Table 2); conversely, the number of growth marks is smaller in the smallest regenerated osteoderms (Fig. 9A,C; Table 2). Unlike the sit-

uation of nonregenerated 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. 9E). 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 nonregenerated osteoderms but is similar to that described by Buffr enil 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 anguid plesiomorphic character.

DISCUSSION

Our results clearly show that intraindividual and intrageneric morphological variability of

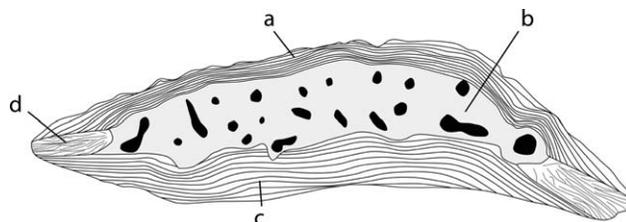


Fig. 8. Schematic representation of a section of a large regenerated tail osteoderm. (a) Superficial cortex, (b) woven-fibered core cortex, (c) basal plate, (d) Sharpey's fiber bone.

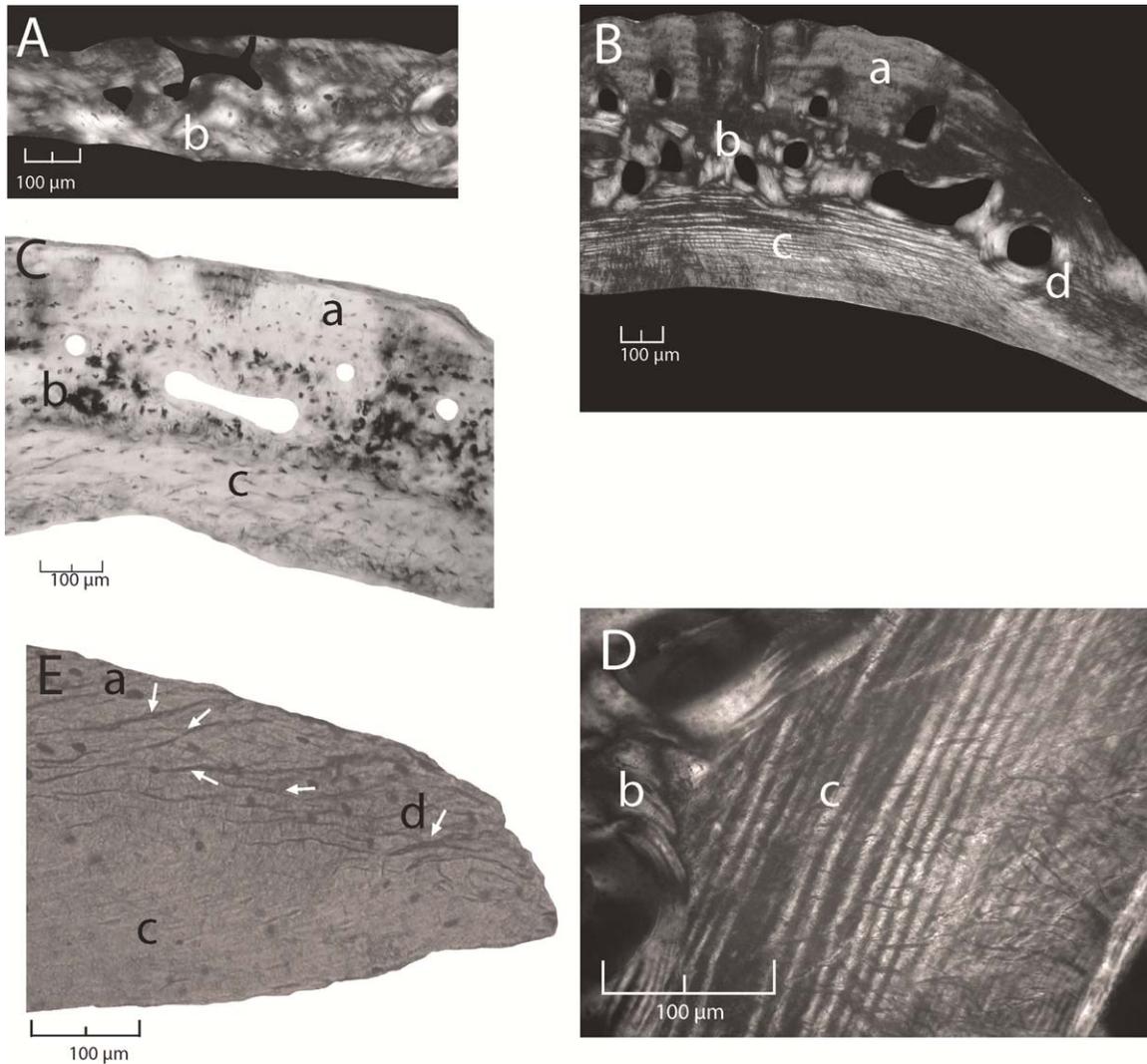


Fig. 9. (A) Osteoderm of the distal third of a regenerated tail (*C. bivittatus* SMF 79022)—transverse section in polarized light. (B and D) Osteoderm of the mid-length of a regenerated tail (*C. occiduus* MNHN-RA 0.1227)—transverse section in polarized light. (C) Osteoderm of the distal third of a regenerated tail (*D. monotropis* SMF 93787)—transverse section in transmitted light. (E) Osteoderm of the distal third of a regenerated tail (*D. monotropis* MNHN-RA 1906.0229)—transverse section in transmitted light. (a) Superficial cortex similar to the previously described on the nonregenerated 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). (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).

