Long bone histology of basalmost and derived Sauropodomorpha: the convergence of fibrolamellar bone and the evolution of giantism and nanism

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Erklärung

Hiermit erkläre ich an eides statt, dass ich für meine Promotion keine anderen als die angegebenen Hilfsmittel benutzt habe, und dass die inhaltlich und wörtlich aus anderen Werken entnommenen Stellen und Zitate als solche gekennzeichnet sind.

Koen Stein

Bottomless wonders spring from simple rules that are repeated without end

Benoit Mandelbrot 1985

Nothing in biology makes sense except in the light of evolution

Theodosius Dobzhansky 1973

Preface

My first contact with bone histology was in 2005 during my MSc studies in Palaeobiology in Bristol. I did not have a clue how much potential this field of research really has. In fact, back then I thought it was a bit boring, mostly based on ignorance. Strangely enough, most subjects I initially find boring (my geology diploma thesis involved brachiopods and multivariate statistics), as soon as I discover its possibilities, I end up studying with much enthousiasm and fascination.

Three years (and a couple of months) is a short time to study all the bone histological sections that Martin Sander, Nicole Klein and I collected. There are a couple of projects that I would have liked to include in this dissertation, but time did not allow for. Time consuming (and too often fruitless) sampling applications, manuscript preparation, submission and peer review processes left no time to include descriptions of the bone histology of some basal sauropods. For example, the Lower Jurassic sauropods *Spinophorosaurus* and *Jobaria* from the collections of the Natural History Museum in Braunschweig were sampled and studied but not included as they did not provide revolutionary data, but do show the typical neosauropod bone microstructure. However, a manuscript with a description of the material is in preperation. Other samples of new material from the Upper Triassic of Thailand, possibly belonging to *Isanosaurus*, have not been included in the thesis either. The histology possibly points towards a complication of the taxonomy of this basal sauropod, with different taxa with aberrant histologies being present in the sample set. However, publication of these results will have to wait for further preparation and morphological description.

I would also have liked to include some more Pleistocene island dwarf bone histology, to compare with the island dwarf sauropods *Europasaurus* and *Magyarosaurus*. Samples of *Myotragus*, the dwarfed goat from Mallorca were obtained from Jelle Reumer in Utrecht, but have not been studied because of lack of time. Since islands are small laboratories with accelerated rates of evolution, understanding the ecological principles leading to small body size on islands may provide insights into what drives body size on the mainland. Hence understanding the bone histology of island dwarf mammals may help us better understand bone histologies, paleobiology and evolution of island dwarf sauropods.

The chapter on osteocyte lacuna densities (Chapter 4) only developped in the final months before submission, and I would have liked to include some data on theropods, birds, and ectotherms, to fully test the convergence hypotheses in this chapter. Unfortunately this will be another topic for further research. Nonetheless, the first steps have been made, and this is only the start of a scientific career. So much to do, so few time to do it.

A final note: Chapters 2 and 5 have been written in American English, as they were published in American journals. The other chapters have been written in British English, and have been or will be published in European journals.

Bonn, Oktober 2010,
Koen Stein

General acknowledgements

Each individual chapter sports its list of acknowledgements, but here I would like to thank some people without whom this entire study would not have been possible.

First of all, I would like to express my deepest gratitude to my supervisor Prof. Dr. Martin Sander for giving me all the opportunities to explore the path to a PhD freely, and especially for putting me back on track each time I drifted to far. Nicole Klein is greatly acknowledged for additional support, for the heated scientific discussions on what was actually visible under the microscope, and her sometimes alternative perspective on matters. I thank Ragna Redelstorff for organising the first sampling trip to Budapest, and introducing me to the coring technique.

I am very thankful for the crucial support of the technical staff at the Steinmann Institut. Olaf Dülfer, who taught me how to make thin sections, and prepared several sections himself, and Georg Olechinski who photographed selected specimens and their histology, are hereby greatly acknowledged.

I especially thank the numerous researchers and curators that permitted cutting or drilling a hole in their specimens. I would personally like to thank Lazslo Kordos for granting access to *Magyarosaurus* in the collection in Budapest; Zoltan Csiki for access to *Magyarosaurus* in the collection in Bucharest; Tim Rowe and Ernie Lundelius for access to *Alamosaurus* in Austin, Texas; Ulrich Joger and his team in Braunschweig for access to *Spinophorosaurus* and *Jobaria*; Varavudh Sutheethorn, Eric Buffetaut, Tida and Romain and the entire staff at the Sirindhorn Museum in Phu Khum Kao, Thailand for access to new early sauropod (?*Isanosaurus*) material; Jelle Römer from Utrecht University for access to *Myotragus* specimens from Mallorca; Max Langer from Sao Paolo University and Steve Perry for sending me a sample of *Saturnalia*. Without these people, there would have been no data to analyse.

Greatly thanked are my coauthors of the published chapters. Zoltan Csiki, Kristi Curry Rogers, David Weishampel, Ragna Redelstorff, and Jose Carballido are greatly thanked for their contribution to the manuscript on *Magyarosaurus* bone histology. Mike Benton and Zoltan Csiki are thanked for inviting me to contribute to the review paper on dwarfism of the Hatzeg dinosaurs. I thank Steve Brusatte and Chris Organ for motivating me to measure osteocyte sizes in Sauropodomorpha. This study got me thinking about osteocyte lacuna densities and metabolic rates. Also Matthew Brown and Sarah Werning were so kind to invite me to write a contribution explaining Martin Sander's histological coring technique for the paleontological techniques volume of *Lithodendron*.

I would also like to thank Kevin Padian for the invitation to the bone histology workshop at NESCENT, Duke University, NC. Meeting some of the principal researchers in this field was an excellent stimulation to try new things.

I would like to thank all my colleagues, friends and fellow students at the Steinmann Institut for stimulating discussions over coffee and lunch, logistic support (thanks Kay!), office pranks and jokes, and keeping a light atmosphere in a serious environment.

Last but not least, möchte ich meine liebe Freundin Hyunseng You danken für ihre Unterstützung, Geduld und Veto als ich wieder spät Nachts arbeitete. 우리 애기 감사합니다

Mijn ouders wil ik vooral bedanken voor alle kansen die ze mij gegeven hebben om mijn droom waar te maken, maar ook voor motivatie, emotionele en financiele steun in mindere tijden.

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Long bone histology of basalmost and derived Sauropodomorpha

The convergence of fibrolamellar bone and the evolution of giantism and nanism

Summary

This study reports on long bone histology and growth in sauropodomorph dinosaurs. Derived members of Sauropodomorpha, the sauropods, were the largest terrestrial animals our planet has ever seen. New methods have recently allowed a much better sampling coverage, and provided a uniform view of uninterrupted laminar fibrolamellar bone (FLB) in neosauropods, but more basal sauropodomorphs remained unclear. Results in the current work indicate that FLB was already present in the earliest sauropodomorphs. *Saturnalia*, one of the most basal sauropodomorphs, clearly shows FLB in its long bones, albeit with growth marks. These marks represent interruptions of the animal's growth, and are also present in other basal sauropodomorphs like *Thecodontosaurus* and *Plateosaurus*. This confirms earlier studies that sauropods accelerated their growth rates, most likely made possible by the preadaptive presence of high basal metabolic rates. FLB in mammals probably evolved as a convergent feature. Osteocyte lacuna densities and sizes suggest that sauropodomorphs and mammals employed different cell strategies to build bone tissue. However, a conclusion that FLB originated in a single archosaur or archosauromorph ancestor of sauropodomorphs and saurischians in general needs further testing. Long bone microstructure thus partially accounts for how these animals grew to giants, or became dwarfs in some cases. Sauropods namely secundarily evolved small sizes on islands. Here we provide strong evidence that the titanosaur *Magyarosaurus dacus* from the Cretaceous of Romania is not a juvenile of the larger '*M.' hungaricus* . *M. dacus* individuals are histologically mature and have a different ontogenetic trajectory than *'M.' hungaricus*. The histology of *M. dacus* is different from *Europasaurus,* a basal titanosauriform island dwarf from the Late Jurassic of Germany. The more intense secondary remodelling of *M. dacus* may be explaned by peramorphic heterochrony, possibly coupled with increased pneumatization in Titanosauria. The selective forces behind the progenetic dwarfing (a form of paedomorphosis) probably include the absence of large predators, and insufficient resources to provide for a healthy population of very large sauropods, and dinosaurs in general. Other dwarfed dinosaur species from the Hatzeg Basin like the ornithopods *Zalmoxes* and *Telmatosaurus* confirm this hypothesis

Chapter 1

Sauropod gigantism and bone growth

1. Introduction

Sauropods are the largest animals that ever walked on the surface of the earth. Their iconic size has inspired palaeontologists and evolutionary biologists to think about the consequences and limits of large body size. Recently, sauropod anatomy, biomechanics, growth dynamics and evolutionary ecology have been the subject of entire volumes (Curry Rogers and Wilson 2005; Tidwell and Carpenter 2005; Barrett and Batten 2007), and any dinosaur treatise will unquestionably bear a chapter on sauropods (McIntosh 1990; McIntosh et al. 1997; Upchurch et al. 2004; Fastovsky and Weishampel 2005). The DFG (German Research Foundation) Research Group 533 'Biology of the sauropod dinosaurs, evolution of gigantism' is entirely devoted to the body size evolution of sauropods (Sander and Clauss 2008; Sander et al. 2010; Sander et al. *in press*; this thesis). It would be impossible to provide an entire review of sauropod and sauropodomorph biology and evolution here, but as an introduction, the current chapter provides a short overview of sauropod biology and evolution, long bone growth and sauropodomorph bone histology. For further information the reader is referred to aformentioned publications.

2. Evolutionary and biological background of sauropod gigantism

The evolutionary development and diversification of a species or group of organisms are commonly studied with phylogenetic analyses. Phylogenetic analyses of dinosaurs are usually based on anatomical, osteological landmarks and other morphological characters of the bones of the taxa of interest. The results of such analyses, called cladograms or phylogenetic trees, provide the most parsimonious affinities and interrelationships of the taxa that were analyzed. Before analyzing the evolution of bone microstructure and gigantism in Sauropodomorpha, it is important to be aware of the phylogenetic framework and interrelationships of Sauropodomorpha, in addition to understanding the forces driving sauropod biology and gigantism.

2.1 Phylogenetic framework: small to large and back again

Sauropodomorph phylogeny has been debated in the last decade (cf. Wilson 2002; Upchurch et al. 2007; Yates 2007a,b; Sereno 2007; Allain and Aquesbi 2008; Remes et al. 2009), with some parts of the tree still not being resolved (e.g. basalmost sauropodomorphs, Titanosauria). However, it is unequivocal that sauropods evolved from much smaller sauropodomorphs (for a current review, see Langer et al. 2010) (Fig. 1). Body weights of basalmost sauropodomorphs like *Saturnalia*, *Panphagia* and *Pantydraco* are estimated around 10 to 20 kg (Langer et al. 1999; Martinez and Alcober 2009; Galton et al. 2007). They were slender built, and facultative bipedal (Remes 2006; Remes 2008; Fechner 2009). Within basal sauropodomorphs, a size increase is noted, but uncertainties in the phylogeny is this part of the tree make the interpretation of body size evolution difficult. Some core-prosauropods, like the Late Triassic *Plateosaurus* from Central Europe, and *Riojasaurus* from Argentina reached masses well over 2 tons (Sander 1992; Peczkis 1994). *Anchisaurus*, a prosauropod with an estimated body mass below 50 kg is more closely related to sauropods than several taxa exceeding 1 ton (Yates 2004; 2007). This suggests that size increase was not linear in prosauropods (Sander et al. 2010; Fig. 2).

