General palaeontology, systematics and evolution (Taphonomy and fossilisation)

Palaeohistology and preservation of tetrapods from Las Hoyas (Lower Cretaceous, Spain)

Paléohistologie et préservation d'ossements de tétrapodes de Las Hoyas (Crétacé inférieur, Espagne)

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A R T I C L E   I N F O

A B S T R A C T

The Upper Barremian (Lower Cretaceous) site of Las Hoyas (Spain) is an important *Konservat Lagerstätte* that has a yielded well-preserved biota. Both the palaeohistology and the preservation of six tetrapods were investigated in this study. Chemical analysis (Ca/P ratio, fluorine, strontium, barium and REE amounts) reveals an alteration of the original apatite of the bone in fluoroapatite by postmortem substitutions, a late Sr incorporation during fossilization, and a rather moderate postmortem REE incorporation in the freshwater environment of Las Hoyas. However, the conditions of fossilization have limited the dissolution-recrystallisation processes and preserved the microstructure of the bones. Bone growth rate estimations have been made according to Amprino's rule. The Discoglossidae indet, the Centrocryptodira indet. and *Scandensia ciervensis* are characterized by low growth rates and an ectothermic metabolism. *Concornis lacustris*, *Pelecanimimus polyodon* and the Archosauria indet. are characterized by a faster growth and probably higher resting metabolic rate. All of the specimens are adults of small-sized species. This leads to the hypothesis of an ecological filter favoring small-sized species in this ecosystem, strengthening the taphonomical results. The combination of geochemical and histological studies seems to offer a more precise overlook of life history traits and preservation of these fossils from Las Hoyas.

R É S U M É

Le site de Las Hoyas datant du Barrémien supérieur (Crétacé inférieur, Espagne) est un *Konservat Lagerstätte* très important. Dans ce travail, six tétrapodes ont été étudiés par une double approche chimique et histologique. L'analyse chimique des os (rapport Ca/P, teneurs en fluor, strontium, baryum et terres rares) révèle des substitutions ioniques postmortem conduisant à la formation de fluoroapatite, une incorporation tardive de Sr au cours de la fossilisation et une incorporation postmortem de terres rares, plutôt modérée au sein de l'environnement dulcicole de Las Hoyas. Les conditions de fossilisation ont cependant limité

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les phénomènes de dissolution-recristallisation et préservé la microstructure osseuse. Les taux de croissance osseux ont été estimés selon la loi d'Amprino. Le Discoglossidae indét, le Centrocryptodira indét. et *Scandensia ciervensis* sont des individus à croissance très lente et à métabolisme ectotherme. *Concornis lacustris*, *Pelecanimimus polyodon* et l'Archosauria indét. ont une croissance plus rapide et probablement des taux métaboliques plus élevés. Ils sont tous caractérisés par une petite taille adulte. Une hypothèse émise est la présence d'un filtre écologique au sein de l'écosystème de Las Hoyas qui aurait favorisé les espèces de petites tailles. L'intégration des études géochimiques et paléohistologiques devrait offrir une vision plus complète des traits de vie et de préservation des fossiles de Las Hoyas.

1. Introduction

Las Hoyas (Lower Cretaceous, Spain) is a *Konservat Lagerstätte* in which freshwater, terrestrial and aerial organisms occur together in the same sedimentary horizon (Sanz et al., 1988, 2001). It has provided specimens of all main eukaryote clades: Fungi, Chlorophyta, Bryophyta, Pteridophyta, Spermatophyta, Arthropoda (Ecdysozoa), Mollusca (Lophotrochozoa), and Vertebrates (Deuterostomia) (Buscalioni and Fregenal-Martinez, 2010). The last taxonomical update gathered 132 genera, and this number is increasing yearly (Buscalioni and Fregenal-Martinez, 2010). Prokaryotes involved in the fossilization processes have also been identified (Briggs et al., 1997; Gupta et al., 2008). The palaeobiodiversity and the palaeoecology of the ecosystem have been analyzed in detail (see the review by Buscalioni and Fregenal-Martinez, 2010). However, only a few studies dealt with the functional significance of morphological structures of vertebrates. These include the analysis of Sanz et al. (2002) on the functional significance of the pygostile in *Iberomesornis* and the alula in *Eoalulavis*, as well as the phylogenetic affinities of these Mesozoic birds, and the analysis of Poyato-Ariza (2005) on ecomorphotypes of the Las Hoyas fish assemblage.

Microscopic structure of bone tissue can be preserved during hundreds of millions of years. Ever since the beginning of palaeohistology in the first third of the 20th century (see the review by Higham, 1963), scientists have tried to discover the secrets of fossilized bone microstructure. The histological structure of bone tissue records parameters such as life history traits (age at sexual maturity, longevity, and growth rate), lifestyle (habitat, e.g. terrestrial, aquatic, amphibious; Germain and Laurin, 2005), thermometabolism, biomechanics, etc. (Cubo et al., 2005; Montes et al., 2010; Ricqlès et al., 2001). At Las Hoyas, only two studies have been realized using the palaeohistological approach. Cambra-Moo (2006) presented in his PhD thesis an overview of the histological structure of bone tissue of some vertebrates, but his analysis was exploratory and the conclusions, preliminary. Only the histology of *Concornis lacustris* (Enantiornithes, Aves) has been analyzed in detail, and used to estimate life-history traits of this Mesozoic bird (Cambra-Moo et al., 2006). Thus the detailed analysis of the remaining samples described by Cambra-Moo (2006) is necessary to obtain a maximum of information on these organisms.

In parallel with the palaeohistological approach, the chemical composition, including trace elements (i.e., Sr/Ca and Ba/Ca) and stable light isotopes ratios of bone tissue may provide valuable information on the biology of extinct species, such as thermometabolism, using the $\delta^{18}O$ on PO_4 (Amiot et al., 2002), past diet and environments (i.e., Balter et al., 2001; Burton et al., 1999; Ségalen and Lee-Thorp, 2009). Our ability to make such inferences depends on the quality of preservation of the fossil remains. The mineral component of bones is composed of biological apatite $[Ca_5(PO_4,CO_3)_3(F,OH,Cl)]$, and during the fossilization processes (depending on burial environments and conditions), fossil structures may be modified in ionic exchange and rearrangements in the primary structure throughout incorporation of foreign ions in the crystal lattice, such as F^- for OH^- or rare earth elements and trace elements for Ca^{2+} in Ca sites (Bryant et al., 1994; Burton et al., 1999). In some cases, geochemical analyses of bone tissue may still provide valuable complementary information on the palaeobiology of extinct organisms. The palaeohistological and geochemical analyses are usually performed in different research groups, and on different fossil materials, but deal with similar palaeobiological problems. Integrative analyses including both the palaeohistological and the geochemical approaches may yield deeper insights into the problems under analysis. We advocate for this kind of study and it is a main goal of this paper to show the virtues of this integrative approach. In this study, we performed both palaeohistological and geochemical analyses in a sample of bone tissues from Las Hoyas, to study the preservation of these fossil remains and to acquire a deeper understanding of the palaeobiology of these intriguing organisms.

2. Material and methods

2.1. Material and geological settings

We studied a sample of tetrapods (Table 1) collected during successive field seasons that have taken place

Table 1
List of the material studied (introduced in Cambra-Moo, 2006).
Tableau 1
Liste du matériel étudié (introduit par Cambra-Moo, 2006).

Taxon	Specimen number	Material
Anura - Discoglossidae indet.	LH-394a	Tibiofibula/Femur
Chelonia -Centrocryptodira indet.	LH-21901	Ulna/Femur
Scandensia ciervensis	LH-22399	Tibia/Fibula/Femur
Concornis lacustris	LH-21006a/b	Tarsometatarsal
Pelecanimimus polyodon	LH-7777	Rib
Archosauria indet.	LH- 17325	Humerus

in Las Hoyas, an Upper Barremian (Lower Cretaceous) *Konservat lagerstätte*, located in the Serranía de Cuenca (southwestern Iberian Ranges), in central Spain. The fossils are preserved in finely laminated limestones composed of calcium carbonate with a small fraction of clays and organic matter (Fregenal-Martínez, 1998). The palaeoclimatic reconstruction for the Barremian at the Serrania de Cuenca suggests an extensive subtropical (seasonal winter wet), continental (freshwater) wetland system, dominated by carbonate sedimentation that overlaid a low-relief karstic terrain. A flat and smooth topography with scattered elevations of Jurassic limestones at the edge of the subbasins may have been the foreground landscape that accompanied sedimentation (Fregenal-Martinez and Buscalioni, 2009). The Las Hoyas subbasin is filled with sediments of the La Huérguina Limestone Formation. This formation is widespread on its central area and the facies indicate deposits in distal alluvial and palustrine-lacustrine environments (Fregenal-Martínez, 1998; Fregenal-Martínez and Meléndez, 2000). Fossil-bearing rhythmically laminated limestones are found in the Rambla de Las Cruces II Sequence. This sequence is entirely composed of carbonates, marlstones and marly limestone. Palustrine-lacustrine facies dominate over floodplain facies.

Taphonomically, Las Hoyas is characterized by fully articulated and exceptionally preserved fossils. A broad range of organic compounds have been preserved: mineralized muscles, tissue imprints, chitin, cellulose, calcium carbonate and apatite skeletons (Fregenal-Martinez and Buscalioni, 2009). Microbial mats were presumably the main agents involved in the exceptional preservation of organisms. Moreover, microorganisms were also responsible for the organic and inorganic tissue degradation (Gupta et al., 2008). The mechanisms that may explain the taphonomic features of Las Hoyas are, in decreasing order of importance: inclusion in microbial mats with rapid burial and anoxia (Fregenal-Martinez and Buscalioni, 2009; Iniesto et al., 2009). These agents and mechanisms have allowed the preservation of the microstructure of bone and made our palaeohistological analysis possible.

2.2. Histological observations

Histological thin sections (with their associated sediments) were prepared according to the general protocol (see for instance Montes et al., 2007). Sections were digitized with a digital camera mounted on a binocular microscope NIKON ECLIPSE E600 POL. The thin sections were also carbon coated and observed with a JEOL JSM 840A scanning electron microscope (secondary electrons and backscattered electrons).