diploglossine osteoderms is much greater than previously supposed. We reported morphology of regenerated and nonregenerated osteoderms of the tail and pointed out a probable reappearance of plesiomorphic anguid characters in regenerated osteoderms.

Morphological Variability of Diploglossine Osteoderms

We reported a previously unknown intraindividual morphological variability in diploglossine osteoderms. Nonregenerated 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. In addition, the cloud-like radix system that was thought to be a distinctive character between *Diploglossus* and *Celestus* (Strahm and Schwartz, 1977) occurs on all observed osteoderms from the anterior-third of the tail although being only present on trunk osteoderms from *Diploglossus*. This character should, thus, be redefined 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 is formed of two fused rectangular osteoderms linked by two small hooks on their basal side (Fig. 3A). This could indicate the occurrence of osteoderms of different morphology in the more distal part of the nonregenerated 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, this osteoderm was transversally arched rather than flat, and lacked the “cloud-like” radix system and the posterior projection characteristic of Diploglossinae that occurred on all the others nonregenerated osteoderms (Fig. 3B). This *Celestus* osteoderm did not bear any of the diploglossine characters except that it was not beveled along its lateral edges.

Histologically, all nonregenerated osteoderms present the same basic organization with three different types of tissues: a superficial cortex composed of an intermediate tissue type between parallel-fibered and lamellar bone, a core of woven fibered bone, and a basal plate of parallel-fibered bone presenting CGMs (Figs. 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: *A. 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 nonregenerated osteoderms share the absence of the Sharpey’s fiber bone and a parallel-fibered basal plate with *A. 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 (Figs. 4 and 5). However, clear general differences with the nonregenerated osteoderms can be noted (Table 1). They tend to be thicker and bear orna-

mentation 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 nonregenerated osteoderms (Figs. 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 *Dasybus novemcinctus* 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 (Hoffstetter, 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 (atavisism) after regeneration.

Barbour and Stetson (1929) hypothesized a similar phenomenon when comparing the scaling pattern of the regenerated tail of extent *Sphenodon* sp. Gray, 1831 to scaling pattern of the fossil sphenodontid *Homeosaurus maximiliani* Wagner, 1853. In *Ophisaurus gracilis*, 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 nonregenerated osteoderms.

The Use of Osteoderms in Systematic and Paleontology Studies

Many characters are traditionally used to separate anguid genera by their osteoderms (Hoffstetter, 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 intrageneric variability depending of the position, age and regenerated or nonregenerated 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 (Hoffstetter, 1962; Meszoely, 1970; Strahm and Schwartz, 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 to subrectangular 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

Histological sections of the osteoderms revealed that, in polarized light, the basal plate cortex shows an alternation of light and dark strata progressively decreasing in thickness towards bone periphery. Such deposits are undoubtedly CGMS 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 CGMs is roughly stable in the nonregenerated osteoderms of a single specimen (Table 2) and that they could possibly be yearly deposits. Nonregenerated osteoderms seem therefore potentially useful for skeletochronology. However, the number of CGMs observed in regenerated osteoderms differs depending on the “maturity” of the regenerated osteoderm (Table 2). In addition, our observations show a clear difference in growth cyclicity between nonregenerated and regenerated osteoderms. Taking account of this bias, we suggest that that regenerated osteoderms cannot be used in any manner to estimate the age of a specimen.

In conclusion, our results show that diploglossine osteoderms display an important morphological variability at both intraindividual and intraspecific levels according to their location on the body (trunk, nonregenerated 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 nonregenerated 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 nonregenerated condition. We have also shown that regenerated and nonregenerated tail osteoderms probably do not follow the same growth rates and that the application of skeletochronology should be limited to nonregenerated osteoderms.

ACKNOWLEDGMENTS

The authors are thankful to people and institutions who provided us the specimens essential to this study: Gunther Köhler and Krister Smith (Senckenberg Museum of Frankfurt, Germany), José P. Rosado (Museum of Comparative Zoology, Harvard, USA), and Jean-Claude Rage, Annelise Ohler, and Laure Pierre. The authors are also grateful to Anthony Herrel, Thierry Descamps and UMR 7179 MECADEV (CNRS/MNH) for the X-ray images of the specimens. Finally the authors thank Robert V. Hill and the anonymous reviewer who help us to improve the quality of this article.

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