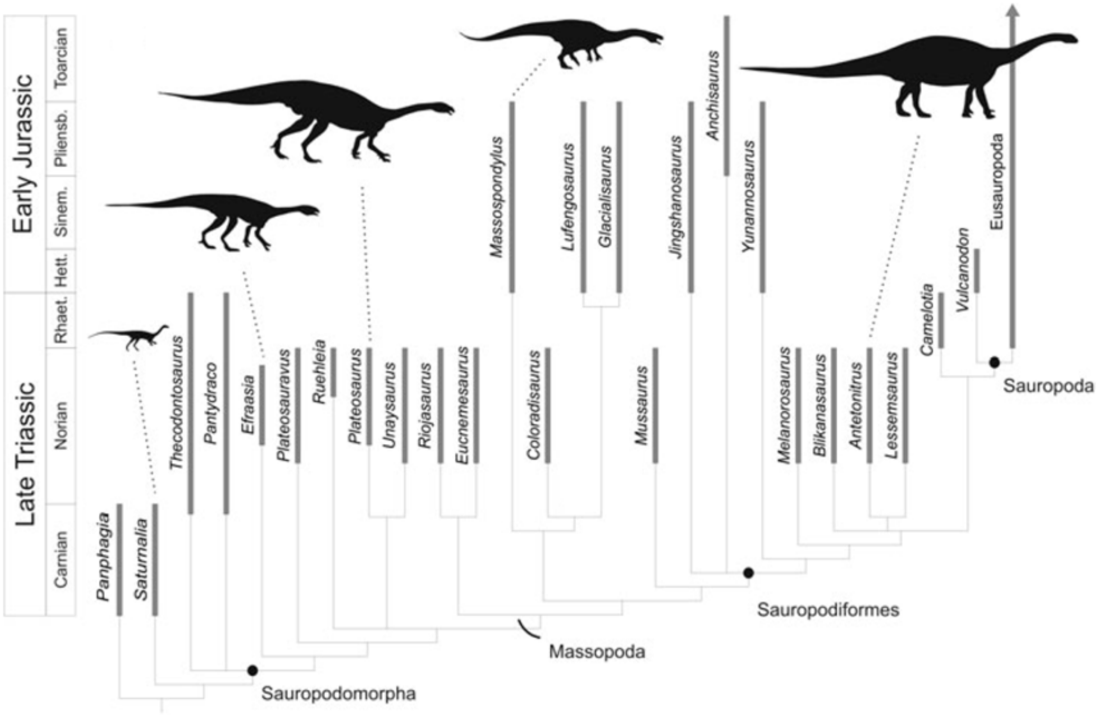

Fig. 1. Phylogenetic relationships of basal members of the sauropodomorph lineage. This time-calibrated phylogeny depicts ''core prosauropods'' as a paraphyletic group. Cladogram based on analyses by Yates (2007a, b), Smith & Pol (2007), and Martinez & Alcober (2009). From Langer et al. (2010).

The first sauropods already show very large size. *Isanosaurus* from the Upper Triassic Nam Pong Formation of Thailand documents this very rapid body size increase compared to more basal sauropodomorphs (Buffetaut et al. 2000; Buffetaut et al. 2002). Lower Jurassic basal sauropods from India (*Kotasaurus* and *Barapasaurus*), and Middle and Late Jurassic forms from China (*Shunosaurus*, *Mamenchisaurus*) all show large body sizes. Theropods may also have rapidly evolved large body size, as suggested by *Allosaur*-sized footprints from the Late Triassic (Thulborn 2003; Lucas et al. 2006) and remains of an *Allosaurus*-sized coelophysoid from the Late Triassic of Germany (Rauhut in Sander et al. 2010). However, all other dinosaur lineages show a gradual body size increase over tens of millions of years (Sander et al. 2004; Hone et al. 2005; Carrano 2006).

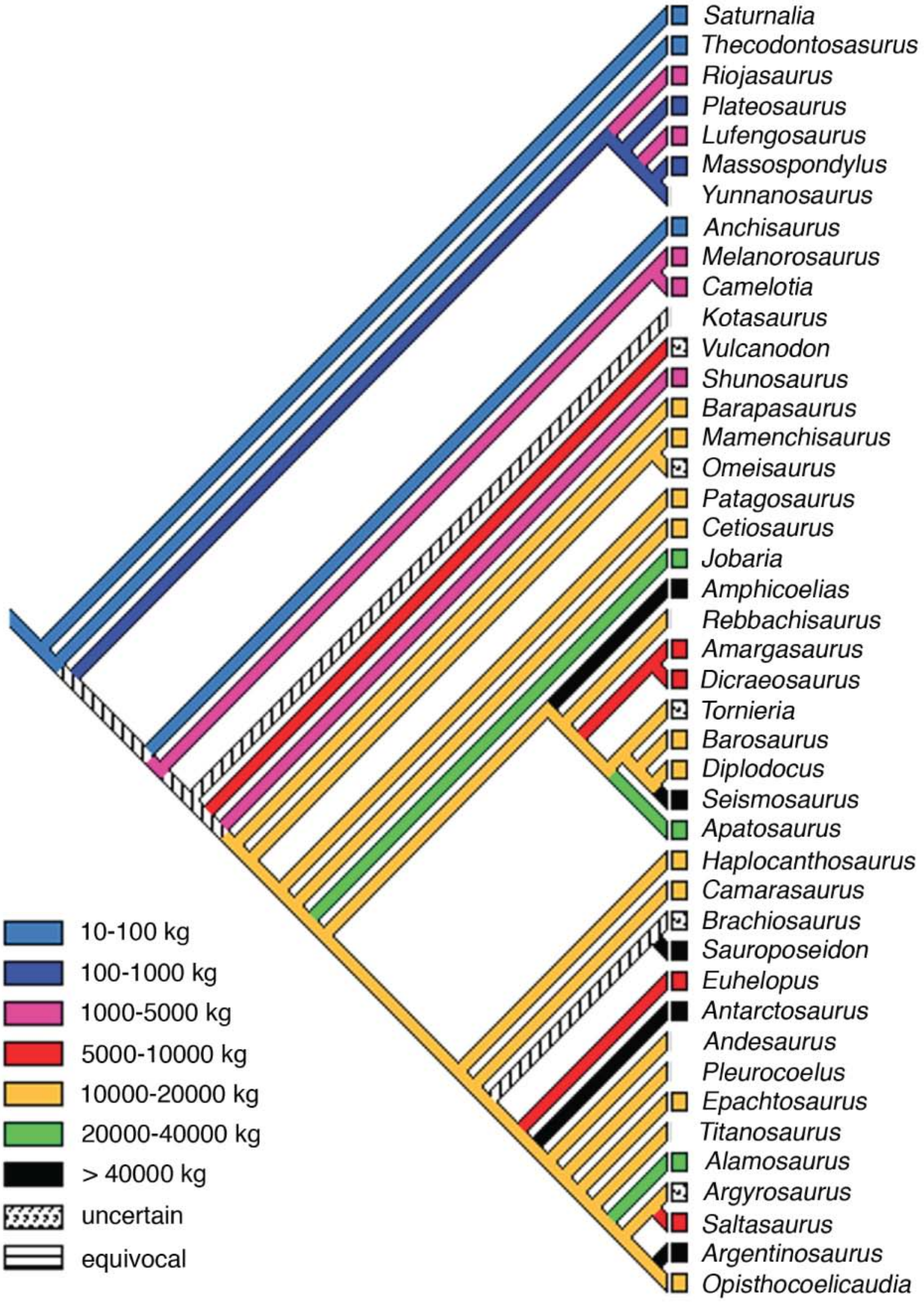

Fig. 2. Optimization of body size on a sauropodomorph phylogeny (phylogeny based on Lloyd et al. 2008). Evolution of gigantic body size (>40 t body mass) occurs convergently in several lineages of Sauropoda. Other large species like *Turiasaurus*, *Paralititan*, *Puertasaurus*, *Futalognkosaurus*, and *Huanghetitan* are not listed because they were not covered by this phylogeny. Lack of a colored box in front of the genus name indicates a lack of mass data. From Sander et al. 2010.

Neosauropoda are characterized by large to giant body size, and may have independently evolved super-gigantism in several lineages (Sander et al. 2010, Fig. 2). Some of the largest sauropods evolved among the Titanosauria (Bonaparte & Coria 1993; Novas et al. 2005; Calvo et al. 2007). Within diplodocoids, the dicraeosaurids are characterized by relatively small body sizes (Rauhut et al. 2005). Exceptionally small are the island dwarf *Europasaurus* from the Upper Jurassic of Central Germany (Sander et al. 2006) and putative dwarf *Magyarosaurus* from the Upper Cretaceous of Romania (Nopcsa 1914; Jianu and Weishampel 1999, Leloeuff 2005). Island ecosystems provide the basis for exceptional evolutionary experiments, and also cause dwarfing in other tetrapod groups, the classic example being the Pleistocene dwarfed elephants of the Mediterranean. *Magyarosaurus* lived in an area that was part of an island arc during the Cretaceous (Dercourt et al. 2000). Other dinosaurs from the area also have a generally small body size, suggesting they may be island dwarfs as well. However, recent controversy around this putative island dwarf sauropod, led to the suggestion that it is of juvenile status (Leloeuff 2005).

Some taxa, mostly belonging to Titanosauria, also show an evolutionary body size reduction with no apparent island effects (Hone et al. 2005; Carrano 2006). Carrano (2006) did not explain these more numerous relatively small forms. Sander et al (2010) hypothesized that palaeogeographic change from the Middle Jurassic to the end of the Cretaceous combined with the area-body size relationship established by Burness et al. (2001) caused the body size spectrum of sauropods to broaden. They observed that both the breakup of Pangea and the sea level rise since the Triassic resulted in a fragmentation of land masses and an increased number of islands. With sauropod body size, as the largest inhabitants of the land masses, being closely tied to land mass size (Burness et al. 2001), the evolution of smaller forms was the result. However, no truly small sauropods are known.

Even sauropods like *Europasaurus* were animals with an adult body mass well in excess of 500 kg (Peczkis 1994; Sander et al. 2006; Stein et al. 2010), a size which is reached by less than 10% and probably more like 1% of modern mammal species (Hotton 1980).

2.2 Sauropod biology

The typical sauropod bauplan consists of a long neck with a small head, a bulky body with four pillar-like legs, and a long tail. Sauropods were obligate quadrupeds, mostly imposed by their own size (Remes 2008; Fechner 2009). Some sauropods like *Argentinosaurus* and *Puertasaurus* (with body mass estimates up to 100 tons) may have reached the upper limit of terrestrial body size, which essentially is determined by the physical constraints of the bauplan of the organism, and by ecological constraints of the environment (Clutton-Brock & Harvey 1983; Peters 1983; Schmidt-Nielsen 1984; Alexander 1998; Hunt & Roy 2005; Makarieva et al. 2005; Bonner 2006). Burness et al. (2001) showed that maximal terrestrial body size correlates well with resource availability, expressed as land surface area. The supercontinent Pangea remained intact during the first half of the Mesozoic, with breakup starting in the Middle Jurassic, thus already more resources were available. However, sauropods were able to exploit the resources in their environment more efficiently than any other terrestrial vertebrate lineage because they possessed a combination of plesiomorphic characters (i.e. derived from their ancestors) and evolutionary innovations, called apomorphies (Sander and Clauss 2008; Sander et al. 2010). Plesiomorphic characters were the lack of mastication, the lack of a gastric mill and having many offspring. Innovations were a complex avian-style respiration system with pneumatization of the skeleton (Wedel 2003; Perry et al. 2009) and a high basal metabolic rate (Sander and Clauss

2008). Sander et al. (2010) suggested that the long neck is the foundation to the energy-efficient food uptake, because it permitted grazing over a large volume with a stationary body. A small head and extensive pneumatization of the axial skeleton lightened the neck. The lack of mastication allowed a small head (and thus long neck) and together with the lack of a gastric mill, it increased food uptake rate. Many small offspring and a high growth rate, fueled by a high basal metabolic rate reduced the extinction risk (by predation or environmental factors) of populations. Current evidence places the origin of sauropods in the Late Triassic (Buffetaut et al. 2004), and during the Late Triassic and Early Jurassic, the combination of aforementioned biological features, pressured by predation from theropod dinosaurs, drove an evolutionary cascade towards ever larger body size in the sauropodomorph dinosaur lineage (Sander et al. 2010; Fig. 3).