2.3. Geochemical analysis

2.3.1. Electron microprobe analysis

To investigate microscale diagenesis, the polished and carbon coated thin sections were analyzed with an electron microprobe Cameca SX 100 (Camparis analysis center UPMC). It consists in firing a beam of electrons onto a sample, which causes each element to emit X-rays at a characteristic frequency. Specific X-ray wavelengths were

selected and counted by wavelength dispersive microscopy (WDS) and allowed the measurement of elemental concentrations. Elemental amounts (in ppm) were measured as quantitative single points along a transect across the bone compacta under vacuum conditions of $<10^{-5}$ mbar with 15 kV high voltage, and 15 nA beam current. Not only the cortex, but also the crystals in the porous network of the bone and in the medullary cavity were analyzed, as well as the sediment surrounding the thin sections (when possible). Major elements (Ca, P and F), were analyzed by a beam with spot diameter of 3 μm. Minor elements (Mn, Fe, Ba, Sr) and rare earth elements (La, Yb, Sm) were analyzed by a beam with spot diameter of 20 μm. The analytical precisions of chemical content was less than 5% for the Ca, Sr, P, F, Ba, Fe, 15% for the Mn, and 20% for the rare earth elements. The relatively high variations are related to the low concentrations observed in the fossils materials and the detection limits.

The concentration of these elements was compared to those in actual fresh bones to evaluate the quality of preservation of each sample. We used the Ca/P ratio that generally ranges from 1.99 to 2.33 in living vertebrates (LeGeros and LeGeros, 1984), the fluorine concentration, generally inferior to 0.2% in living vertebrate bioapatites (LeGeros, 1981), and the REE concentrations, which is nearly absent in living vertebrates (Hinners et al., 1998). The Ba/Ca and Sr/Ca ratios could potentially be used to assess the dietary intakes of these fossils, depending on the quality of preservation.

2.3.2. Optical cathodoluminescence

In parallel to these methods, the samples have been observed in optical cathodoluminescence. This method has been mostly used to identify diagenetic phases and alteration zones in sediments (Machel and Burton, 1991; Machel et al., 1991; de Rafélis et al., 2000) or biogenic materials (Balter et al., 2002; Ségalen et al., 2008; Williams and Henderson, 1997). Cathodoluminescence is based on to photon emission in the visible range resulting from excitation of a sample by high-energy electrons. Mn^{2+} is the main activator for luminescence phenomena, and also REE. The luminescence exists only when the activators are substituting in the Ca sites and not when the elements are adsorbed onto the crystals (Gaft et al., 1996). Fe is an inhibitor of luminescence (Machel et al., 1991). Cathodoluminescence examinations of the thin sections of bones were made using optical cathodoluminescence with an OPEA Catodym luminoscope at approximately 15 kV and 300 μA. Cathodoluminescence photographs were performed using a Nikon D70 digital camera.

3. Results

3.1. Geochemistry

3.1.1. Major and minor elements

Amongst the six samples (Figs. 1–6), two groups can be distinguished according to their alteration: *Pelecanimimus polyodon* (Fig. 5) and the Archosauria indet. (Fig. 6) are fairly altered, whereas the other samples are less altered (Discoglossidae indet. Fig. 1; Centrocryptodira indet. Fig. 2; *Scandensia ciervensis*, Fig. 3; *Concornis lacustris*, Fig. 4). The
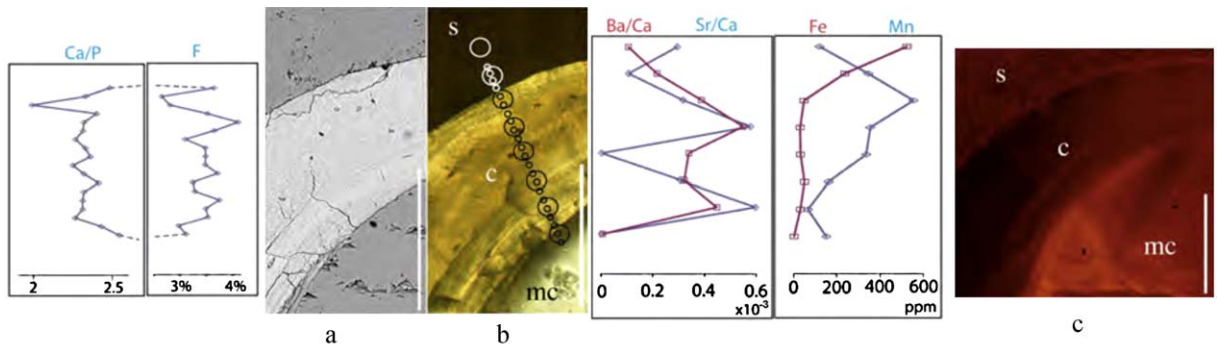
Fig. 1. Elementary profiles obtained by electron microprobe on the Discoglossidae indet. Photo (a) was taken in scanning electronic microscopy (SEM)–Backscattered electrons (BSE). Photo (b) was taken under natural light (NL) and indicates the microprobe spots (small spot diameter for major elements (Ca, P and F): 3 μm; big spot diameter for minor elements (Ba, Sr, Mn, Fe and REE): 20 μm). Photo (c) was taken by optical cathodoluminescence (CL). Abbreviations (same from Figs. 1–6): c: cortex; mc: medullar cavity; s: sediment. Scale bars: 100 μm.
Fig. 1. Profils élémentaires obtenus par microsonde électronique chez Discoglossidae indet. La photo (a) a été prise en microscopie électronique à balayage (MEB)–Mode électrons rétrodiffusés (ER). La photo (b) prise en lumière naturelle (LN) indique les passages de la sonde. Les petits cercles de 3 μm de diamètre, correspondent aux points de mesure des éléments majeurs (Ca, P et F) ; les gros cercles, d'un diamètre de 20 μm, correspondent à ceux des éléments mineurs (Ba, Sr, Mn, Fe et REE). La photo (c) est prise en cathodoluminescence (CL) optique. Abréviations (les mêmes pour les Fig. 1 à 6) : c : cortex ; mc : cavité médullaire ; s : sédiment. Échelle : 100 μm.

latter group presents Ca/P values that range roughly from 2 to 2.5 (Figs. 1–4) and the former shows higher values ranging from 3 to 5 (Figs. 5 and 6). All of these values are similar to those observed in some altered dinosaur and plesiosaur bones of the Late Cretaceous and Early Jurassic (Goodwin et al., 2007).

The fluorine concentration of each sample is fairly high (higher than the biological range), ranging approximately from 3 to 4% (Figs. 1, 3–6). Only the Centrocryptodira indet. (Fig. 2) shows slightly higher values (with a maximum of 4.8%).

3.1.2. Optical cathodoluminescence

The bones are not luminescent compared to the sediments and the crystals in their medullary cavities (Figs. 1c, 2c, 3b, 4c). This is due to a relatively low Mn concentration, mostly between 0 and 20 ppm for each specimen (Figs. 2–6). This does not apply to the Discoglossidae indet. that has higher values, approaching 300 ppm (Fig. 1). In concert with the low Mn values, this could also be due to a higher Fe concentration that inhibits the luminescence, especially visible in the Archosauria indet. where the Fe amount is much higher than the Mn amount (approximately 230 ppm vs. less than 20 ppm; Fig. 6). The yellow-bright orange luminescence observed in the medullary cavities indicates the presence of secondary calcite (Figs 2c, 3b, 4c and 6b). In addition to this, recrystallisations and sediment filling are noticeable in vascular canals (Fig. 6b), fractures (Figs. 2c and 6b), and in the osteocytic lacunae (Figs. 2c and 4c).
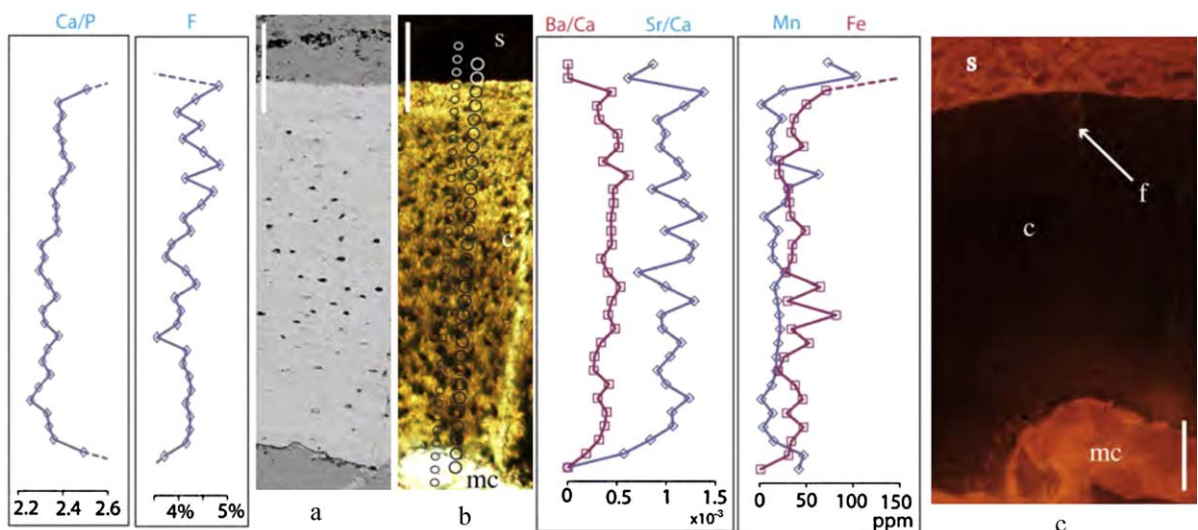

Fig. 2. Elementary profiles obtained by electron microprobe on the Centrocryptodira indet. Photo (a) was taken in SEM-BSE, photo (b) in NL and photo (c) in optical-CL. Supplementary abbreviation: f: fracture. Scale bars: 100 μm.
Fig. 2. Profils élémentaires obtenus par microsonde électronique chez Centrocryptodira indet. La photo (a) est prise en MEB-ER, la photo (b) en LN et la photo (c) en CL-optique. Abréviation supplémentaire : f : fracture. Échelle : 100 μm.
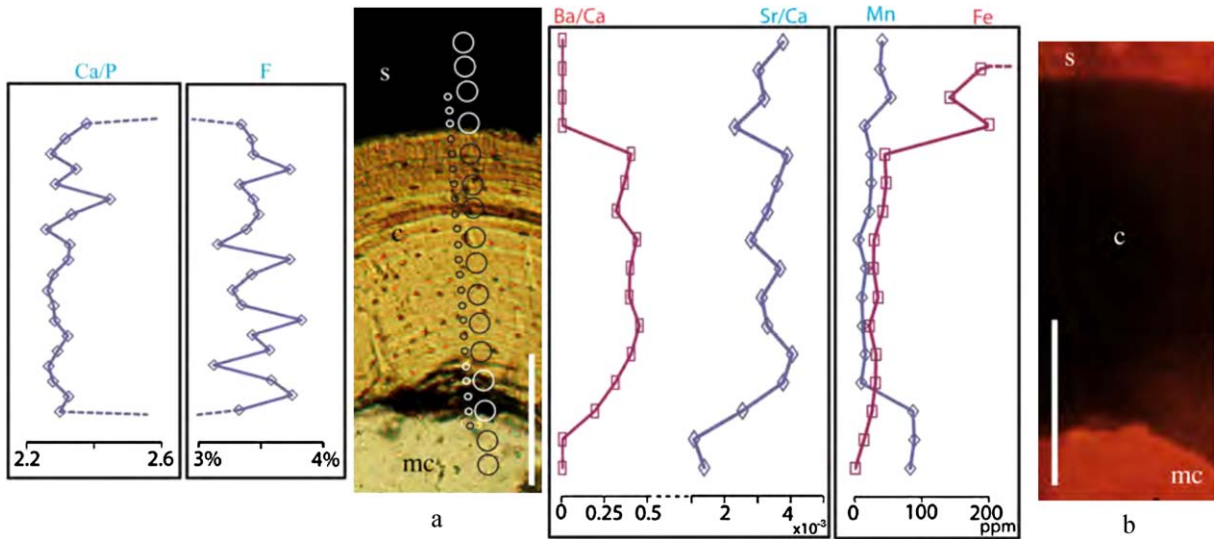
Fig. 3. Elementary profiles obtained by electron microprobe on *Scandensia ciervensis*. Photo (a) was taken in NL and photo (b) in optical-CL. Scale bars: 100 μm.