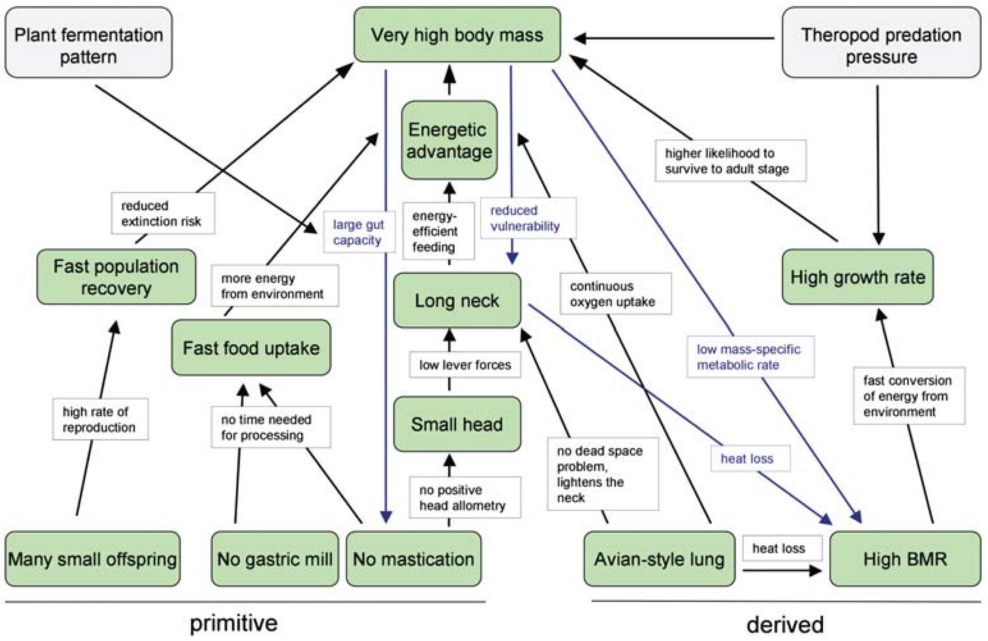

Fig. 3. Sander et al.'s (2010) flow chart of the evolutionary cascade leading to sauropod gigantism. Green boxes contain the biological properties of sauropods, and the black arrows indicate primary evolutionary causation. Theropod predation pressure is depicted as a representative selection factor for body size increase. In addition to primary evolutionary causation, sauropod gigantism was also driven by evolutionary feedback loops (blue arrows). The blue boxes indicate the selective advantage in the feedback loop. The boxes on the black arrows show the selective advantages conferred on sauropods by the biological properties. BMR, basal metabolic rate.

3. Bone growth

For the purpose of this dissertation, to gain better understanding of the evolution of sauropod fibrolamellar bone, growth rates and gigantism, long bones were the preferred focus of study. Mainly because long bones are the fastest growing elements in the tetrapod skeleton, but also because they are most abundant in the fossil record, easy to sample, and because curators are less reluctant to allow destructive sampling (for more on sampling, see Chapter 2; Stein and Sander 2009). Therefore, this short introduction will be limited to general long bone growth in tetrapods. For more extensive treatise on bone growth in the tetrapod skeleton, please refer to Ricqles (1973), Francillion-Viellot et al. (1990), Currey (2002) or Hall (2005).

Bone can be studied at different levels of organization (Francillon-Viellot et al. 1990). Classically, four hierarchies are recognized: molecules, cells, tissue and morphology. For the purpose of brevity, some of these levels will be discussed in combination.

3.1 Bone matrix: molecules crystallites and fibres

Bone is a living composite tissue. This means, it is maintained throughout an animals' life, and can be modified according to changes in load, strain and it can even repair breakage. It is composed of a mineral phase, hydroxyapatite, and an organic phase, collagen. The orientation of collagen fibres within the matrix is determined by osteocytes. Fibres that are slowly deposited in thin parallel lamellae form lamellar bone tissue. When fibres are rapidly deposited, they have a random orientation, and form unorganized anisotropic woven bone. Parallel fibred bone is intermediate between lamellar and woven bone. The hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$ or $[Ca_3(PO_4)_2]_3.Ca(OH)_2$) crystallites are interwoven with collagen fibres. In fossil bone, collagen fibres decompose and are replaced by phosphate mineral, i.e., apatite.

3.2 Bone cells and histology

3.2.1 Epithelial growth

The biomineralising cells in epithelial membranes that form bone matrix are called osteoblasts. Once these cells become encased in the bone matrix, they are referred to as osteocytes, which are non secretive, but remain alive and interconnected with canaliculi. This network of interconnected osteocytes allows cells to communicate, for example signalling defects or cell death in a process called mechanosensing. A third type of cell has the capability to dissolve bone. They are called osteoclasts, and play an important role in bone remodelling. Typical periosteal appositional (vascular) bone growth is schematically explained in Fig. 4a.

Strong endogenous biorhythms may cause disruptions in bone formation, and can form a line of arrested growth (LAG). LAGs represent periods of temporary complete or near-complete cessation of appositional growth and represent the external surface of the bone at the time the LAG is formed. The length of time represented by a LAG may vary from a few days up to six months (Castanet and Smirina 1990). LAGs occur in poikilotherms, but also in many dinosaurs (including birds) and mammals, which suggests they are not an exclusively bradymetabolic feature (Chinsamy 1990; Sander and Andrassy 2006).

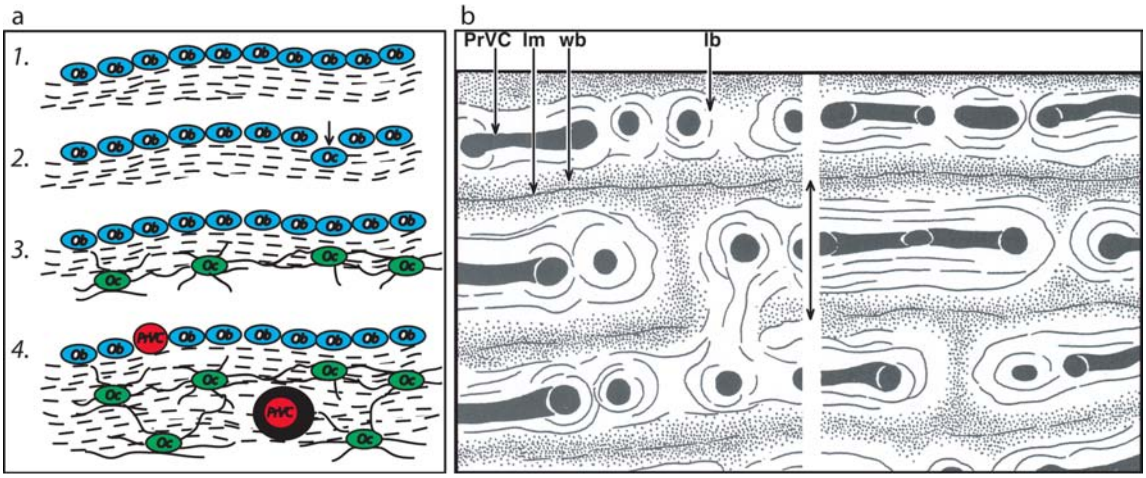

Fig. 4. Bone apposition and primary osteons. a Schematic diagram of epithelial bone apposition: *1.* epithelium (with osteoblasts) secretes matrix. *2.* The epithelium continues to secrete, but some cells seize secreting and become incorporated in matrix as osteocytes. *3.* More cells become incorporated in the bone, turning into osteocytes, with the bone matrix they have secreted in between them. *4.* The epithelium is also provided with bloodvessels, which become incorporated into the matrix. Blood vessels remain functional within the bone. In fossil bone, such spaces are referred to as vascular space or vascular canal. These spaces may have contained nerves and lymph vessels as well. b. primary osteons in fibrolamellar bone. Symbols: Ob, osteoblast; Oc, osteocyte; PrVC, primary vascular canal; wb, woven bone matrix; lb, lamellar bone matrix; lm, lamina mark, a hypermineralized region of fibrous bone and early site of deposition of a new lamina. (b. modified from Francillon-Viellot et al. 1990; c. modified from Curry Rogers and Erickson 2005)

3.2.2 Fibrolamellar bone, vascular canals and osteons

During periosteal deposition, vascular canals from the periost become integrated into the bone matrix in vascular spaces. As these spaces in a woven bone matrix mature, they become filled up with slower deposited lamellar bone, and are then referred to as primary osteons. The lamellar bone matrix is centripetally deposited in the primary vascular spaces (Fig. 4b). Primary osteons can be oriented longitudinally, circularly, radially or oblique to the long axis of the long bone. However, vascular canals in bone tissues usually run in multiple directions, commonly in one of three major patterns (Fig. 5). Reticular vascularisation is an oblique and irregular arrangement of vascular canals. Plexiform vascularisation is characterised by interweaving longitudinal, circular and radial vascular canals. Laminar vascularisation has mainly circumferential and longitudinal vascular canals, superimposed in laminae. This is the most common bone tissue in subadult to adult sauropods.

The duality of a woven bone framework, compacted with slow growing lamellar bone is called the fibrolamellar complex, or fibrolamellar bone (FLB).

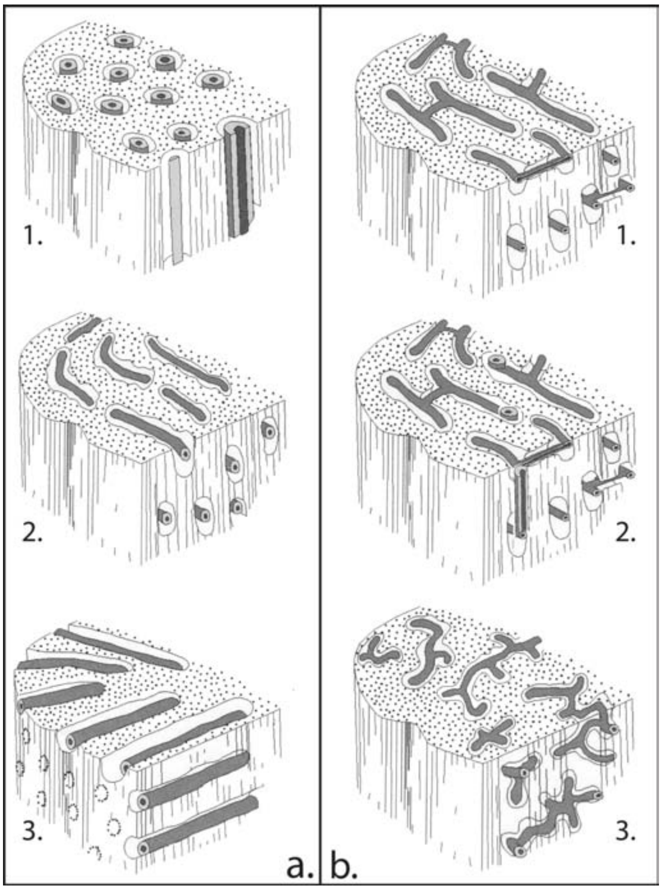

Fig. 5. Major organisation patterns of vascular canals in compact bone. a. Canal orientation: 1. longitudinal, 2. circumferential or circular, 3. radial. b. Major vascularisation patterns: 1. laminar 2. plexiform 3. reticular. From Curry Rogers and Erickson (2005).

3.2.3 Remodelling

Bone can be eroded by osteoclasts (modified mononuclear phagocytes, i.e. different origin than osteoblasts) brought in through blood vessels. Osteoclasts eat away the bone matrix by secreting acids, removing old or dead bone, leaving erosion cavities. Subsequently,

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new osteoblasts come in through the blood vessels, forming new bone matrix in the blood vessel, called secondary bone. Blood vessels filled up with secondary bone are called secondary osteons or haversian systems. The original bone matrix is called primary bone, recording the original growth record. Secondary osteons can be discerned from primary ones by the presence of a cementing line (also called resting line or erosion line), and are usually oriented longitudinally. The whole process of erosion and deposition of secondary lamellar bone is called secondary remodelling (Fig. 6), and is believed to be triggered by disruptions in the mechanosensing network. However, recent studies suggest that healthy osteocytes can also contribute to local remodelling (reviewed in Qing and Bonewald 2009). When primary bone has been completely replaced by secondary osteons (usually visible by crosscutting relations forming interstitial laminae), the bone is called haversian bone (see also Chapter V).

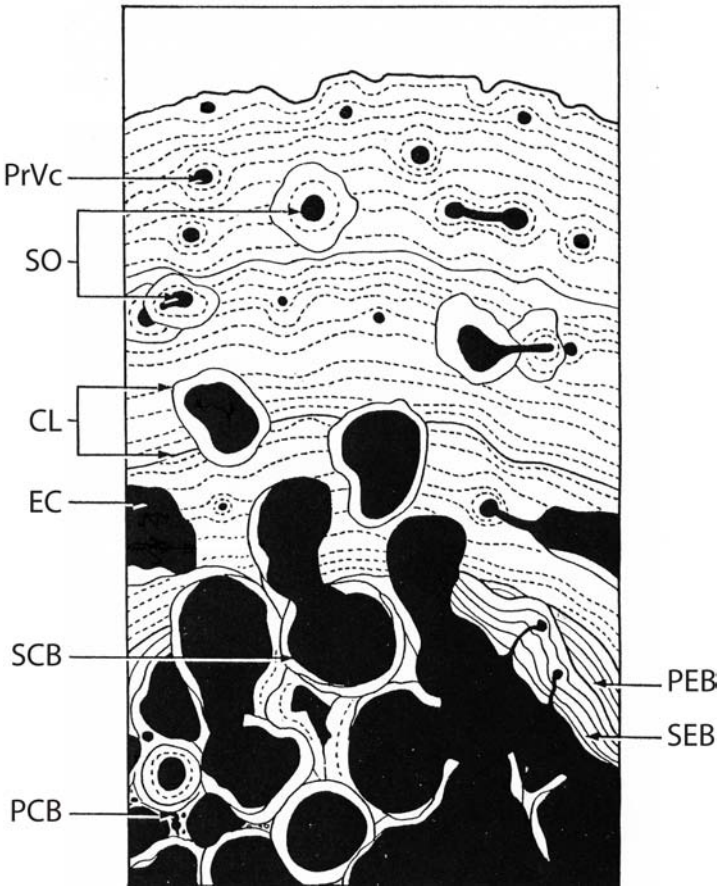

Fig. 6. Secondary bone formation. Erosion cavities are formed by osteoblasts brought in through blood vessles. Secondary bone deposition by osteoblasts secreting matrix in the newly formed erosion canals. Symbols: PrVC, primary vascular canal in a primary osteon; SO, secondary osteon; CL, cementing line/erosion line; EC, erosion cavity; SCB, secondary cancellous bone; PCB, primary cancellous bone; PEB, primary endosteal (i.e. deposited by the endosteal epithelium) bone; SEB, secondary endosteal bone. Modified from Francillon-Viellot et al. (1990).

3.3 From microstructure to morphology: macroscopic bone growth

In general, tetrapod long bone growth (Fig. 7) starts with a cartilage precursor of the bone. The cartilage core then starts to ossify by periosteal deposition of bone by osteoblasts brought in by a major blood vessel. This periosteal bone forms the diaphysis. Longitudinal growth occurs by cartilage forming chondrocytes. The cartilage core of the bone disappears, forming the initial medulary cavity, while at the ends new chondrocytes keep producing cartilage at the epiphyses. As the bone grows longitudinally and periosteally, cartilage in the metaphysis is replaced by endochondral bone (also replacement bone). Cartilage being replaced by bone along the vascular canals in the epiphyses, is referred to as cancellous bone. Compact bone is only produced by periosteal growth. Erosion processes will expand the medullary cavity increasingly, destroying some of the primary bone in the process.

To summarize, a long bone consists essentially of two cones of endochondral bone with cartilage (the epiphyses) and a compact bone shaft, the diaphysis. In sauropods, bone growth slows down increasingly after the animal reaches sexual maturity, but it does not stop completely. This is a typical reptilian growth pattern. Mammals are different in that they have 3 centres of ossification, including an ossified epiphysis cap, forming the joint. The mammal is fully grown when the epiphysis is completely ossified/fused with the bone.

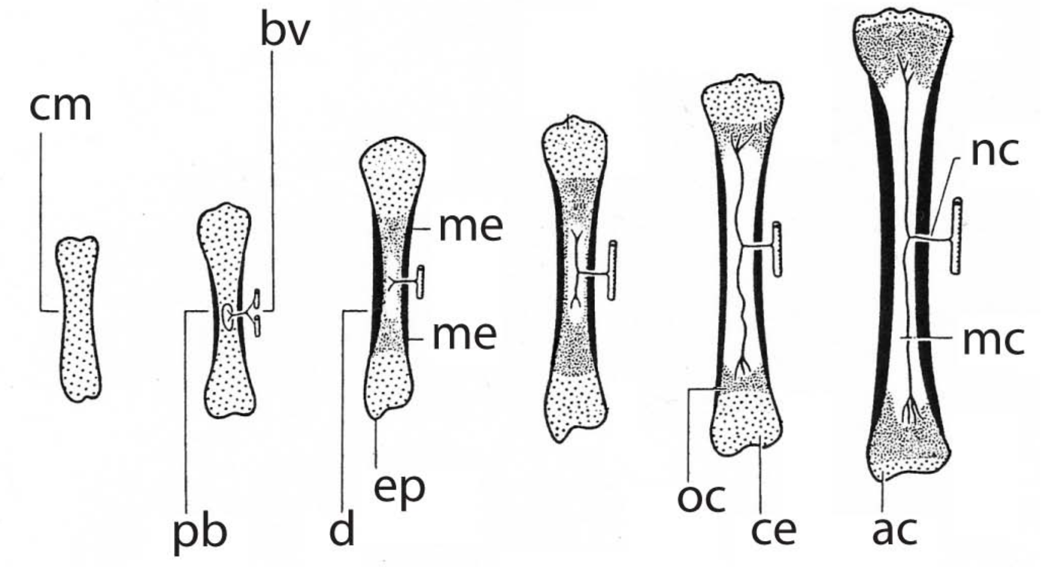

Fig. 7. Macroscopic bone growth. CM: cartilage precursor model of the bone. Ossification starts under influence of external forces (cf. Wolff's law), and periosteal bone (pb) is deposited by osteoblasts brought in through blood vessels (bv) entering the bone through a nutrient canal (nc). Chondrocytes in the epiphysis (ep) produce cartilage, and cause the bone to grow longitudinally. Periosteal growth by osteoblasts along the diaphysis (d) increase bone thickness. The cartilage precursor of the bone is resorbed by osteoclasts, the created space forms the medullary cavity (mc). As the bone grows longitudinally and periosteally, cartilage in the metaphysis (me) is replaced by endochondral bone (oc) (also replacement bone). Cartilage being replaced by bone along the vascular canals in the epiphyses (ce), is referred to as cancellous bone. Compact bone is only produced by periosteal growth. The bone now essentially consists of two cones of endochondral bone with articular cartilage (ac) and a compact bone shaft, the diaphysis. In sauropods, bone growth slows down increasingly after the animal reaches sexual maturity, but it does not stop completely. Modified from Francillon-Viellot et al. (1990).

4. The evolution of sauropod gigantism and long bone histology

Evolution is documented by osteological studies, which allow us to reconstruct the rise and demise of major lineages. Any bone morphological changes that occur within a lineage thus allow us to model changes in body shape, muscle function, and posture. The internal architecture of fossil bones, however, records patterns of bone deposition and remodelling, and allows us to observe changes in bone growth through ontogeny, and quantify and compare basal sauropodomorph and sauropod growth rates and life histories.

4.1 Sauropod long bone histology

FLB is the dominant tissue in sauropods (Ricqles 1968a; Ricqles 1968b; Rimblot-Baly et al. 1995; Curry 1999; Sander 1999; Sander 2000; Sander and Tückmantel 2003; Sander et al. 2004; Curry Rogers and Erickson 2005; Klein and Sander 2008; Lehman and Woodward 2008; Klein et al. 2009; Woodward and Lehman 2009). Throughout ontogeny, FLB is highly vascular, with canals organized in laminar, reticular, and or plexiform patterns (cf. Klein and Sander 2008). Late in ontogeny, cortical bone becomes avascular, and comprised of parallel fibred or lamellar bone, and LAGs may occur. This avascular bone with LAGs is called an external fundamental system (EFS), and indicates that growth slowed down significantly at this point. Untill late in ontogeny, LAGs are rare in sauropod long bones. Moreover, the fibrillar and vascular organization of sauropod bone is comparable to extant rapidly growing mammals and birds, and inconsistent with a reptilian growth model. Sander et al. (2004) reported uninterrupted FLB in *Isanosaurus*, a basal sauropod from Thailand. They suggested that the loss of growth marks in Sauropoda marks a growth rate acceleration compared to prosauropods, and the loss of developmental plasticity (cf. Sander and Klein 2005).

4.2 Basal sauropodomorph long bone histology

Basal sauropodomorph bone histology is known from limited taxa. Chinsamy (1993) studied *Massospondylus*, a prosauropod from the Lower Jurassic of South Africa. Sander and Klein (2005) and Klein and Sander (2007) provide a detailed account of the bone histology of *Plateosaurus*, a prosauropod from the Upper Triassic of Germany and Switzerland. Chinsamy (1993) and Sander and Klein (2005) found similar histological patterns in these prosauropods, consisting of zones of fibrolamellar bone, alternating with annuli of lamellar bone and/or lines of arrested growth. The presence of fibrolamellar bone suggests periods of rapid growth, and high basal metabolic rates in these animals. However, the strong variation in adult body size in *Plateosaurus* compared to *Massospondylus* suggests a strong developmental plasticity in the former (Sander and Klein 2005). *Massospondylus* shows a better correlation of body size and age, but lacks the EFS that is seen in many *Plateosaurus* individuals. Chinsamy (1993) interpreted the lack of an EFS as a feature of indeterminate growth in *Massospondylus*, but later suggested that adult *Massospondylus* individuals that had stopped growth may not have been sampled (Chinsamy-Turan 2005). Nonetheless, current data suggest that *Plateosaurus* and *Massospondylus* had different growth strategies (Klein and Sander 2007).

The presence of a lamellar zonal bone pattern in at least one basal dinosaur taxon, the basal thyreophoran *Scutellosaurus*, renders the precise origin of FLB in Sauropodomorpha unclear (Padian et al. 2004). However, it is clear that most ornithodirans (pterosaurs+dinosaurs) had lost developmental plasticity, that FLB had evolved in some basal dinosaurs and that the ancestral condition most likely was a poikilothermic vertebrate with a lamellar-zonal bone pattern (Ricqles et al. 2000; Padian et al. 2001; Starck and

Chinsamy 2002; Padian et al. 2004; Ricqles et al. 2003; 2008). FLB also evolved in the therapsid-mammal lineage, but from parsimony arguments probably did so independently from dinosaurs because the indedependant evolution of FLB in two lineages involves much fewer evolutionary step than the single evolution of FLB in the earliest amniotes and its multiple loss or even re-evolution afterwards (Ricqles et al. 2003; 2008; Padian et al. 2004; Ray et al. 2004; Ray et al. 2009; Clarke and Pörtner 2010).

5. Goals of the study

This thesis is the result of project E1 from the second three-year funding period of research group FOR533, and focusses on the evolution of sauropod growth rates and body size from the perspective of bone microstructure. New techniques (Chapter 2) have made it possible to compare large numbers of bone histological samples from a wide phylogenetic range, but also to compare different ontogenetic stages within one taxon.

Sauropodomorphs cover an extreme range of body sizes, starting from dog sized or even smaller animals, up to the largest terrestrial animals ever known, and back to cow or horse sized forms. The evolutionary cascade towards giant body size in sauropods really started once the general saurpod body plan was more or less established. This sudden acceleration of growth in Sauropoda was most likely made possible by the preadaptive presence of FLB and high metabolic rates in a sauropod ancestor. *Where does FLB in sauropods come from?* To investigate the early origins of FLB in Sauropodomorpha, basal sauropodomorph long bone histology needs to be sampled. *Is FLB in Sauropodomorpha really convergent with that in mammals?* We also hypothesise that the convergent nature of FLB in Sauropodomorpha and mammals can be observed on a smaller hierarchical level.

Osteocyte lacunae generally preserve relatively well in fossilised bone and bone cells should provide deeper insight in the nature of the bone tissues in both mammal and sauropodomorph clades. Therefore, cell-level histological analyses will be performed to test this.

Was Magyarosaurus a dwarf? The hypothesis that *Magyarosaurus* was a dwarf form can also be tested with long bone histology. *What causes dwarfing of large animals on islands?* If *Magyarosaurus* is indeed a dwarf form, understanding the mechanisms of dwarfing, growth rate decrease or shortening of growth will also provide feedback of information on the evolution of gigantic forms.

The main objective is thus to understand of the evolution of FLB in Sauropodomorpha and the origins of their high growth rates, basal metabolic rates and tachymetabolic endothermy. It requires further sampling of more basal sauropodomorphs, but also study of already published materials, and a comparison with other tachymetabolic groups, like mammals. This objective is taken on in Chapter 3 and 4.

Chapter 5 and 6 deal with island dwarfism in sauropods and other dinosaurs, with the purpose of better understanding the mechanisms that drive or limit terrestrial body size. Finally, Chapter 7 aims to provide a synthesis on sauropodomorph bone microstructure and body size evolution and provides prospects for further research.

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Chapter 2

Histological core drilling: a less destructive method for studying bone histology

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Abstract

This paper describes a new method for obtaining histological core samples. The Histological Coring Method (HCM) involves drilling a small core at a standardized location on the bone chosen for study. The core is collected using diamond studded drill bits mounted on a standard household power drill. The drill is stabilized in a drill press to control the vertical drilling speed. Long bones are preferably sampled because of their abundance and relatively simple growth pattern and morphology, but any vertebrate hard tissue can be sampled. Using an appropriately sized drill bit means any specimen ranging in size from small to extremely large can be sampled. After a core is obtained, it is processed into histological thin sections, and or polished sections with standard histological thin sectioning methods.