Fig. 3. Profils élémentaires obtenus par microsonde électronique chez *Scandensia ciervensis*. La photo (a) est prise en LN et la photo (b) en CL-optique. Échelle : 100 μm.

3.1.3. Rare earth elements

As proposed by Reynard et al. (1999) and Lécuyer et al. (2003), La/Yb and La/Sm ratios were calculated and normalized to the standard Post Archean Shale Composite, PAAS (McLennan, 1989). These ratios allow characterizing REE incorporation domains in different postmortem environments (Fig. 7). All analyzed samples are located within the domain of REE-bearing non-marine fluids (river freshwater). The (La/Yb)N values range from 0.61 to 0.34

and the (La/Sm)N values from 0.20 to 0.44 (Fig. 7). These results suggest rather a moderate postmortem REE incorporation to freshwater pattern than a recrystallisation in the presence of groundwater fluids during a multi-stage late diagenesis (Reynard et al., 1999; Lécuyer et al., 2003).

3.1.4. Sr and Ba

Concerning Sr (Fig. 8), 3 groups are distinguished, one with high concentrations (from 1300 to 2000 ppm), one
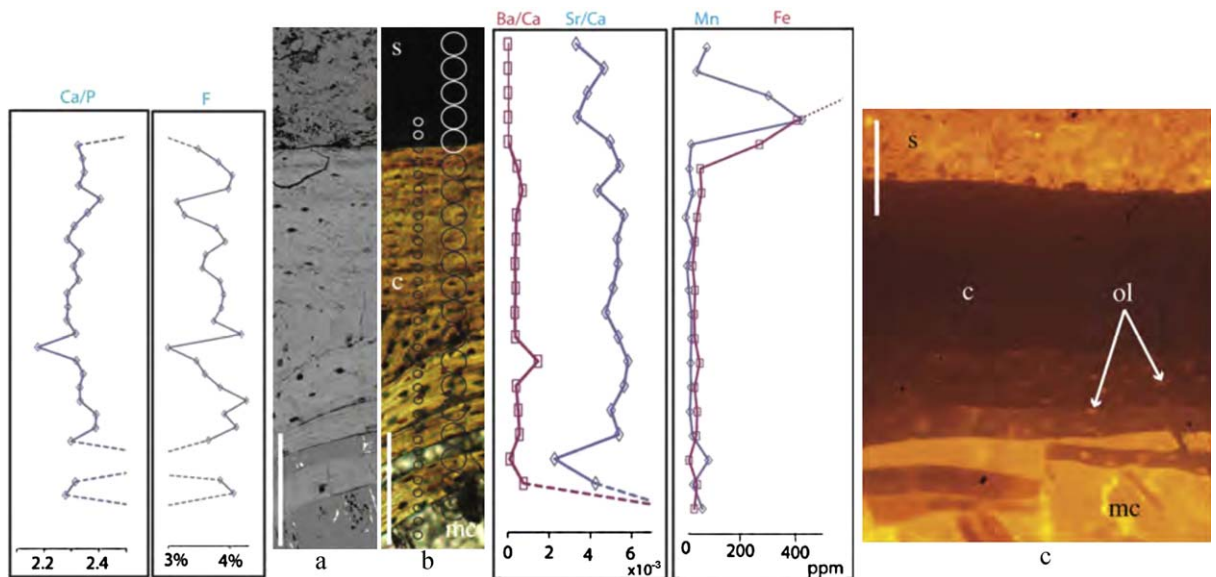

Fig. 4. Elementary profiles obtained by electron microprobe on *Concornis lacustris*. Photo (a) was taken in SEM-BSE, photo (b) in NL and photo (c) in optical-CL. Supplementary abbreviation: ol: osteocytic lacunae. Scale bars: 100 μm.

Fig. 4. Profils élémentaires obtenus par microsonde électronique chez *Concornis lacustris*. La photo (a) est prise en MEB-ER, la photo (b) en LN et la photo (c) en CL-optique. Abréviation supplémentaire : ol : logette ostéocytaire. Échelle : 100 μm.
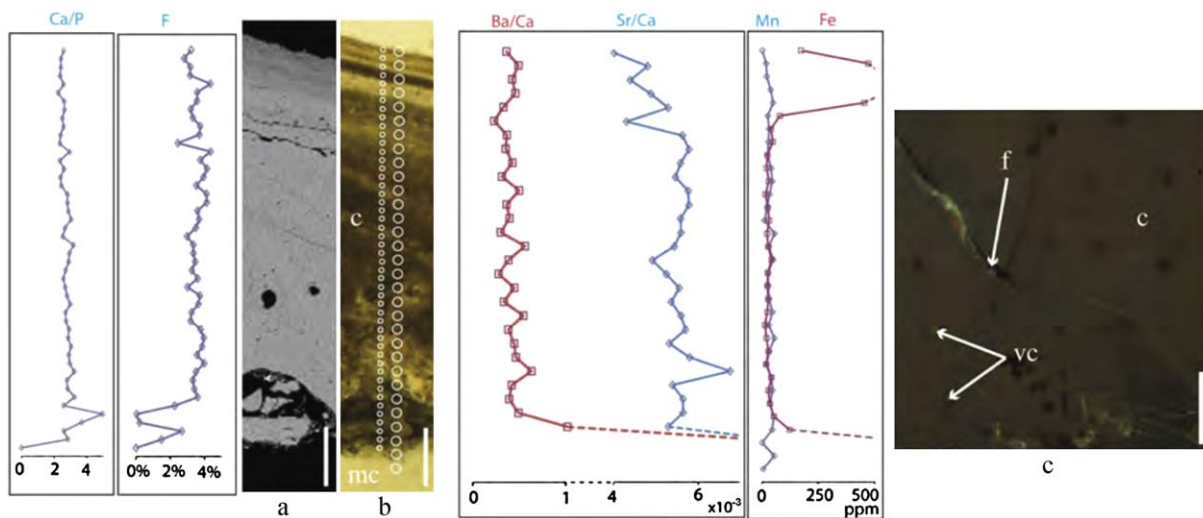
Fig. 5. Elementary profiles obtained by electron microprobe on *Pelecanimimus polyodon*. Photo (a) was taken in SEM-BSE, photo (b) in NL and photo (c) in optical-CL. Supplementary abbreviation: vc: vascular canals. Scale bars: 100 μm.
Fig. 5. Profils élémentaires obtenus par microsonde électronique chez *Pelecanimimus polyodon*. La photo (a) est prise en MEB-ER, la photo (b) en LN et la photo (c) en CL-optique. Abréviation supplémentaire : vc : canaux vasculaires. Échelle : 100 μm.

specimen showing a range from 800 to 1200 ppm and the last group with lower concentrations (from 100 to 300 ppm). These distributions are in agreement with the Sr concentration observed for the sediments (Poyato-Ariza et al., 1998). Therefore, these 3 groups may rather reflect a late Sr incorporation during the fossilization than a true palaeobiological signal. The small variations observed in

the Sr/Ca profiles may also be linked to the occurrence of some sediment in the osteocyte cavities inducing perturbations during the analysis. Hence, the Sr/Ca ratios (Figs. 1–6) cannot be used to infer any biological trait. According to Talbot et al. (1995), who investigated the chemistry of the waters of the Las Hoyas environment, these Sr uptakes might be due to a contamination from three sources: an
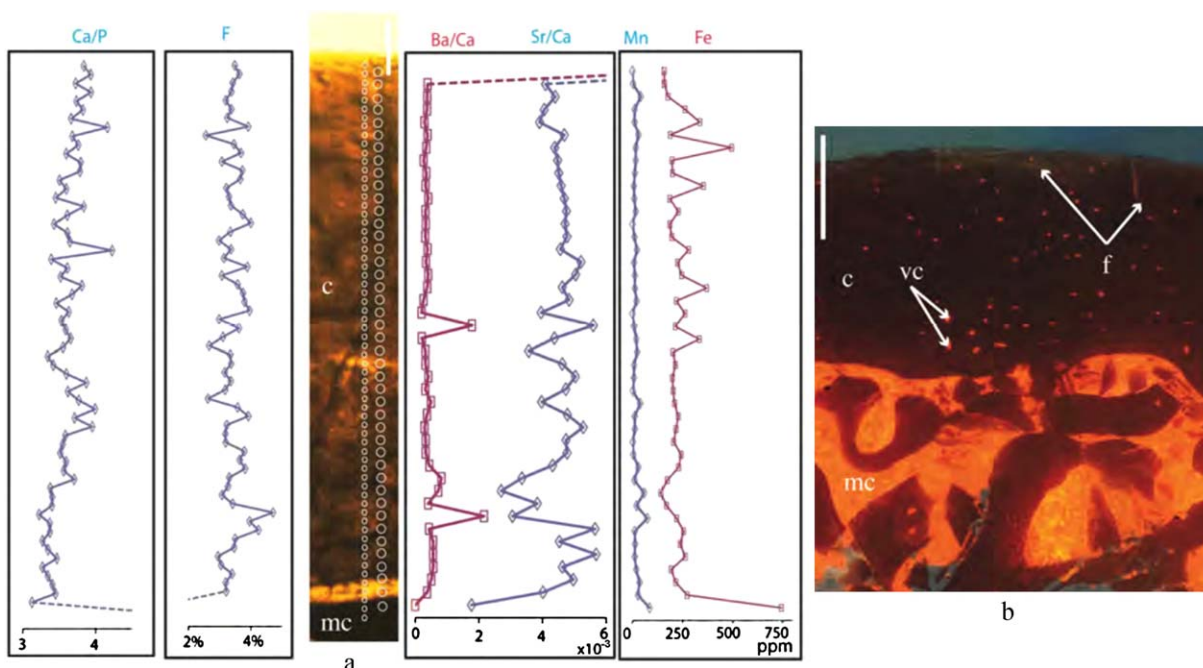