Compared to classical histological techniques, the HCM is a much less destructive method for sampling vertebrate hard tissues. This new method will therefore allow continued conservation of rare and valuable specimens while simultaneously permitting access to unique biological information.

1. Introduction

Paleohistology is the science that studies the internal microscopic structure of fossilized vertebrate tissues like bone, ossified tendon, eggshell, teeth and dermal scutes. Bone paleohistological studies have recently produced an array of information on extinct animals, more particularly on growth strategies (Horner *et al.*, 2000; Sander, 2000; Erickson *et al.* 2001; McFadden, 2004; Sander and Klein 2005; Erickson, 2005; Klein and Sander 2008; Lehman and Woodward, 2008), reproduction (Schweitzer *et al.* 2005; Erickson *et al.* 2007; Lee and Werning, 2008), and evolution (Chinsamy and Elzanowski, 2001; Sander *et al.* 2004, 2006; Ricqlès *et al.* 2008).

Unfortunately, the usual practice of bone histological studies involves the destructive sectioning of complete bones, teeth, scutes or other preserved hard tissues (Wells 1989, Chinsamy and Raath, 1992, Wilson, 1994). Understandably, museum and collection curators are reluctant to give up rare or type specimens to these destructive methods. This has limited paleohistological studies in the last century mainly to isolated and or fragmented specimens and, with a few exceptions (e.g. Enlow and Brown 1956-58; Riqclès 1968, 1969, 1974, 1976, 1977, 1978, 1980, 1981), prevented substantial comparative analyses.

In repsonse, a less destructive method was developed by one of the authors (MS) to study long bones of sauropod dinosaurs (Sander, 1999, 2000). Instead of sectioning entire sauropod long bones, a small core sample was taken at a specified location on the long bone shaft, akin to a medical biopsy. This allowed a large number of bones to be sampled, expanding the study into a more quantitative analysis instead of a mere qualitative description, providing many insights into sauropod biology, and even allowing taxon discrimination based on their histology. Sander (2000) only gave a brief description of the method, which has since been developed further and will be explained in detail here.

Histological sampling is commonplace in archaeology and archaeozoology as well (e.g. Chan *et al.* 2007, Zedda *et al.* 2008) but this subject is beyond the scope of this paper.

2. Bone choice and sample location

Choosing the right bone for osteohistological studies requires knowledge about bone growth. Bones do not have mere appositional growth like the trunk of a tree, but they are formed in a process of constant primary bone deposition, resorption, and remodeling (Currey, 2002; Hall, 2005). This causes the bone to keep its original shape as it grows, but it also means that the earlier growth record is progressively being destroyed. When studying the primary growth record, it is therefore important to choose a bone with as little resorption and remodeling as possible.

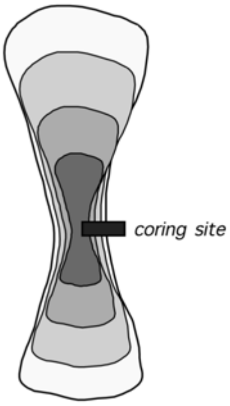

Fig. 8. Idealized growth scheme of a long bone. Two epiphysial cones of endochondral bone are surrounded by a mantle of periosteal bone that is thickest at or near the middle of the diaphysis (shaft). The optimal sampling location is the narrowest part of the diaphysis, which is usually the middle of the bone shaft or slightly distal to it, as a core taken here will encounter the thickest as well as oldest periosteal bone and thus the most complete growth record.

Sander's initial study (Sander, 1999, 2000) and subsequent sauropod and prosauropod studies (e.g. Sander and Tückmantel 2003; Sander *et al.* 2004; Klein and Sander 2005; Sander *et al.* 2006) focused on long bones because of their simple morphology and relative abundance. Sauropod long bones do not have distinctive bends, crests, or trochanters that would require significant remodeling during growth to maintain the shape of the bone. This means that they approximate the general growth scheme of two epiphysial cones of endochondral bone surrounded by a mantle of periosteal bone that is thickest at or near the middle of the diaphysis (shaft) (Sander, 1999, 2000; Sander *et al*. 2004), which makes them very suitable for histological studies (Fig 1).

On theoretical grounds, the optimal sample location is thus the narrowest part of the diaphysis, which is usually the middle of the bone shaft or slightly distal to it. A core taken here will encounter the thickest as well as oldest periosteal bone. To further minimise any effect of shape change of the long bones during growth, they are best drilled on the convex side. In anterior limb bones, this would be the posterior side, and in posterior limb bones the anterior side. However, when the surface of the bone is too damaged or unstable at these sites, it is best to choose an alternative drill location. Furthermore, Werning *et al*. (2008) describe a statistical method for determining which transects or coring locations best represent mean radial growth across the entire cross section. The specific histological sample location on long bones stresses the need for complete to near complete specimens. Isolated epiphyses or diaphyses provide little to no control on sample location, and are thus unsuited for comparative studies.

Apart from these theoretical considerations and general experience, the optimal location for drilling is best determined in a cross section of a medium-sized specimen of the taxon to be sampled. The cross section will reveal the location of the best growth record which is influenced by both the local apposition rate of primary bone and the patterns of remodeling and medullary cavity expansion.

3. Description of the coring device

The coring method involves a portable histological coring device. Setting up the coring device is straightforward. The three most crucial tools in the drill setup (Fig. 2) will be discussed in more detail. Most important are the drill bits (Fig. 2A), but also needed are a portable drill press (Fig. 2B) and a normal household electric drill. All the equipment required for the coring method is listed in Table 1.

Fig. 9. Histological coring set-up. A: Drill bits. From left to right, 6 mm drill bit, 5/8 inch (15.9 mm) diameter bit, custom-made extension piece for 5/8 inch (15.9 mm) drill bit, and a 1/2 inch (12.7 mm) diameter bit. B: Drill press for normal sized power drill. C: Complete drill set-up. Here a large sauropod humerus is being sampled. Notice the plasticine dam and water being added to cool the drilling site. D: Close-up of the drilling process. Notice that incomplete bones also qualify for sampling, as long as the standard location can be sampled. E: Retrieving the core, after it has been broken from its base with a small screwdriver. Note the mark of the bone long axis on the core, which is important for the correct orientation of the thin section.

Table 1. List of equiment or how to put a tool kit together.

Equipment
drill bits
extension piece and allen key
electric drill
drill press
large flower pot dish or tray
water spray bottle
tape measure
empty cloth bags or equivalent
small screwdriver (to break the core from its base)
tweezers

3.1 Drill bits

The drill bits are available from different companies and in different sizes (Fig. 2A). We have mainly used 5/8 inch (15.9 mm) and ½ inch (12.7 mm) diameter bits. The bits should be covered with fine to very fine diamonds, as medium diamonds are too rough and cut away too much bone. We cannot provide exact data on diamond grit size because this information is usually not released by the manufacturers. However, diamonds the size of 80 to 100 grit sand paper are best. High-quality bits have the diamonds sintered to the crown while in cheaper ones have the diamonds galvanized to the crown. Custom ordering of drill bits from lapidary companies is also an option, as well as resurfacing worn-down drill bits. Alternatively to buying finished drill bits, many university or museum machine shops should be able to turn out bits, and the diamond cover could then be added by a lapidary company. The coring bits should have a thin wall, less than 0.3 mm in thickness. This maximizes the size of the sample compared to the size of the hole. The length of the crown should exceed its diameter by several times because otherwise the recovered core will be too short. We use mostly bits with a crown length of about 45 mm, but other sizes exist. Most mid-range household electrical drills have a chuck size of 3/8 inch (9.5 mm) in the US and up to 13 mm in Europe, so the shank of the drill bit should also fit these requirements. Miniature drills (see next section), of course, require a smaller shank size.

The 5/8 inch bits are good for bones roughly over 60 cm in length (Fig. 2C); the ½ inch ones are good for bones from 30 to 60 cm long (Fig. 2D). Smaller bones can be sampled with smaller drill bits, but then the thickness of the wall becomes critical, and the diamond cover may become too coarse and cut away too much bone during the drilling process. Therefore, it is best to make a complete cross section when dealing with very small bones. When dealing with very large bones, the length of the drill bit may not be sufficient to drill all the way through the bone wall into the medullary cavity. In this case, an extension piece can be used (Fig 2A). This extension piece allows drilling to greater depths within the same hole. The extension piece has to be custom-made by a metal workshop and should consist of a short rod of brass or aluminum with a whole drilled into the center to receive the shank of the drill bit. To fasten the shank, a small sunken bolt with an Allen wrench head is added.

3.2 Electric drill

The drill should be a common household electrical drill. It should be able to run very slowly and with little torque, with torque decreasing at lower speeds. This means the drill should be of medium quality; the high-quality drills tend to be too powerful with torque being more constant at different speeds. For bones smaller than 30 cm, a low voltage miniature drill such as those made by Dremel® or Proxxon® may be used. These run with less power and fewer vibrations.

3.3 Drill press

A small and portable drill press is optimal (Fig. 2B). Manufacturers of the miniature drills discussed above also provide smaller presses for their tools. When visiting collections, the coring device can be set up on a table in a preparation lab or collections room. The press is then set in a bed of sand, or stabilized with bags of sand in a photo tray, or large flowerpot dish. Sand is available in most preparation laboratories, but on occasion we have also used rice to stabilise the drill press and the specimens. In a domestic laboratory, the drill press can be mounted on a table top for added stability. When dealing with extremely large and heavy specimens, like sauropod long bones, the specimen will extend over the edges of the tray, but the large weight of the specimen will add stability to the entire setup. If the diameter of the specimen is too large, a vertical extension of the drill press may be needed. In this case, the setup can be placed on a table top, with the drill on the opposite side of the drill press platform, hanging over the edge of the table top. The specimen can then be placed under the drill, on a lower table top.

It is important when attaching the drill to the press to make sure that the axis of rotation of the drill bit is exactly parallel to the direction of the up and down motion of the press. If this is not the case, the core will break off prematurely. The drill press should also be well lubricated to allow smooth movement.

3.4 Traveling abroad

When traveling to foreign countries, be sure to take along the appropriate power adapter for the drill. We have usually checked-in the drilling device in a small suitcase as regular baggage when flying. The suitcase may be searched by customs or airport security, so always carry any freshly drilled specimens in your hand luggage, along with a letter of consent from the specimens’ repository institution and perhaps any governmental permits. So far we have not had any major problems, but if you are visiting multiple countries on your trip, it is probably best to use a reliable international package delivery service. Taking specimens across a border of country that is not your homeland may lead to unfounded suspicion from security personnel.

Practical problems may rise when flying. Some airlines have a limited baggage allowance, and will charge exorbitantly for any excess kilos. Therefore it is important to know that a disassembled drill kit will easily weigh around 10 kg when setting a budget for traveling.

4. Drilling

4.1 Supplies needed and the drilling process

Supplies
sample boxes
adhesive tape (to seal sample boxes)
lubricants (water for cooling the drill bit oil for lubricating the drill press, and the drill bit in some cases)
plasticine or clay
cyanoacrylate glue (e.g. Paleobond®)
tissue paper
permanent marker

Lubrication of the drill bit is an essential part of the drilling process. Water or light oil are good lubricants. Water is usually less messy, cheaper and directly available, but some bones are damaged by water such as the *Plateosaurus* material from Trossingen (Germany) and Frick (Switzerland), because they are clay cemented and desintegrate in water. Under such circumstances, oil is a more appropriate lubricant. See Sander and Klein (2005) and Klein and Sander (2007) for a histological description of the *Plateosaurus engelhardti* material, and more details of the methods used for sampling. A practical way of keeping the drill site permanently lubricated consists of building a circular plasticine or clay dam around it and filling it with 20 to 30 ml of lubricant. It is wise to change the lubricant periodically, or each time a core segment is taken out. This will avoid build up of mud in the drill hole and on the coring site, providing optimal lubrication. Once the core is finished, the reservoir can be drained with paper towels or a suction device. The bone can then be cleaned, and the dam removed and applied on the next specimen.

For further processing, thin-sectioning, and describing the bone histology, it is necessary to orient the sample. For comparative reasons, sections in long bones are usually cut perpendicular to the bones length axis. Therefore, the long axis of the bone is marked on the drill site on the bone before the core is drilled. Be sure to use a permanent marker that will not dissolve in the lubricant and the epoxy resin used to embed the samples.

The drilling process begins with the drill and drill bit in place above the drill site, with the drill bit in the basin filled with lubricant. The drill is then started and lowered onto the specimen. If the drill bit is placed on the specimen and then switched on, the outer part of the cortex may be damaged by shearing forces. Slow and careful drilling is in order until the bit has cut a complete circular groove. The electric drills used in this method do not have an accurate rpm-setting, but the intial drilling is usually done at the lowest speed, with only gentle pressure applied on the drill press. The speed dial may be increased one step once the drill bit has sunk about 5 mm into the specimen. After that, drill speed and pressure is adjusted, depending on how well-mineralised the specimen is, so that good progress is made but without overloading the drill motor or damaging the sample.

The drilling process continues until the medullary cavity is reached. The sedimentary fill of the medullary cavity usually differs in hardness and color from the cortical bone, and the color of the drill mud as well as the pressure necessary for drilling may change upon reaching the medullary cavity. If the thickness of the cortex is greater than the length of the crown of the bit, the core may have to be broken off prematurely. However, commonly, the core shears off earlier, anyway. If the cortex is thicker than the length of the crown of the drill bit, the already drilled core is retrieved, and the drilling is continued with the extension piece in place. Our 5/8” bit recovers a core of maximally 40 mm in length, and the extension adds another 30 mm. This takes care of the cortex of even the largest sauropod long bones, such as a 1.8 m femur of *Apatosaurus* (OMNH 4020) with a cortex thickness of 60 mm, and an ulna of *Supersaurus* (BYU 725-13744) with a cortex thickness of 70 mm.

It is possible to automate the drill feed by putting weights on the handle (e.g. sand bags), but the power necessary to depress the handle increases gradually, mainly because of the increasing compression of the spring holding the handle up, but also because of the changing geometry of the mechanism. It is thus best to drill by manually pressing down the lever of the drill press, to keep a constant coring rate.

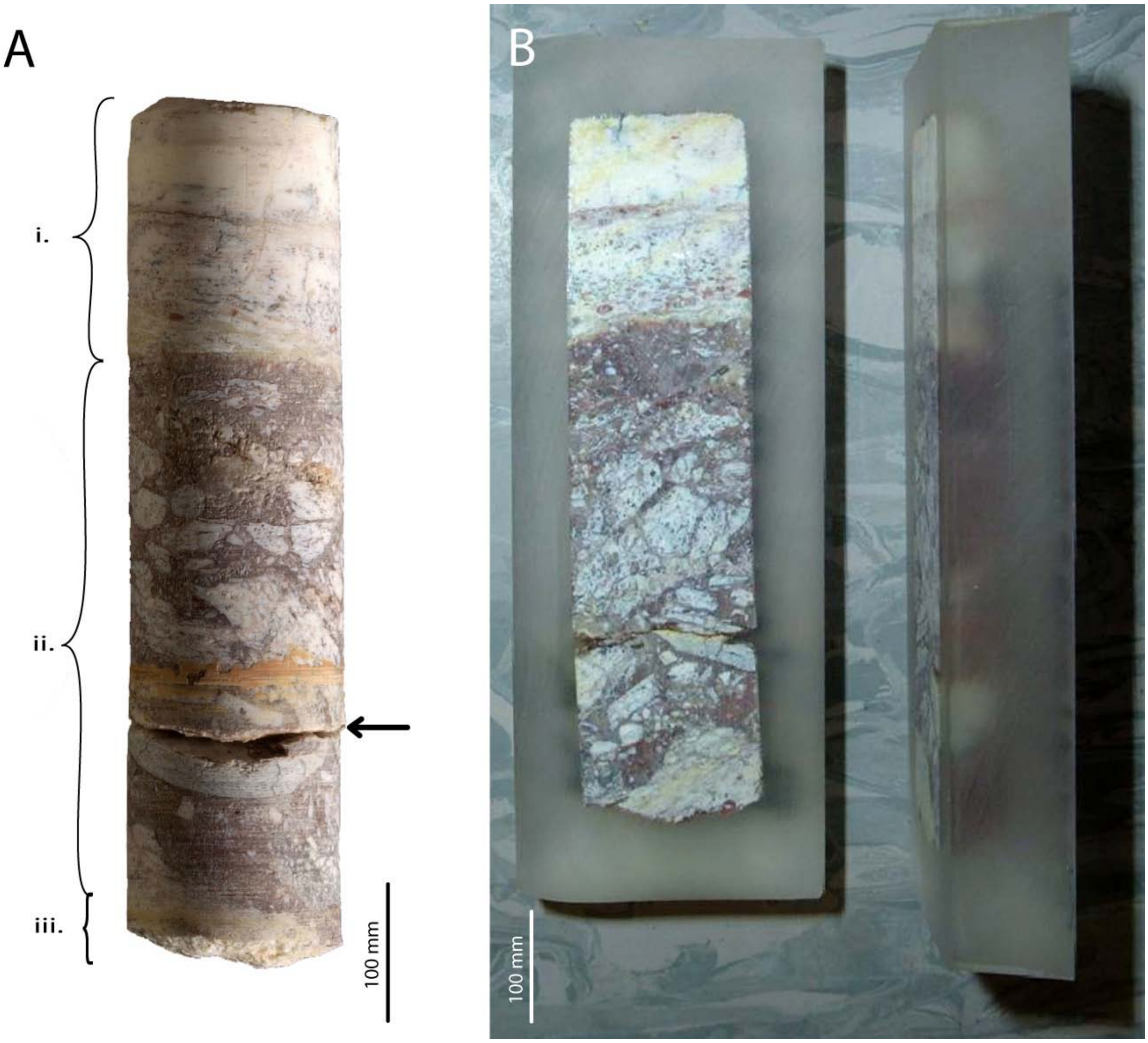

Fig. 10. A: Core taken with a 5/8 (15.9 mm) drill bit. i. Cortex posterior side. Drilling commenced on this side; ii. Medullary cavity with matrix infill and crushed trabeculae; iii. Part of the cortex of the anterior side. This is where the core was broken off its base. Note that the lower part of the core had broken off in the drilling process (arrow). This is usually easily glued back together. B: The same core as in A embedded in epoxy resin and cut perpendicular to the long axis of the bone. These sections are further processed into a polished section and thin sections.

The cores shear of frequently and in most cases are not recovered in one piece. When a core segment has sheared off in the drilling process, it is important to stop drilling and retrieve it, or it may be damaged. However, core segments that have broken off are usually easily glued back together (Fig.3A). We usually apply a cyanoacrylate glue such as

Paleobond ™. Cyanoacrylate has the advantage of fast curing and a refraction index close to 1, and it will not interact with the epoxy resin used for embedding the cores. However, other glues that will not obscure the thin sections or interact with the resin may also be used. Retrieved cores should immediately be stored in secure sample boxes with the information of the particular specimen, and preferably sealed with adhesive tape. Retrieving cores, or core segments that have not sheared off prematurely may require a thin screwdriver, a pair of tweezers and some skillful fingers (Fig. 2E). The screwdriver is inserted in the drilled cavity and used as a lever to break the core off its base. Then the tweezers are used to retrieve the core from the hole. It is almost impossible to avoid occasional core segments getting stuck in the drill bit. If this happens, the bit needs to be removed from the drill, so the core segment can carefully be pushed out with a thin rod or a blunt nail through the hole in the shank of the drill bit. Poorly mineralised specimens can be and have been destroyed in this action, so rince with plenty of water to avoid any friction caused by buildup of mud in the bit.

If further drilling is required (e.g. when the medullary cavity has not been reached), the process may be continued within the same hole with the same core bit, or with an extension piece if the maximum drill depth of the core bit has been reached.

4.2 Timing

The time required per core is strongly dependant on the specimen that is being sampled. On one hand, silicified bones, such as some specimens from the Carnegie Quarry at Dinosaur National Monument, may take a long time and are best drilled with a drill bit studded with medium grained diamonds to speed up the process. On the other hand, subrecent specimens, such as Pleistocene mammals, have to be drilled slower (lowest setting on the drill speed dial) and more careful than usual, as they have not yet (completely) undergone the mineralization process, but have already begun degrading, and are generally more fragile than fossil and recent bones. Recent bone is relatively tough and can be drilled faster, providing there is no overheating. On average, about an hour per core sample is needed.

When desired, the small hole left at the drill site can be filled up with plaster, putty or other materials, so the original shape of the bone is restored. However, we prefer to leave the hole open for several reasons: the sampling site is immediately obvious and preserved for later workers, the existing hole can be use to obtain additional bone tissue samples, e.g. for isotope geochemistry, and the core sample can be extended by deeper drilling, e.g. to study the medullary region and the cortex of the other side of the bone.

5. Thin sectioning and polished sections

Histological cores are ultimately processed into petrographic thin sections (Fig.3B), following standard methods (Enlow and Brown, 1956; Wells, 1989; Chinsamy and Raath, 1992; Wilson, 1994; Lamm, 2007). The cores are usually embedded in polyester resin that cures water-clear (e.g. Araldite® or Silmar 41®) and then cut along the long axis of the core, perpendicular to the surface mark indicating the long axis of the long bone. The freshly cut surfaces are impregnated with resin in a vacuum chamber to reduce the risk of air bubbles that may obscure the slide. The sectioned surfaces are then ground with grinding powder until smooth and all saw marks having been obliterated. In our lab, we use a sequence of 400, 600 and 800 grit sizes for grinding, but depending on availability, other grit sizes may be used. Specimens should be thoroughly rinsed when changing grit size. Once the embedded core is smooth enough, it is glued with resin onto a frosted glass slide of desired size, and left to dry. Other glues or epoxies may also be used, providing they have a refraction index that is near that of water and are strong enough to withstand the forces of sectioning and grinding. The use of polyester resins is not recommended, as they are not very stable and may desintegrate after a number of years. It is also best to avoid the use of dessicators or glue presses, as these increase the risk of bubbles. It usually takes 24 hours for the sample to cure. Then, it is cut to a thickness of a few milimeters, using an automatic rock saw, and further manually ground to a thickness of about 120 to 150 µm. The desired thickness can be approximated by repeated control with a polarizing microscope. Finally, a cover slip is put on the section to increase the contrast and protect the sample. We usually apply a UV light curing adhesive to glue the cover slip. Two initial thin sections are made, one from each core half. The residual core part can then be used for polished sections, microprobe or isotope analysis and/or further thin sectioning. Alternatively, one of the core sections can be polished into a polished section *sensu* Sander (2000). Polished sections are polished to a high gloss, similar to polished ore or coal samples, to facilitate study in incident light. This allows observing polish lines, a growth line in fibrolamellar bone, which are only visible under bright field illumination, and not in thin sections (Sander 2000). When making polished sections, one should aim for a ‘decorative polish’. Advanced polishing machines create an overpolish, making the polish lines invisible. In the process, a simple polisher, aluminiumoxide, and a synthetic polishing cloth are used. Polish lines will appear where differences in hardness exist in the bone matrix, as softer regions will be ground away faster. For a detailed description of polish lines in sauropod bones, see Sander (1999, 2000).

Literature on making geological and histological thin sections is extensive, but expertise in preparing fossil bone thin sections can only be acquired by experience. In general, thin sections from fossil bone are more difficult to cut than regular rock thin sections because the brittle nature of fossil bone. Examples of more detailed descriptions of the thin sectioning process for paleontological specimens can be found in Enlow and Brown (1956), Wells (1989), Chinsamy and Raath (1992), Wilson (1994), or Lamm (2007).

6. Advantages and limitations of the coring method

The most important advantage of the histological coring method described here is its less destructive nature compared to the cross-sectioning of whole bones. Comparable to a biopsy on living tissue, only a small fragment of the tissue is taken, and the morphology of the specimen is preserved. The coring method may also be used as an alternative to serially sectioning bones, taking cores at designated places along the long axis of the bone. Although the specimen would then have to be reinforced by filling up the drilled holes with putty or plaster, it is not entirely lost for morphological studies. When performing growth studies, incomplete specimens also qualify for core sampling, as long as the standard mid-shaft location can be sampled, and the full size of the specimen can be estimated.

The less destructive aspect of this method is more appealing to most museum curators and collections managers than traditional sectioning methods, and thus sampling is more easily approved. As more and more samples become available, the way for comprehensive comparative, skeletochronological and other quantitative studies is being paved.