Fig. 6. Elementary profiles obtained by electron microprobe on the Archosauria indet. Photo (a) was taken in NL and photo (b) in optical-CL. Scale bars: 100 μm for (a) and 500 μm for (b).
Fig. 6. Profils élémentaires obtenus par microsonde électronique chez Archosauria indet. La photo (a) est prise en LN et la photo (b) en CL-optique. Échelle : 100 μm pour (a) et 500 μm pour (b).
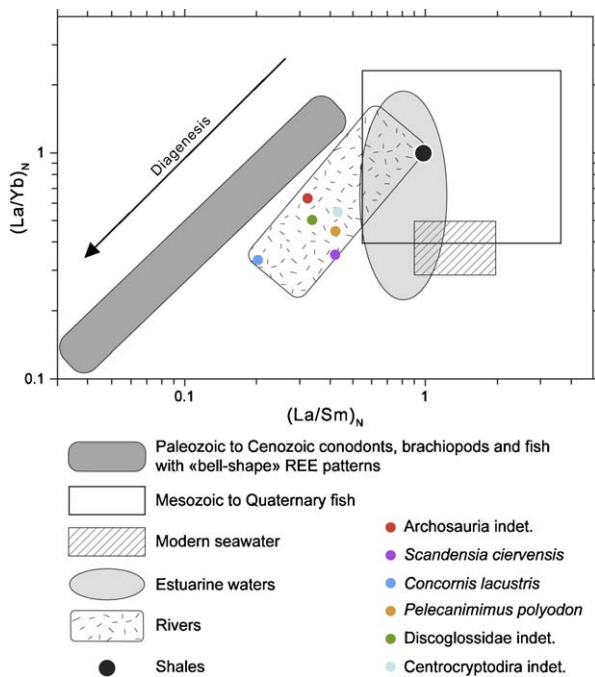
Fig. 7. Normalized La/Yb ratios versus La/Sm ratios of the Las Hoyas samples reported in the diagram proposed by Reynard et al., 1999. Modified from Lécuyer et al., 2003.
Fig. 7. Rapports normalisés La/Yb et La/Sm des fossiles de Las Hoyas, reportés dans le diagramme proposé par Reynard et al., 1999. Modifié d'après Lécuyer et al., 2003.

inflow from the dominating Jurassic limestones; the karst surface of the lakes; and Triassic clastic rocks located in the south-east of the Las Hoyas basin. The Ba values are much lower than the Sr values, ranging from about 50 to 300 ppm (Fig. 8), and are different from the sediment (almost Ba-free). This may reflect a true biological signal, especially since the values are located within the biological range observed in living organisms (Burton et al., 1999; Hinners et al., 1998). However, the Ba/Ca ratios (ranging approximately from 3×10^{-4} to 4×10^{-4}, Figs. 1–6) do not allow to significantly distinguishing the specimens by their diet.
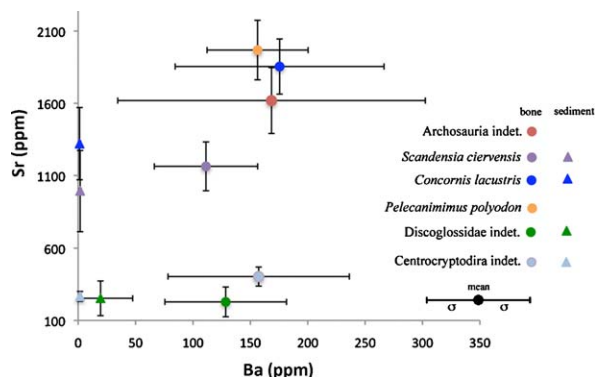

Fig. 8. Sr and Ba concentrations (mean and standard-deviation) in bones and their associated sediments.
Fig. 8. Teneurs en Sr et en Ba (moyennes et écart-types) dans les ossements et les sédiments associés des échantillons.

In other words, the range of Ba/Ca values regroups all the specimens within a single dietary intake category, which in this case would be the herbivorous category, according to studies made on actual mammalian ecosystems (Balter et al., 2002; Burton et al., 1999). This is very unlikely to reflect a true biological signal and may rather be a post-mortem modification that have biased and homogenized the Ba content.

3.2. Palaeohistology

Amphibia - Anura–Discoglossidae *incertae sedis*.
This small amphibian has been attributed to the family Discoglossidae. The medullary cavities of its bones are filled with calcite (Fig. 9). The primary cortex is formed of parallel-fibered avascular bone and shows few osteocytic lacunae (Fig. 9b). A small crista is noticeable and presents a higher cellular density and oblique sharpey's fibers (Fig. 9c). The outermost layer is composed of lamellar bone (Fig. 9b). Lamellar endosteal bone lines the perimedullary border (Fig. 9b). There is no visible sign of cyclical growth.

Chelonia–Centrocryptodira *incertae sedis*.
The medullary cavity of the femur is partially filled with cancellous bone and the primary cortex is almost avascular (Fig. 10). The lateromedial sides are composed of pseudolamellar bone (the fibers are parallel and show a stratification with successive layers that have a global circumferential orientation). The posterior side is thicker and is composed of parallel-fibered bone (Fig. 10b). The cellular density in this area is high, with round osteocytes and many radiating canaliculi (well visible in the ulna, Fig. 10c). There is again no visible sign of cyclical growth. No resorption, endosteal bone or remodeling traces are observable at the perimedullary border (Fig. 10a, c).

Lepidosauria–Squamata - *S. ciervensis*.
This small lizard probably belongs to the species *S. ciervensis*, whose osteology was described by Evans and Barbadillo (1998). The medullary cavity is filled with calcite (Fig. 11). The primary cortex is essentially composed of pseudolamellar avascular bone. The osteocytic lacunae are numerous (Fig. 11a–d). There is an alternance of zones (dark, rapidly growing) and annuli (light, with a less rapid growth), which characterize lamellar-zonal bone (Fig. 11c). At the metaphyseal region of the femur, we can observe a drifted resorption of the periosteal cortical bone by the perimedullary border (Fig. 11d). Endosteal lamellar bone was secondarily laid down on the less resorbed side. This phenomenon is called bone drift (Enlow, 1963). With the help of scanning electronic microscopy, a calcified cartilage matrix being replaced by bone (Ricqlès et al., 1991) can be observed (Fig. 11e).

Theropoda–Aves–Enantiornithe–*C. lacustris*
This specimen of *C. lacustris* (Sanz et al., 1995) is an Enantiornithine (a clade of bird which disappeared at the end of the Cretaceous) and its histology was recently described by Cambra-Moo et al. (2006). A tibiotarsal and
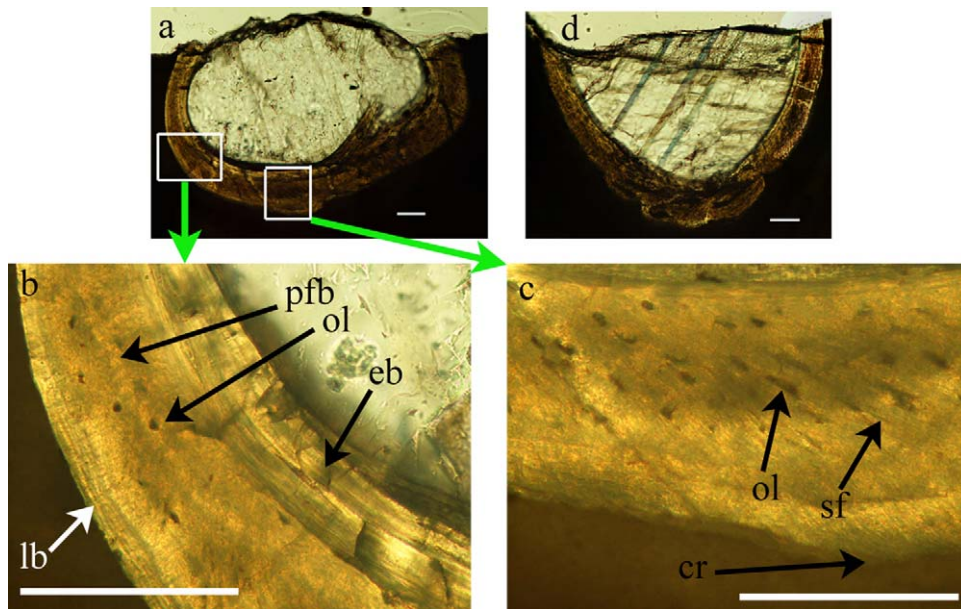
Fig. 9. Metaphyseal sections of the Discoglossidae indet. natural light. a: tibiofibula. b and c: enlarged view of a. d: femur. Abbreviations: cr: small crista; eb: endosteal bone; lb: lamellar bone; ol: osteocytic lacuna; pfb: parallel-fibered bone; sf: sharpey's fibers. Abbreviations will be the same for all figures. Scale bars: 100 µm.

Fig. 9. Sections métaphysaires de Discoglossidae indét., lumière naturelle. a : tibiofibula. b et c : vue élargie du tibiofibula. d : fémur. Abréviations : cr : petite crête ; eb : os endostéal ; lb : os lamellaire ; ol : lacune ostéocytaire ; pfb : os à fibres parallèles ; sf : fibres de sharpey. Les abréviations seront les mêmes pour toutes les figures suivantes. Échelle : 100 µm.
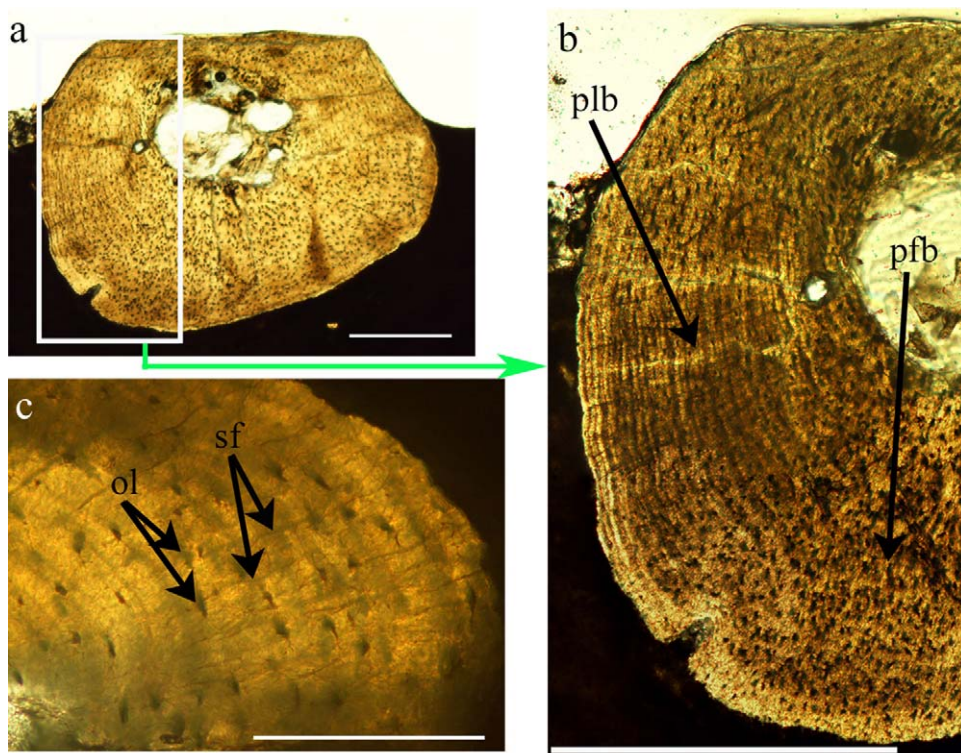

Fig. 10. Mid-diaphyseal sections of the Centrocryptodira indet. natural light. a: femur. b: enlarged view of a. c: ulna. Other abbreviation: plb: pseudolamellar bone. Scale bars: 500 µm for a and b; 100 µm for c.

Fig. 10. Sections mi-diaphysaires de Centrocryptodira indét., lumière naturelle. a : fémur. b : vue élargie du fémur. c : ulna. Abréviation supplémentaire : plb : os pseudolamellaire. Échelle : 500 µm pour a et b ; 100 µm pour c.
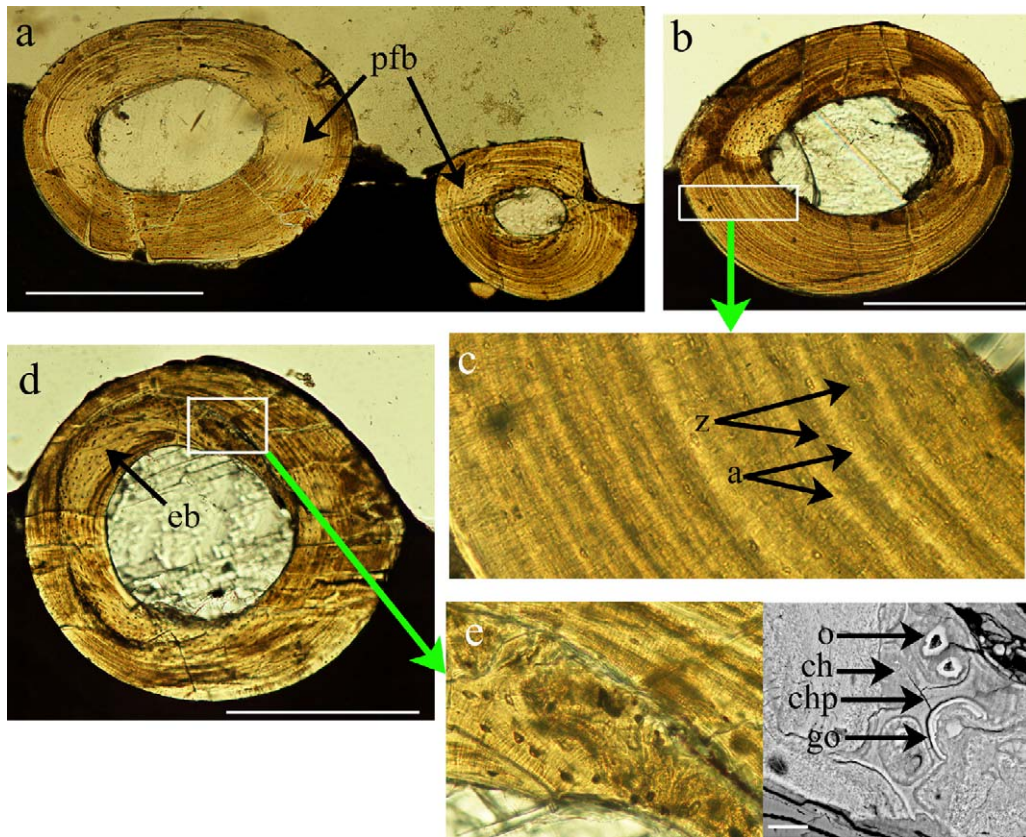
Fig. 11. Mid-diaphyseal (a, b, c) and metaphyseal sections (d et E) of *Scandensia ciervensis*, natural light. a: tibia on the left and fibula on the right. b (with an enlarged view in c) and d (with an enlarged view in e), femur. e shows a natural light photography on the left and a SEM photography of the same area on the right (Backscattered electron mode). Other abbreviations: a: annuli; ch: hypertrophied chondrocyte; chp: chondroplasm; go: *globuli ossei;* o: osteocyte; z: zones. Scale bars: 500 μm for a, b and d; 10 μm for e.

Fig. 11. Sections mi-diaphysaires (a, b, c) et métaphysaires (d et e) de *Scandensia ciervensis*, lumière naturelle. a : tibia à gauche et fibula à droite. b (avec une vue élargie en c) et d (avec une vue élargie en e), fémur. La photo e montre une photographie en lumière naturelle à gauche, et une photographie de microscopie électronique à balayage à droite. Abréviations supplémentaires : a : annuli ; ch : chondrocyte hypertrophié ; chp : chondroplasme ; go : *globuli ossei* ; o : ostéocyte, z : zones. Échelle : 500 μm pour a, b et d ; 10 μm pour e.
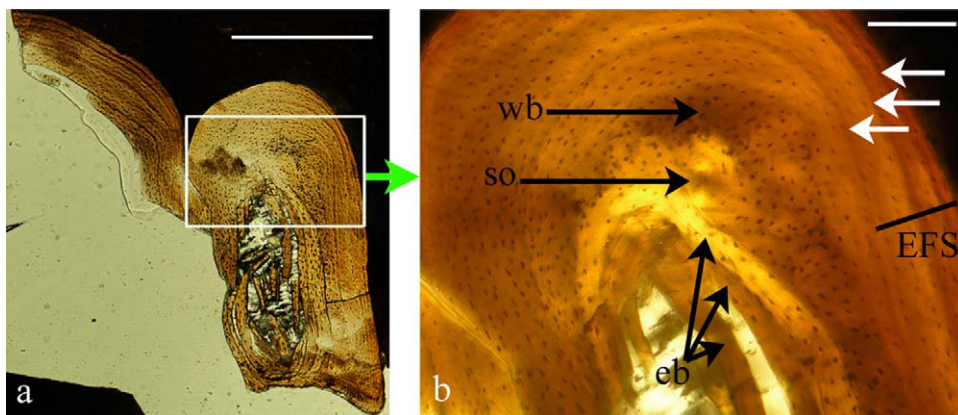

Fig. 12. Section in the tarsometatarsal IV of *Concornis lacustris*, natural light. Other abbreviations: EFS: external fundamental system; so: secondary osteon; wb: woven bone. The white arrows indicate LAGs. Scale bars: 500 μm for a and 100 μm for b.

Fig. 12. Section du tarsométatarse IV de *Concornis lacustris*, lumière naturelle. Abréviations supplémentaires : EFS, système fondamental externe; so : ostéones secondaires ; wb : os à fibres enchevêtrées. Les flèches blanches indiquent les LACs. Échelle : 500 μm pour a et 100 μm pour b.

fused 23 mm long tarsometatarsals were analyzed. Only the tarsometatarsal IV will be presented here (Fig. 12). The perisoteal cortex is composed of two distinct parts (Fig. 12b): (1) an internal layer of woven bone with numerous rounded osteocytes, which seems to have undergone secondary reconstructions (indicated by the presence of what could be secondary osteons); and (2) an External Fundamental System (EFS; Cormack, 1987) formed of lamellar-zonal bone, with at least 3 lines of arrested growth (LAGs). The osteocytes are typically more flattened in the EFS.

Theropoda–Ornithomimosauria–*P. polyodon*

The studied bone belongs to a detached fragment from *P. polyodon*, the first Ornithomimosaur (theropod dinosaur) found in Europe. Its histology was described by Perez-Moreno et al. (1994). Only the anterior part of the fully articulated specimen was preserved (the skull, the cervical and dorsal vertebra, the ribs, the pectoral girdle, the sternum and the forelimbs). A noticeable particularity is its high number of teeth (approximately 220). Unlike the other Ornithomimosaurs whose teeth were used to cut and rip, *Pelecanimimus*'s teeth might have functioned as a beak (Perez-Moreno et al., 1994). The studied material comes from one of its ribs (Fig. 13). The medullary cavity is partially filled with an endosteo-endochondral spongiosa (Fig. 13b). Ribs are ventrally curved bones; consequently, they possess a convex ventral part with a relatively thick cortex, and a concave dorsal part with a thinner cortex (Fig. 13a). Because of this morphogenesis and these particular growth processes, bone typologies and remodeling degrees are highly variable locally. In a highly remodeled area (Fig. 13b), we observe an internal cortical bone made of Haversian systems. The most external cortical bone is formed of a fibrolamellar complex (primary osteons in a woven bone matrix) with variably isolated vascular canals. A line of arrested growth in the proximity of the bone surface probably marks the beginning of an external fundamental system.

Archosauria *incertae sedis*

The studied bone is a 4 cm-long fragment of isolated proximal end of a humerus. It is attributed to an Archosauria indet. The medullary cavity is partially filled with fractured endosteal trabeculae (Fig. 14). Its very complex cortex shows remodeling and modeling processes. Two tissue types are observable:

- A primary cortex composed of a highly vascularized fibrolamellar complex (Fig. 14c, d). The vascularisation pattern is laminar (Fig. 14d). The osteocyte lacunae are rounded with well-developed canaliculi. The outermost layer is composed of avascular parallel-fibered bone with at least two LAGs. The perimedullar border is lined by endosteal bone (Fig. 14c);
- Compacted coarse-cancellous bone with an endosteal origin (Enlow, 1963; Fig. 14b, c). This type of tissue is due to the general relocalisation process: during the growth in length and width of a bone, some areas are automatically relocated in different regions, relatively

to their original location. In our case, cancellous bone originally located in the metaphysis was progressively relocated and compacted in the diaphysis. This metaphyseal remodeling (Enlow, 1963) is followed by resorption and redeposition of endosteal bone. Thus this tissue is composed of old bony trabeculae (coming originally from the medullary cavity), sinuous lamella of endosteal bone, and secondary endosteal osteons (Fig. 14b). We can also observe a bone drift phenomenon. The primary cortex (synthesized secondarily to increase the width of the diaphyseal cortex) is not evenly distributed (Fig. 10a), which indicates a differential growth associated to morphogenesis (probably linked to the synthesis of the deltopectoral crest).