In addition to long bones, other bones may also qualify for the coring method. Sauropod ribs usually tend to show extensive remodeling and few to no growth lines. However, serial sectioning in our lab of complete *Camarasaurus* ribs from the Morisson Formation showed the proximal region and the rib neck preserve the original growth record. Other species may also preserve their growth record in this region, and so in further investigations rib specimens can be sampled using a coring device as well. Other researchers have already succesfully studied histology in a variety of bone types, which may also qualify for histological coring. Curry (1999) found growth marks in scapulae of *Apatosaurus* sp., which was grossly different from what could be observed in *Apatosaurus* long bones, where no growth marks were observed in the typical primary fibrolamellar cortex. Erickson *et al.* (2004) and Horner and Padian (2004) performed multi-element histological analyses on tyrannosaurid bones. These studies revealed that non-weight bearing bones (e.g. pubis, fibula, ribs, gastralia and some post-orbital skull bones) usually exhibit a better growth mark record than weight bearing bones, which tend to have more extensive remodeling.

The coring method also has some practical advantages. The drill press, drill, and other components will fit together in a small travel case, with a total weight of around 10 kg, which makes it possible to take the drilling equipment along and sample bones directly in the collection rooms, transport to preparation lab being not necessary. This is a major advantage in the case of large sauropod long bones that may weigh several hundred pounds. The samples obtained by core drilling are small enough to carry a large number in a small backpack or travel case. Additionally, the method works equally well for extremely large as well as for small bones, only requiring the mounting of a smaller drill bit when drilling smaller specimens.

The most important limitation of the method is the restricted view of the cortex. The core is a sample taken at a controlled location on the bone shaft, but it is only a narrow segment of the cortex in a large bone. Any localized variation in cortical histology (e.g. pathologies, cortical drift, medullary bone, differential remodeling) along the circumference of the bone will not be observed, which could seriously affect any histological interpretations. This emphasizes again the importance of a standard sampling location when comparing histologies of different individuals and taxa.

Other problems may arise when sampling subrecent or other poorly preserved specimens. If there has been no infilling or cristallization of the medullary cavity, coring can destroy the trabecular bone. Therefore, it is necessary to decide which features should be studied, before starting the drilling, and evaluate the risk of destroying those features or the entire core in the process. Extremely fragile bones are best embedded in resin to stabilize them, and subsequently completely sectioned instead of core drilled. Nevertheless, if the coring method is chosen because the bone is too large, or because complete sectioning is not allowed, slow drilling is in order. Additionally, if the bone has not been consolidated already, it may help to locally impregnate the drill site with an appropriate product (e.g. polyvinyl alcohol or polyvinyl acetate). Finally, it should be mentioned that this is a general description of the coring method, and that this description is not a definite working procedure. Many aspects described here can be modified to workers' and technicians' preferences and wishes, and most difficulties will probably surface finding adequate drill bits.

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Chapter 3

Sauropod Dinosaurs Evolved Moderately Sized Genomes Unrelated to Body Size

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Abstract

Sauropodomorph dinosaurs include the largest land animals to have ever lived, some reaching up to ten times the mass of an African Elephant. Despite their status defining the upper range for body size in land animals, it remains unknown whether non-avian theropods evolved smaller sized genomes than sauropodomorphs, their sister taxon, or whether a relationship exists between genome size and body size in dinosaurs, two questions critical for understanding broad patterns of genome evolution in dinosaurs. Here we report inferences of genome size for 10 sauropodomorph taxa. The estimates are derived from a Bayesian phylogenetic generalized least squares approach that generates posterior distributions of regression models relating genome size to osteocyte lacunae volume in extant tetrapods. We estimate that the average genome size of sauropods was 2.02pg (range of species means: 1.77pg - 2.21pg), values in the upper range of extant birds (mean = 1.42pg, range: 0.97pg - 2.16pg) and near the average for extant non-avian reptiles (mean = 2.24pg, range: 1.05pg - 5.44pg). These results suggest that the variation in size and architecture of genomes in extinct dinosaurs was lower than the variation found in mammals. A substantial difference in genome size separates the two major clades within dinosaurs, Ornithischia (large genomes) and Saurischia (moderate-small genomes). We find no relationship between body size and estimated genome size in extinct dinosaurs, which suggests that neutral forces did not dominate the evolution of genome size in this group.

1. Introduction

Little is known about the molecular biology and evolution of extinct organisms. The absence of molecular information in long extinct species limits the use of molecular data in phylogenetic analyses that include fossil species, though recent research suggests that such endeavors may be possible (Shedlock, 2006). More profoundly, pervasive extinction obscures large-scale patterns of molecular evolution through deep time.

An emerging route around the latter problem is the estimation of genome size in extinct species, a genomic parameter analogous to organismal body size, which is largely determined in animals by introns and various forms of repetitive elements (Gregory, 2001). There is a tight correlation between cell size and genome size in extant vertebrates (Organ et al., 2007; Organ and Shedlock, 2009), which allows the estimation of genome size in extinct species from the size of osteocyte lacunae in paleohistological thin sections (Gregory, 2005). Such estimations provide critical insights about the genome biology of extinct species and about the macroevolution of the animal genome. For example, how are genomes maintained over long periods of time (Lynch and Conery, 2003)? Is genome size adaptive or non-adaptive (Andrews et al., 2008; Organ and Shedlock, 2009)? Are the well established correlations among genome size and certain adaptations or behaviors, such as flight (Vinogradov and Anatskaya, 2006) or endothermy (Organ et al., 2008) due to historical constraints? Why is there so much variation in genome size among species (Sereno, 1999)? Like many questions in evolutionary biology, these are difficult to fully answer without recourse to the fossil record.

Dinosaurs are a promising extinct group for palaeogenomic investigation. Dinosaurs were the preeminent vertebrates in most terrestrial ecosystems during a 160-million-year span from the Late Triassic until the end of the Cretaceous (Brusatte et al., 2008), evolved into a wide array of shapes and sizes, and filled many ecological niches (Padian and Chiappe, 1998). Mesozoic dinosaurs also gave rise to birds, and thus occupy a critical position as ancestors and close relatives of birds, the most speciose group of extant terrestrial vertebrates (Erickson, 2005). Practically, dinosaurs are well-studied and known from a bounty of specimens, many of which have been investigated histologically (Organ et al., 2007). Indeed, dinosaurs were the first long extinct amniotes studied in a genomic context (Organ et al., 2007). However, these analyses were aimed at two specific issues: the evolution of genome size as related to the evolution of avian flight and the timing of the genomic contraction that led to the small and constrained genomes of birds.

Previous work on dinosaur paleogenomics (Packard et al., 2009) unevenly sampled Dinosauria, with only one specimen from Sauropodomorpha, one of the three main dinosaur subgroups and the sister group to the carnivorous theropods (which include birds). The largest terrestrial animals to ever live are sauropods, even if many estimations of body mass for these species are inflated due to statistical artifacts (Packard et al., 2009). Sauropods include iconic creatures such as *Brachiosaurus* and *Apatosaurus* that reached masses of at least 16 tonnes (Sander and Clauss, 2008) and perhaps as much as 80 tonnes with body lengths up to 40 meters (a non-phylogenetic analysis has found such a relationship in birds`; Gregory, 2005). Organ *et al.*(2007) hypothesized that non-avian theropods evolved smaller genomes than sauropodomorphs, despite their immense size, but were unable to address this question because of poor sampling within Sauropodomorpha. We address this hypothesis in the current study as well as the hypothesis that no relationship between genome size and body size exists in terrestrial vertebrate species (Peczkis, 1994; Packard et al., 2009). Because of their tremendous body sizes (for example`, Reid, 1996; Horner et al., 2001), sauropodomorphs provide a critical source of data to the ongoing debate about genome size evolution. Moreover, sauropodomorphs must be considered to comprehensively evaluate hypotheses concerning the diversity of the dinosaur genome, the timing of the contraction of genome size along the lineage leading to birds, and the neutral theory of genome evolution in extinct dinosaurs.

2. Materials and Methods

Samples of fossil bone were thin sectioned for the following sauropodomorph taxa: *Massospondylus carinatus*, *Barosaurus lentus*, *Janenschia robusta*, *Europasaurus holgeri*, *Phuwiangosaurus sirindhornae*, *Plateosaurus engelhardti*, *Thecodontosaurus antiquus*, *Dicraeosaurus sattleri*, and *Saturnalia tupiniquim*. Phylogenetically, this group contains basal (e.g. *Saturnalia* and *Thecodontosaurus*) and derived (e.g. *Europasaurus* and *Janenschia*) sauropodomorphs, that range in body size from small to colossal. The specimens used for sectioning were subadults and adults. Preparation of thin sections followed standard protocols (2007). Cell volumes (lacunae volume) were estimated by measuring the length and width of the largest lacunae within a given thin section (Figure 1), assuming an ellipsoid shape ($4/3 \times \pi \times$ width axis radius^2 $\times$ length axis radius), as detailed in Organ et al. (see Organ and Shedlock, 2009). These data (Table 1) were combined with cell volume and haploid genome size data (www.genomesize.com) from 38 extant species (2007). Several extinct archosauromorph species were also included from Organ et al. (2009) and Organ and Shedlock (2009) so that the clade Archosauria was sampled evenly.

Construction of the phylogenetic framework followed Organ and Shedlock (2004; 2007), with Sauropodomorpha constructed from Upchurch et al. (Maddison and Maddison,

2007) using Mesquite v2.01 (Faure et al., 2006) and the StratAdd package (Gradstein et al., 2004). Branch lengths are in units of time and follow the standard geologic timescale (Christiansen and Farina, 2004).

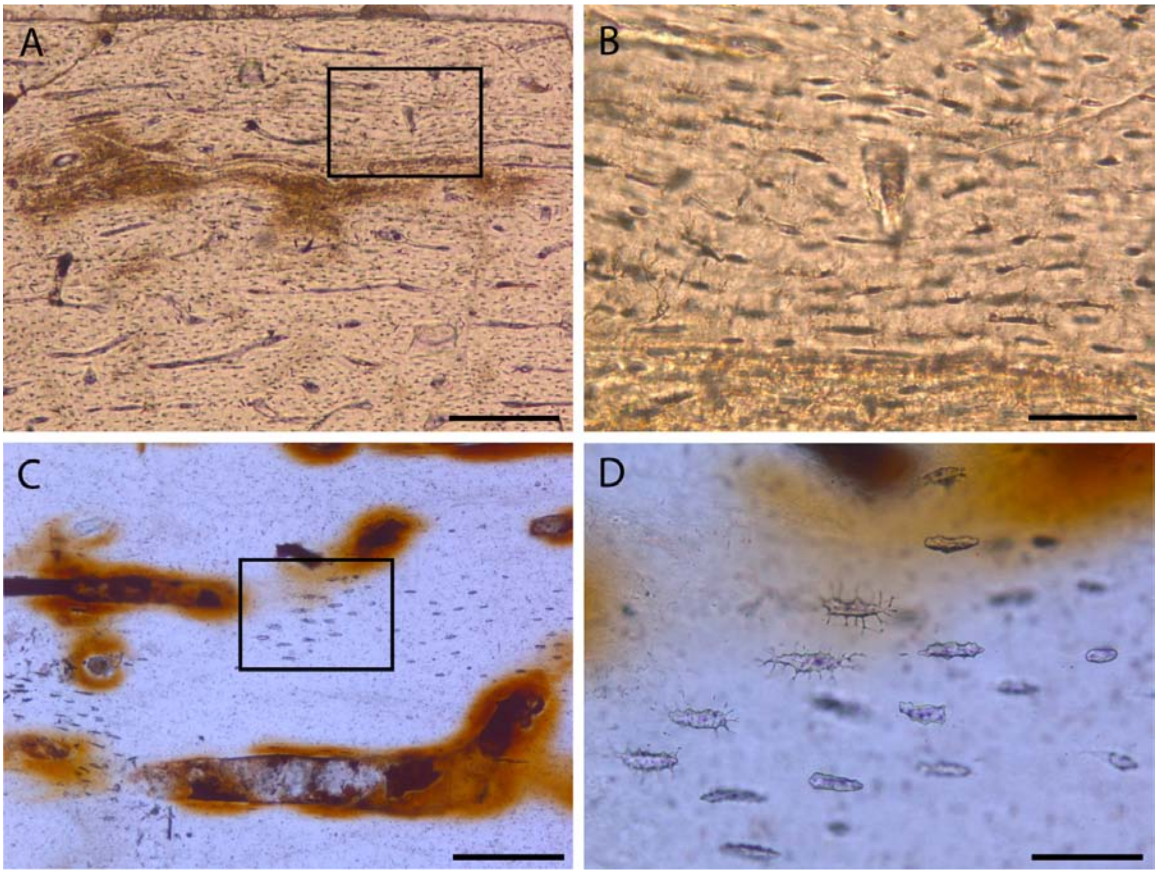

Fig. 11. Thin sections of fossil dinosaur bone with preservation of vascular spaces and osteocyte lacunae. A: transverse section of a femur of the basal sauropodomorph *Thecodontosaurus antiquus*. Scale bar equals 200 µm. B: magnification of selected area in A. Scale bar equals 50 µm. C: longitudinal section of a *Barosaurus lentus* femur. Scale bar equals 200 µm. D: magnification of selected area in C. Scale bar equals 50 µm.