4. Discussion

Bony tissues record the ontogenetic history of fossil vertebrates (Ricqlès et al., 1991). The growth rate estimations of such animals, based on Amprino's rule (1947), have been investigated not too long ago (Curry, 1999; Erickson, 2005; Horner et al., 1999, 2000, 2001; Padian et al., 2001, 2004; Ricqlès et al., 2003, 2008; Sander, 2000). The body (and bone) growth rates are considered because they may have an important selective value (Montes et al., 2010). For example, high growth rates are adaptive in King penguin chicks because they confer a higher evolutionary fitness than lower growth rates (Margerie et al., 2004). Indeed, after hatching, these animals grow really fast in order to get ready for winter, where only chicks that have reached large sizes can survive (Margerie et al., 2004). This is also similar in non-colonial nesting birds that have to grow rapidly in order to escape predators as soon as possible (Cubo et al., 2000). Amprino's (1947) rule allowed us to estimate the local growth rate of each studied bone. This information related to the growth dynamics of each specimen is important to understand the vertebrate assemblage of Las Hoyas. The presence of endosteal bone, bone remodeling, EFS (Figs. 11–14), or an external thin layer of lamellar bone (for the Discoglossidae indet.; Fig. 9), indicate that all of the specimens were adults, except for the Chelonia indet. Its lack of secondary bone and the low resorption of the perimedullary border suggest a subadult, or even a juvenile (Fig. 10). The Discoglossidae indet., *S. ciervensis* and the Chelonia indet. show pseudolamellar avascular or parallel-fibered avascular cortices (Figs. 9–11). Even though their osteocytic lacunae have different shapes and densities, this avascularity indicates a (local) slow growth, inferior to 2 μm/day. This estimation is based on extant amphibians (Francillon, 1979), chelonians and lepidosaurians (Castanet, 1985). Their slow growth must have been a consequence of low resting metabolic rates (Montes et al., 2007). The microstructure of the other specimens (*C. lacustris*, *P. polyodon* and the Archosauria indet.) indicates higher growth rates. The first layer of the primary cortex of *C. lacustris* (Fig. 12b) indicates a relatively rapid growth, between 2 and 10 μm/day. The lamellar-zonal complex then implies a slowdown of growth (probably inferior to 2 μm/day). This lamellar-zonal complex presenting LAGs is observable in other Enantiornithes (Chinsamy et al., 1995) but absent in the
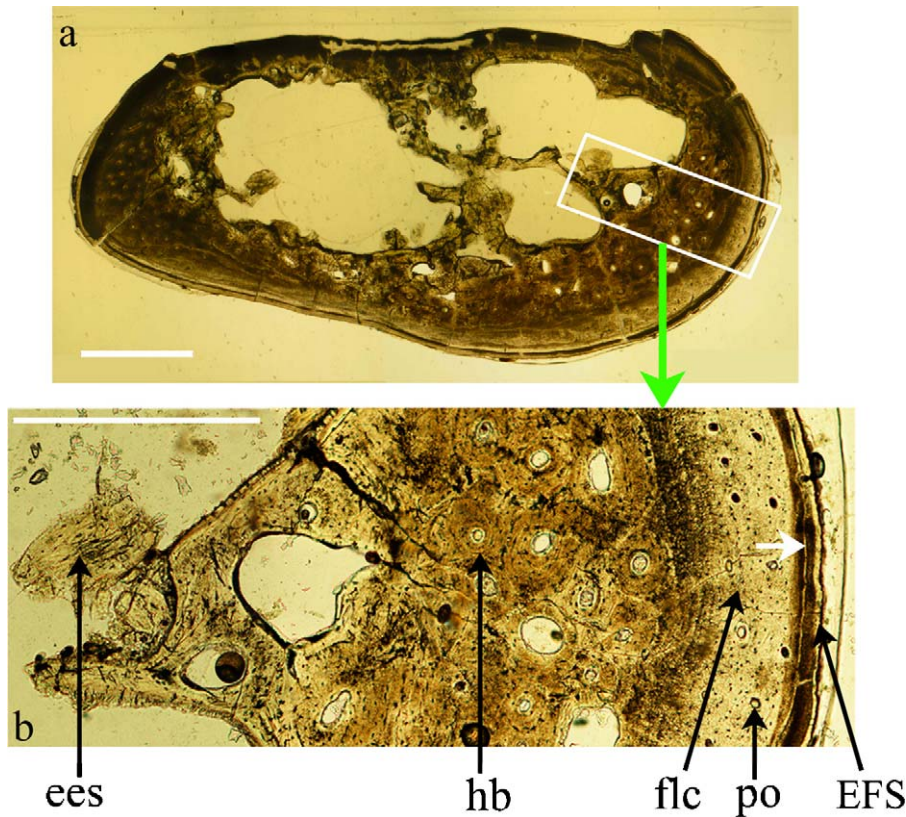
Fig. 13. Section in a rib of *Pelecanimimus polyodon*, natural light. Other abbreviations: ees: endosteo-endochondral *spongiosa*; flc: fibrolamellar complex; hb: haversian bone; po: primary osteon. The white arrow indicates a LAG. Scale bars: 1 mm for a and 500 μm for b.
Fig. 13. Section d'une côte de *Pelecanimimus polyodon*, lumière naturelle. Abréviations supplémentaires : ees : *spongiosa* endostéo-endochondrale ; flc : complexe fibrolamellaire ; hb : os haversien ; po : ostéone primaire. La flèche blanche indique une LAC. Échelle : 1 mm pour a et 500 μm pour b.

majority of extant birds, in which it has been observed only in the kiwi (Bourdon et al., 2009). This suggests that Enantiornithes had lower growth rates than most nesting living birds (Chinsamy-Turan, 2005; Ricqlès et al., 2003). The 3 LAGs indicate an age of at least 3 year, which contrasts the 12-months long growth of the majority of extant birds (Starck and Ricklefs, 1998). It is tempting to hypothesize that the low growth rate in this specimen is due to an early flight (because part of the energy would be allocated to locomotion). Such a small species would perform only flapping flight, highly energy consuming, which suggests an endothermic metabolism. The typology of *P. polyodon* (Fig. 13b) also suggests a relatively high growth rate (approximately 10 μm/day). It is hazardous to discuss the metabolism of this specimen on the basis of only one section, but the important vascularization may reflect an endothermic metabolism (Cubo et al., 2008), which is also consistent with the conclusions of palaeohistological studies on non-avian dinosaurs (Padian et al., 2001; Ricqlès, 1980). The high vascularization of the Archosauria indet (Fig. 14) suggests a fast growth (probably superior to 10 μm/day) and therefore a high resting metabolic rate. The laminar pattern is similar to what is observed in some species of dinosaurs (Horner et al., 1999). In addition to growth rates, these specimens also inform

about the palaeoenvironmental conditions of Las Hoyas. Four of the six specimens have a cyclic bone apposition: *C. lacustris*, *P. polyodon* and the Archosauria indet. show cessations of growth (see the LAGs in Figs. 12b, 13b, 14b) while *S. ciervensis* shows a slowdown of growth (Fig. 11c). These marks are the histological expression of a temporary variation of the growth rate (Castanet et al., 1993) and it may very well be due to the strong seasonal (arid and wet) alternance, sedimentologically interpreted at the Serranía of Cuenca Basin during the Barremian (Fregenal-Martínez, 1998; Gómez Fernández and Meléndez., 1991). The size of the specimens is also really interesting to understand the ecosystem of Las Hoyas. Indeed, every specimen belongs to a small-sized species (a few centimeters; except for *P. polyodon*). In fact, the majority of the vertebrates found in Las Hoyas are small (Buscalioni et al., 2008) and affected by "dwarfism". It is worth noting that most of the recovered specimens in the amphibious category are small-sized species. At first glance, this could be due to a preferential preservation of small animals. However, the particular ecological condition of Las Hoyas that might be interpreted as a nesting protected area (Buscalioni and Fregenal-Martinez, 2010), and/or the existence of other ecological filters (Cambra-Moo, 2006; Ricqlès pers. comm.) may favor the abundance of small-sized species.
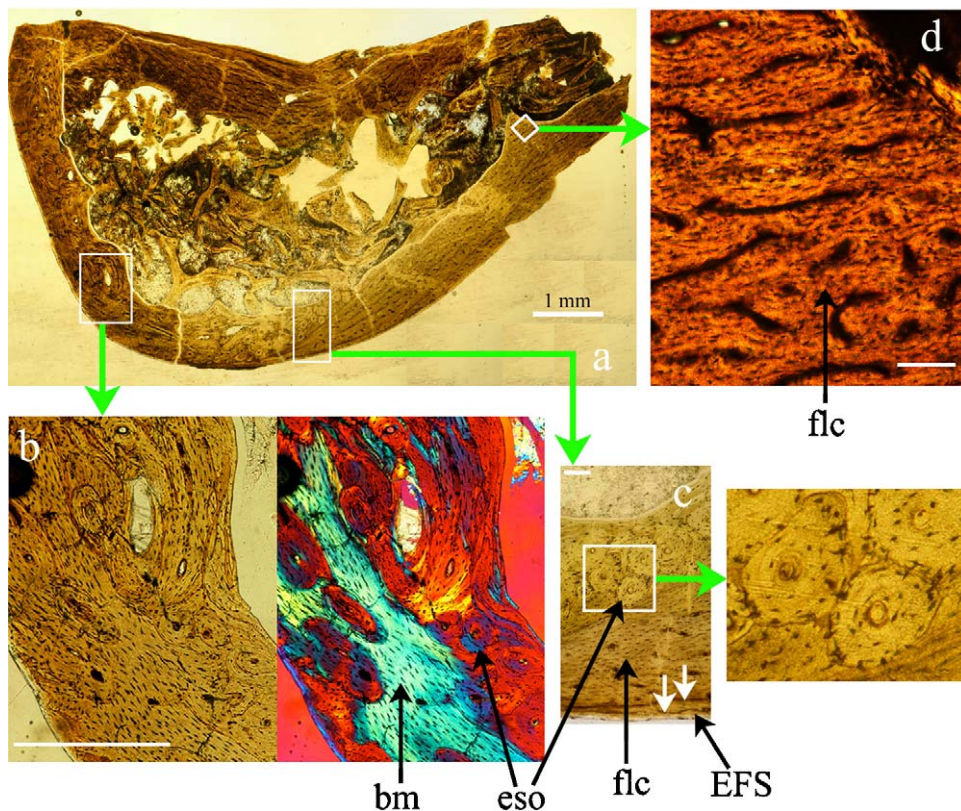
Fig. 14. Metaphyseal section of the humerus of the Archosauria indet. (a), natural light. b shows compacted coarse-cancellous bone in natural light (left) and polarized light (right). c shows the limit between compacted coarse-cancellous bone and the primary cortex (also shown in d). Other abbreviations: bm: bone originally from medulla; eso: endosteal secondary osteons. The white arrows indicate LAGs. Scale bars: 1 mm for a; 500 μm for b; 100 μm for c and d.

Fig. 14. Section métaphysaire de l'humérus de l'Archosauria indét. (a), lumière naturelle. La photo b montre de l'os spongieux secondairement compacté en lumière naturelle à gauche et en lumière polarisée à droite. La photo c présente la limite entre l'os spongieux secondairement compacté et le cortex primaire, lui-même présenté en figure d. Abréviations supplémentaires : bm : os provenant primitivement de la médulla ; eso : ostéones secondaires endostéaux. Les flèches blanches indiquent des LACs. Échelle : 1 mm pour a ; 500 μm pour b ; 100 μm pour c et d.

5. Perspectives

Palaeobiology (i.e., the estimation of ecological, physiological, environmental and life history traits of extinct species) is a developing field with great potential. Bone microstructure and bone chemical composition are valuable sources of palaeobiological information because they record many parameters on the ecology, physiology, environment and life history traits during the lifespan of extinct animals. In a landmark paper, Tütken et al. (2004) used bone histology and oxygen isotope composition to infer palaeoclimatic conditions in the Jurassic Shishugou Formation in the Junggar Basin, NW China. These authors found that seasonal intra-bone oxygen isotope variations are congruent with seasonal histological patterns of bone deposition. Future work integrating palaeohistological and geochemical approaches may allow testing dynamic relationships. For instance, it should be possible to test whether ontogenetic changes in a given individual (calibrated using bone histology, e.g. the change from a juvenile, fast growing stage during which fibrolamellar bone tissue is formed, to a subadult stage, during which a parallel fibered bone is formed) are accompanied by changes in the dietary intake (estimated using the Sr/Ca and Ba/Ca ratios) or in the metabolic rate (using oxygen isotopes).

References

Amiot, R., Lécuyer, C., Buffetaut, E., Escarguel, G., Fluteau, F., Martineau, F., 2002. Oxygen isotopes from biogenic apatites suggest widespread endothermy in Cretaceous dinosaurs. Earth Planet. Sci. Lett. 246, 41–54.
Amprino, R., 1947. La structure du tissu osseux envisagée comme expression de différences dans la vitesse de l'accroissement. Arch. Biol. 58, 315–330.

Balter, V., Bocherens, H., Person, A., Labourdette, N., Renard, M., Vandermeersch, B., 2002. Ecological and physiological variability of Sr/Ba and Ba/Ca in mammals of West European mid-Würmian food webs. Palaeogeogr. Palaeoclimatol. Palaeoecol. 186, 127–143.

Balter, V., Person, A., Labourdette, N., Drucker, D., Renard, M., Vandermeersch, B., 2001. Were Neandertalianns essentially carnivores? Sr and Ba preliminary results of the mammalian palaeobiocoenosis of Saint-Césaire. C. R. Acad. Sci. Paris Ser. IIa 332, 59–65.

Bourdon, E., Castanet, J., Ricqlès de, A.J., Scofield, P., Tennyson, A., Lamrous, H., Cubo, J., 2009. Bone growth marks reveal protracted growth in New Zealand kiwi (Aves, Apterygidae). Biol. Lett. 5, 639–642.

Briggs, D.E.G., Wilby, P.R., Pérez-Moreno, B.P., Sanz, J.L., Fregenal-Martinez, M., 1997. The mineralization of dinosaur soft tissue in the Lower Cretaceous of Las Hoyas, Spain. J. Geol. Soc. London 154, 587–588.

Bryant, J.D., Luz, B., Froelich, P.N., 1994. Oxygen isotopic composition of fossil horse toothphosphate as a record of continental paleoclimate. Palaeogeogr. Palaeoclimatol. Palaeoecol. 107, 303–316.

Burton, J.H., Price, T.D., Middleton, W.D., 1999. Correlation of bone Sr/Ca and Ba/Ca due to biological purification of calcium. J. Archaeolog. Sci. 26, 609–616.

Buscalioni, A.D., Fregenal-Martinez, M., 2010. An holistic approach to the Palaeoecology of Las Hoyas Konservat Lagerstätten (La Huerguina Formation, Lower Cretaceous, Iberian Ranges). J. Iber. Geol. Special Issue Mesozoic Terrestrial Ecosystems and Biotas 36 (2), 297–326.

Buscalioni, A.D., Fregenal-Martinez, M.A., Bravo, A., Poyato-Ariza, F.J., Sanchiz, B., Baez, A.M., Cambra-Moo, O., Marugán-Lobon, J., 2008. The vertebrate assemblage of Buenache de la Sierra (Upper Barremian of Serranía de Cuenca, Spain) with insights into its taphonomy and palaeoecology. Cretaceous Res 29, 687–710.

Cambra-Moo, O., 2006. Bioestatinomía y Fosildiagénesis de Arcosaurios. Aplicación de la Actuotafonomia al estudio de la influencia paleobiologica en el proceso tafonomico, Unpublished PhD thesis, Universidad Autónoma de Madrid, Facultad de Ciencias, 334 p.

Cambra-Moo, O., Buscalioni, A.D., Cubo, J., Castanet, J., Loth, M.-M., de Margerie, E., de Ricqles, A.J., 2006. Histological observations of enantiornithine bone (Saurischia, Aves) from the Lower Cretaceous of Las Hoyas (Spain). C. R. Palevol 5, 685–691.

Castanet, J., 1985. La squelettochronologie chez les reptiles. I : résultats expérimentaux sur la signification des marques de croissance squelettiques chez les lézards et les tortues. Ann. Sci. Nat. Zool. Biol. Anim. 7, 23–40.

Castanet, J., Francillon-Vieillot, H., Meunier, F.J., Ricqlès de, A.J., 1993. Bone and individual aging. In: Hall, B.K. (Ed.), Bone: bone growth, 7. B. CRC Press, Boca Raton, Florida, pp. 245–283.

Chinsamy-Turan, A., 2005. The microstructure of dinosaur bone: deciphering biology with fine-scale techniques. John Hopkins University Press, Baltimore.

Chinsamy, A., Chiappe, L.M., Dodson, P., 1995. Mesozoic avian bone microstructure: physiological implications. Paleobiology 21, 561–574.

Cormack, D., 1987. Ham's Histology. Lippincott, New York.

Cubo, J., Fouces, V., Gonzales-Martin, M., Pedrocchi, V., Ruiz, X., 2000. Nonheterochronic developmental changes underlie morphological heterochrony in the evolution of the Ardeidae. J. Evol. Biol. 13, 269–276.

Cubo, J., Legendre, P., de Ricqlès, A., Montes, L., de Margerie, E., Castanet, J., Desdevises, Y., 2008. Phylogenetic, functional, and structural components of variation in bone growth rate in amniotes. Evol. Dev. 10, 217–227.

Cubo, J., Ponton, F., Laurin, M., de Margerie, E., Castanet, J., 2005. Phylogenetic signal in bone microstructure of sauropsids. Syst. Biol. 54, 562–574.

Curry, K.A., 1999. Ontogenetic histology of *Apatosaurus* (Dinosauria: Sauropoda): new insights on growth rate and longevity. J. Vert. Paleont. 19, 654–665.

Enlow, D.H., 1963. Principles of bone remodeling. C. C. Thomas, Springfield, Illinois.

Erickson, G., 2005. Assessing dinosaur growth patterns: a microscopic revolution. Trends Ecol. Evol. 20, 677–684.

Evans, S.E., Barbadillo, L.J., 1998. An unusual lizard (Reptilia: Squamata) from the Early Cretaceous of Las Hoyas. Spain. Zool. J. Linn. Soc. 124, 235–265.

Francillon, H., 1979. Étude expérimentale des marques de croissance sur les humérus et les fémurs des tritons crêtés (*Triturus cristatus* Laur.) en relation avec la détermination de l'âge individuel. Acta Zool. 60, 223–232.

Fregenal-Martínez, M.A., 1998. Análisis de la cubeta sedimentaria de Las Hoyas y su entorno paleogeográfico (Cretácico Inferior, Serranía de Cuenca). Sedimentología y aspectos tafonómicos del yacimiento de Las Hoyas. Unpublished PhD thesis, Universidad Complutense de Madrid, 354 p.

Fregenal-Martinez, M.A., Buscalioni, A.D., 2009. Las Hoyas Konservat-Lagerstätte: a field trip to a Barremian subtropical continental (wetland) ecosystem. In: Alcala, L., Rayo-Torres, R. (Eds.), Mesozoic terrestrial ecosystems in eastern spain. Fundacion Conjunto Paleontologico de Teruel, Dinopolis, pp. 131–152.

Fregenal-Martínez, M.A., Meléndez, N., 2000. The lacustrine fossiliferous deposits of the Las Hoyas subbasin (Lower Cretaceous, Serranía de Cuenca, Iberian Ranges, Spain). In: Gierlowski-Kordesch, E.H., Kelts, K. (Eds.), Lake basins through space and time, AAPG Stud. Geol., 46, pp. 303–314.

Gaft, M., Shoval, S., Panczer, G., Nathan, Y., Champagon, B., Garapon, C., 1996. Luminescence of uranium and rare-earth elements in apatite of fossil fish teeth. Palaeogeogr. Palaeoclimatol. Palaeoecol. 126, 187–193.

Germain, D., Laurin, M., 2005. Microanatomy of the radius and lifestyle in amniotes (Vertebrata, Tetrapoda). Zool. Scr. 34, 335–350.

Goodwin, M.B., Grant, P.G., Bench, G., Holroyd, P.A., 2007. Elemental composition and diagenetic alteration of dinosaur bone: distinguishing micron-scale spatial and compositional heterogeneity using PIXE. Palaeogeogr. Palaeoclimatol. Palaeoecol. 253, 458–476.

Gómez Fernández, J.C., Meléndez, N., 1991. Rhythmically laminated lacustrine deposits in the Lower Cretaceous of la Serranía de Cuenca basin (Iberian Ranges, Spain). In: Anadón, P., Cabrera, Ll., Kelts, K. (Eds.), Lacustrine Facies Analysis, 13. International Association of Sedimentologists, Special Publication, pp. 247–258.

Gupta, N.S., Cambra-Moo, O., Briggs, D.E.G., Love, G.D., Fregenal-Martinez, M.A., Summons, R.E., 2008. Molecular taphonomy of macrofossils from the Cretaceous Las Hoyas Formation, Spain. Cretaceous Res. 29, 1–8.

Higham, N., 1963. A very scientific gentleman: the major achievements of Henry Clifton Sorby. Pergamon Press, Oxford.

Hinners, T.A., Hughes, R., Outridge, P.M., Davis, W.J., Simon, K., Woolard, D.R., 1998. Interlaboratory comparison of mass spectrometric methods for lead isotopes and trace elements in NIST SRM 1400. Bone Ash. J. Anal. At. Spectrom. 13, 963–970.

Horner, J.R., Ricqlès de, A.J., Padian, K., 1999. Variation in skeletochronological indicators of the hadrosaurid dinosaur *Hypacrosaurus*: implications for age assessment of dinosaurs. Paleobiology 25, 295–304.

Horner, J.R., Ricqlès de, A.J., Padian, K., 2000. The bone histology of the hadrosaurid dinosaur *Maiasaura peeblesorum*: growth dynamics and physiology based on an ontogenetic series of skeletal elements. J. Vert. Paleont. 20, 15–129.

Horner, J.R., Ricqlès de, A.J., Padian, K., 2001. Comparative osteohistology of some embryonic and neonatal archosaurs: implications for variable life histories among dinosaurs. Paleobiology 27, 39–58.

Iniesto, M., López Archilla, A.I., Fregenal-Martinez, M., Peñalver, E., Buscalioni, A.D., Guerrero, M.C., 2009. Experimental simulation of early biostratinomic stages by bacterial sealing and its implications in the genesis of Las Hoyas Konservat Lagerstatte (Lower Cretaceous, Spain). In: 10th Mesozoic Terrestrial Ecosystems and Biota. Universidad Autónoma de Madrid, pp. 273–274.

Lécuyer, C., Bogey, C., Garcia, J.P., Grandjean, P., Barrat, J.-A., Floquet, M., Bardet, N., Pereda-Superbiola, X., 2003. Stable isotope composition and rare earth element content of vertebrate remains from the Late Cretaceous of northern Spain (Lano); did the environmental record survive? Palaeogeogr. Palaeoclimatol. Palaeoecol. 193, 457–471.

LeGeros, R.Z., 1981. Apatites in Biological Systems. Prog. Cryst. Growth Charact. Mater. 4, 1–45.

LeGeros, R.Z., LeGeros, J.P., 1984. Phosphate minerals in human tissues. In: Nriagu, J.O., Moore, P.B. (Eds.), Phosphate Minerals. Springer Verlag, pp. 351–385.

Machel, H.G., Burton, E.A., 1991. Factors governing cathodoluminescence in calcite and dolomite, and their implications for studies of carbonate diagenesis. In: Barker, C.E., Kopp, O.C. (Eds.), Luminescence microscopy: quantitative and qualitative aspects, 25. SEPM short course, pp. 37–57.

Machel, H.G., Mason, R.A., Mariano, A.N., Mucci, A., 1991. Causes and emission of luminescence in calcite and dolomite. In: Barker, C.E., Kopp, O.C. (Eds.), Luminescence microscopy: quantitative and qualitative aspects. SEPM short course, pp. 9–25.

Margerie de, E., Robin, J.-P., Verrier, D., Cubo, J., Groscolas, R., Castanet, J., 2004. Assessing a relationship between bone microstructure and growth rate: a fluorescent labelling study in the king penguin chick (*Aptenodytes patagonicus*). J. Exp. Biol. 207, 869–879.

McLennan, S.M., 1989. Rare earth elements in sedimentary rocks; influence of provenance and sedimentary processes. Rev. Mineral. Geochem. 21 (1), 169–200.

Montes, L., Castanet, J., Cubo, J., 2010. Relationship between bone growth rate and bone tissue organization in amniotes: first test of Amprino's rule in a phylogenetic context. Anim. Biol. 60, 25–41.

Montes, L., Le Roy, N., Perret, M., de Buffrénil, V., Castanet, J., Cubo, J., 2007. Relationships between bone growth rate, body mass and resting metabolic rate in growing amniotes: a phylogenetic approach. Biol. J. Linn. Soc 92, 63–76.

Padian, K., Ricqlès de, A.J., Horner, J.R., 2001. Dinosaurian growth rates and bird origins. Nature 412, 405–408.

Padian, K., Horner, J.R., Ricqlès de, A.J., 2004. Growth in small dinosaurs and pterosaurs: the evolution of archosaurian growth strategies. J. Vert. Paleont. 24, 555–571.

Perez-Moreno, B., Sanz, J.L., Buscalioni, A., Moratalla, J., Ortega, F., Rasskin-Gutman, D., 1994. A unique multitoothed ornithomimosaur dinosaur from the Lower Cretaceous of Spain. Nat. 370, 363–367.

Poyato-Ariza, F.J., 2005. Palaeoecology of the fishes from the Early Cretaceous lake of Las Hoyas, Cuenca, Spain, with a hypothesis of sexual dimorphism for the chanidae Rubiesichthys. Bull. Kitakyushu Mus. Nat. Hist. Hum. Hist. Ser. A Nat. Hist. 3, 153–168.

Poyato-Ariza, F.J., Talbot, M.R., Fregenal-Martínez, M.A., Meléndez, N., Wenz, S., 1998. First isotopic and multidisciplinary evidence for nonmarine coelacanths and pycnodontiform fishes: palaeoenvironmental implications. Palaeogeogr. Palaeoclimatol. Palaeoecol. 144, 64–84.

Rafélis de, M., Renard, M., Emmanuel, L., Durlet, C., 2000. Apport de la cathodoluminescence à la connaissance de la speciation du manganèse dans les carbonates pélagiques. C. R. Acad. Sci., Ser. IIa 330 (9), 391–398.

Reynard, B., Lécuyer, C., Grandjean, P., 1999. Crystal-chemical controls on rare-earth element concentrations in fossil biogenic apatites and implications for paleoenvironmental reconstructions. Chem. Geol. 155, 233–241.

Ricqlès de, A.J., 1980. Tissue structure of dinosaur bone: functional significance and possible relation to dinosaur physiology. In: Thomas, R.D.K., Olson, E.C. (Eds.), A Cold Look at the Warm-Blooded Dinosaurs. Westview Press, Boulder, pp. 103–139.

Ricqlès de, A., Meunier, F.J., Castanet, J., Francillon-Viellot, H., 1991. Comparative microstructure of bone. In: Hall, B.K. (Ed.), Bone: bone matrix and bone-specific products, 3. CRC Press, Boca Raton, Florida, pp. 1–78.

Ricqlès de, A., Padian, K., Horner, J.R., 2001. The bone histology of basal birds in phylogenetic and ontogenetic perspectives. In: Gauthier, J., Hall, L.F. (Eds.), New perspectives on the origin and early evolution of birds: proceedings of the international symposium in honor of John H. Ostrom. Peabody Museum of Natural History, New Haven, CT, pp. 411–426.

Ricqlès de, A.J., Padian, K., Horner, J.R., Lamm, E.T., Myhrvold, N., 2003. Osteohistology of Confuciusornis sanctus (Theropoda: Aves). J. Vert. Paleont. 23, 373–386.

Ricqlès de, A.J., Padian, K., Knoll, F., Horner, J.R., 2008. On the origin of high growth rates in archosaurs and their ancient relatives: Complementary histological studies on Triassic archosauriforms and the problem of a "phylogenetic signal" in bone histology. Ann. Paleontol. 94, 57–76.

Sander, P.M., 2000. Longbone histology of the Tendaguru sauropods: implications for growth and biology. Paleobiology 26, 466–488.

Sanz, J.L., Chiappe, L.M., Buscalioni, A.D., 1995. The osteology of Concornis lacustris (Aves: Enantiornithes) from the Lower Cretaceous of Spain and a reexamination of its phylogenetic relationships. Am. Mus. Novit. 3133.

Sanz, J.L., Fregenal-Martinez, M.A., Melendez, N., Ortega, F., 2001. Las Hoyas. In: Briggs, D.E.G., Crowther, P.R. (Eds.), Palaeobiology II. Blackwell Science Publications, Osney Mead, Oxford, pp. 356–359.

Sanz, J.L., Wenz, S., Yebenes, A., Estes, R., Martinez-Delclos, X., Jimenez-Fuentes, E., Dieguez, C., Via, L., 1988. An Early Cretaceous faunal and floral continental assemblage: Las Hoyas fossil site (Cuenca, Spain). Geobios 21, 611–635.

Sanz, J.L., Perez-Moreno, B., Chiappe, L.M., Buscalioni, A.D., 2002. The birds from the Lower Cretaceous of Las Hoyas (Province of Cuenca, Spain). In: Chiappe, L.M., Witmer, L.M. (Eds.), Mesozoic Birds. Above the heads of dinosaurs. University of California Press, Ltd, Berkeley and Los Angeles, California, pp. 209–229.

Ségalen, L., Lee-Thorp, J.A., 2009. Palaeoecology of late Early Miocene fauna in the Namib based on $^{13}C/^{12}C$ and $^{18}O/^{16}O$ ratios of tooth enamel and ratite eggshell carbonate. Palaeogeogr., Palaeoclimatol., Palaeoecol. 277, 191–198.

Ségalen, L., Rafelis de, M., Lee-Thorp, J.A., Maurer, J.F., Renard, M., 2008. Cathodoluminescence tools provide clues to depositional history in Miocene and Pliocene mammalian teeth. Palaeogeogr. Palaeoclimatol. Palaeoecol. 266, 246–253.

Starck, J.M., Ricklefs, R.E., 1998. In: Starck, J.M., Ricklefs, R.E. (Eds.), Patterns of development: The Altricial-Precocial Spectrum. Oxford University Press, Oxford, pp. 3–26.

Talbot, M.R., Meléndez, N., Fregenal-Martínez, M.A., 1995. The waters of the Las Hoyas lake: sources and limnological characteristics. In: Meléndez, N. (Ed.), Las Hoyas. A Lacustrine Konservat-Lagerstätte, Cuenca, Spain. Field trip guide book of the II International Symposium on Lithographic Limestones. Ediciones Universidad Complutense de Madrid, pp. 11–16.

Tütken, T., Pfretzschner, H.-U., Vennemann, T.W., Sun, G., Wang, Y.D., 2004. Paleobiology and skeletochronology of Jurassic dinosaurs: implications from the histology and oxygen isotope compositions of bones. Palaeogeogr. Palaeoclimatol. Palaeoecol. 206, 217–238.

Williams, C.T., Henderson, P., 1997. The environment of deposition indicated by the distribution of rare earth elements in fossil bone from Olduvai Gorge, Tanzania. Appl. Geochem. 12, 537–547.