Genome size and cell size measurements were logarithmically transformed and analyzed, along with femur length - a proxy for body size (Pagel, 1997; Pagel, 1999), in the program BayesTraits (http://www.evolution.rdg.ac.uk). BayesTraits generates posterior distributions of regression models for continuous characters while using phylogenetic generalized least squares (PGLS) to account for the evolutionary non-independence among the character (1993). Predictions of genome size were made by sampling the posterior distributions of regression models and accounting for the amount of expected covariation among the extinct and extant taxa based on the phylogenies noted above. Details about our character analysis can be found in the electronic supplementary material accompanying this report.

Sauropod Taxon	Specimen ID	Skeletal Element	n	Cell Vol Avg (μm^3)	Cell Vol Stnd Dev	C-value (pg)	C-value Stnd Dev
Apatosaurus	MOR 1996-05	scapula	20	130.49	52.11	2.31	0.44
Barosaurus	T16a	femur	30	57.3	43.04	1.77	0.36
Dicraeosaurus	NHUB O2	femur	30	106.69	60.78	2.21	0.42
Europasaurus	FV495.9	femur	30	95.29	82.31	2.13	0.41
Janenschia	F37a	femur	30	79.02	53.58	1.99	0.39
Massospondylus	Chinsamy (Organ et al., 2007; Organ and Shedlock, 2009)	femur	30	106.65	46.86	2.21	0.7
Phuwiangosaurus	k4-366	femur	30	71.87	54.23	1.93	0.40
Plateosaurus	F14a	femur	30	63.68	39.92	1.82	0.30
Saturnalia	MCPV-3846	femur	37	75.93	33.50	1.94	0.282
Thecodontosaurus	IPB (no ID)	tibia	30	67.56	30.15	1.87	0.31

Table 1: Summary statistics of lacunae volume for 10 sauropodomorph species. These data were used to infer haploid genome (C-value) size using the Bayesian comparative method described above and in further detail elsewhere (Organ et al., 2007). Apatosaurus data (gray) is taken from (Organ et al., 2007). Institutional abbreviations are as follows: MCP (Museu de Ciências e Tecnologia PUCRS, Porto Alegre, Brazil), SMNS (Staatliches Museum für Naturkunde, Stuttgart, Germany), NHUB (Naturkundemuseum of the Humboldt-Universität Berlin, Germany), DFMMh/FV (Dinosaurier-Freilichtmuseum Münchehagen/Verein zur Förderung der Niedersächsischen Paläontologie (e.V.), Germany), PCDMR (Paleontological Collection of the Department of Mineral Resources of the Government of Thailand, Khon Kaen Province, Kalasin, Thailand), MOR (Museum of the Rockies, Bozeman, MT).

3. Results

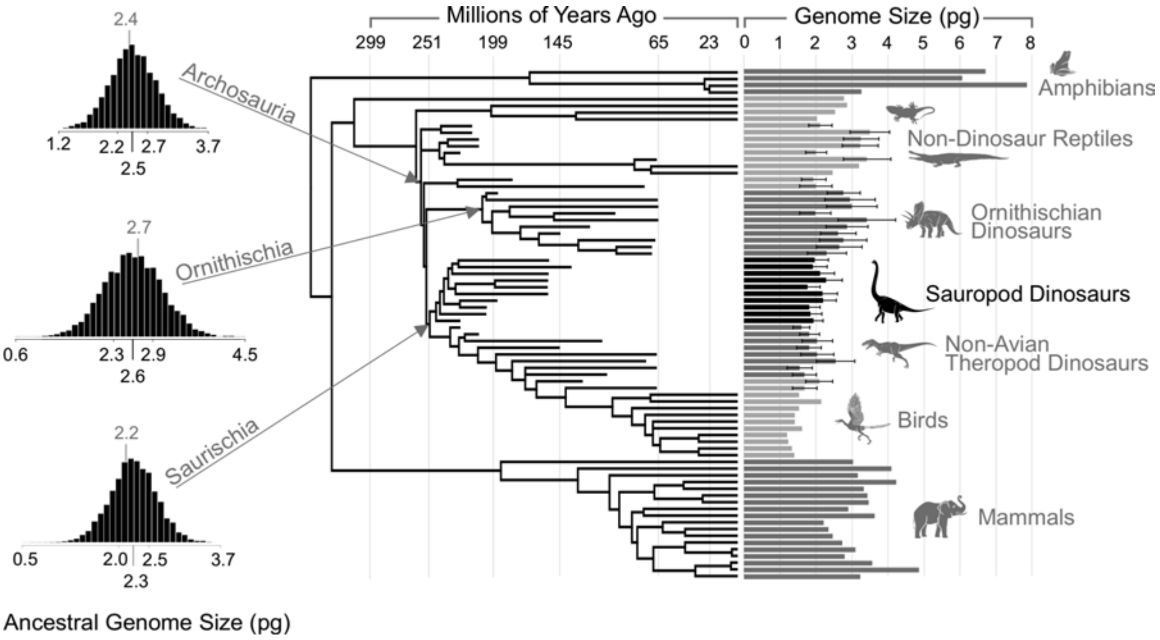

Fig. 12. Estimated haploid genome size for sauropod dinosaurs (black bars). For extinct taxa, the bars are the mean of the posterior prediction and the error bars are one standard deviation. The branches of the phylogeny relating the bars in the graph are drawn in units of absolute time. The distributions in the left panel are ancestral state reconstruction for Archosauria (top), Dinosauria: Ornithischia (middle), and Dinosauria: Saurishcia (bottom). The axes in the left panel are labeled with the minimum, 25% quartile, median, 75% quartile, and the maximum of the distributions. The gray number on top and behind the distributions is the ancestral state point estimate produced by squared change parsimony. As is clear, the two methods give largely congruent answers.

The Bayesian estimation of the correlation between osteocyte cell volume and genome size (Supplemental Figure 2) did not differ from previous work (Organ et al., 2007). The markov chain reached the station phase without an extended burn-in. Moreover, plots of the regression parameters by their log-likelihood show that they are normally distributed so that the mean of the distribution is close to the maximum likelihood estimate.The average estimated haploid genome size for the ten sauropodomorphs in this study (Table 1 and Figure 2) is 2.02pg, with a standard deviation of 0.41. There is no apparent phylogenetic pattern in the distribution of genome size within sauropods. That is, the genome size of basal members of the group, such as *Saturnalia* (mean estimated genome size of 1.94 pg, sd = 0.82), do not differ substantially from more derived members, such as *Dicraeosaurus* (mean estimated genome size of 2.21pg, sd = 0.42). We found no evidence suggesting that genome size evolution evolved in a directional manner in dinosaurs (random walk model (model A) vs. directional model (model B), p-value = 0.14).

The hypothesis that genome size contraction evolved within the theropod lineage can be evaluated using our results. We find that, as originally hypothesized (Organ et al., 2007), theropods have smaller genomes than sauropodomorphs (phylogenetic *t*-test for difference in genome size between theropods and sauropodomorphs, p-value = 0.048). There is also a substantial difference between the genome sizes of Saurischia (Sauropodomorpha and Theropoda) and Ornithischia (phylogenetic *t*-test, p-value = 0.0002).

We find no relationship between body size and genome size in extinct Mesozoic dinosaurs (n = 27, PGLS r^2 = 0.0005, p-value = 0.282) or within sauropodomorphs (n = 10, PGLS r^2 = 0.011, p-value = 0.39). For example, the small extinct bird *Hesperornis* (femur length = 155 mm) is estimated to have had a genome nearly identical in size as the massive sauropod *Barosaurus* (femur length = 1,440 mm).

4. Discussion

Previous work on genome size evolution in non-avian dinosaurs (Gregory, 2009) included only one sauropodomorph taxon (*Apatosaurus*). Because estimation of ancestral states is essentially a weighted mean, taxon sampling can have a large influence on estimated trait values at nodes. This is especially true if taxa near a node are omitted and these taxa have 1) very short branch lengths connecting to the node, 2) trait values far from the mean of the group, and/or 3) the underlying model (Brownian motion or Ornstein-Ulenbeck) poorly fits the data. Several sauropodomorphs used in this study have short branch lengths leading to the node Saurischia, a major subgroup of dinosaurs that includes birds, and these have estimated genome sizes intermediate between theropod and ornithischian dinosaurs (average genome sizes for Ornithischia = 2.75pg, Sauropodomorpha = 2.02pg, and Theropoda = 1.7pg). Our analysis suggests that genome size within Sauropodomorpha differs little from the ancestral saurischian condition (Figure 2, left panel) and we find no evidence for directional evolution that would invalidate the ancestral state reconstructions noted above (a direction model of evolution indicates that the ancestral value may lie outside the range observed in the descendants). The results also indicate that a progressive decrease in genome size occurred along the evolutionary line to birds, with substantial decrease in Saurischia after the origin of dinosaurs and again in the theropod line after saurischians split into sauropodomorphs and theropods. These results agree with Organ *et al.* (2007), who found that the small genomes of extant birds began to contract in non-avian theropods before the origin of flight.

In the wider context of reptile genomics, our results suggest that the genomes of sauropodomorphs lie in the upper range of birds (ostrich, *Struthio camelus*, genome size = 2.16pg) and near the median for non-avian reptiles (2.24pg), which ranges from 1.05pg in the skink *Chalcides mionecton* to 5.44pg in the Greek tortoise *Testudo graeca* (Gregory, 2009). Our results also indicate that genome size variability in extinct dinosaurs, though larger than in extant birds, was not as great as that in mammals, which range from 1.73pg for the bent-winged bat *Miniopterus schreibersi* to 8.40pg, the red viscacha rat

Tympanoctomys barrerae (note that the red viscacha rat is tetraploid). The average genome size for mammals is 3.37pg (Ellegren, 2007). The variability in genome size within extinct Dinosauria is therefore more comparable to the variability found in non-avian reptiles than in mammals, whose diversity and dominance in modern terrestrial ecosystems is often compared with Mesozoic dinosaurs.

One of the most important and debated questions in modern evolutionary genomics is the extent to which the evolutionary forces of selection and drift shape the genome (summarized in Lynch, 2007). For instance, recent work by Lynch (Lynch, 2007) provides important insights into the respective roles that selection and drift have played in forming genome architecture, and therefore genome size. These ideas may be termed “the neutral theory of genome evolution” and they predict that genome size in small populations, in which the efficiency of selection is reduced, should expand due to the accumulation of mutations drifting to fixation. The primary evidence for the neutral theory of genome evolution is the large population sizes and small genomes of prokaryotes in contrast to the small population sizes and large genomes of eukaryotes. This contrast is made feasible because body size, which is roughly inversely proportional to population size, and spans 20 orders of magnitude across prokaryotes and eukaryotes (Peczkis, 1994).

Unlike the prokaryote/eukaryote contrast, in living amniotes differences in body size reach around only four orders of magnitude, well exemplified by the proverbial mouse and elephant. As the largest land animals to have ever walked the earth, weighing up to 80 tonnes (Lynch, 2007), sauropodomorphs provide a critical source of data to the ongoing debate about genome size evolution. The neutral theory of genome evolution predicts that sauropodomorphs should have had relatively small populations, due to their large body size, and therefore large genomes. The genome sizes estimated here for sauropodomorphs would support the neutral theory if genome size and body size are inversely related in animals (Vinogradov and Anatskaya, 2006) or if genome size expands for derived sauropods (species with the largest body sizes) relative to more primitive, smaller basal sauropodomorphs. However, our results do not support a purely neutral process of genome evolution in extinct dinosaurs. We find no correlation between genome size and body size in Mesozoic dinosaurs as predicted by the neutral theory. This agrees with recent findings in which correlations between genome size and body size were absent in extant eukaryotes using standard statistical approaches (Gregory 2005), and in extant tetrapods using phylogenetic comparative methods (Organ and Shedlock 2009). Furthermore, we do not find an expansion of genome size in larger sauropods, but rather the opposite, a slight reduction relative to the saurischian common ancestor (Figure 3). However, these results do not rule out the possibility that drift played an important role in the evolution of amniote or dinosaur genomes, only that the evidence presented here does not match the predictions made by a predominately neutral model.

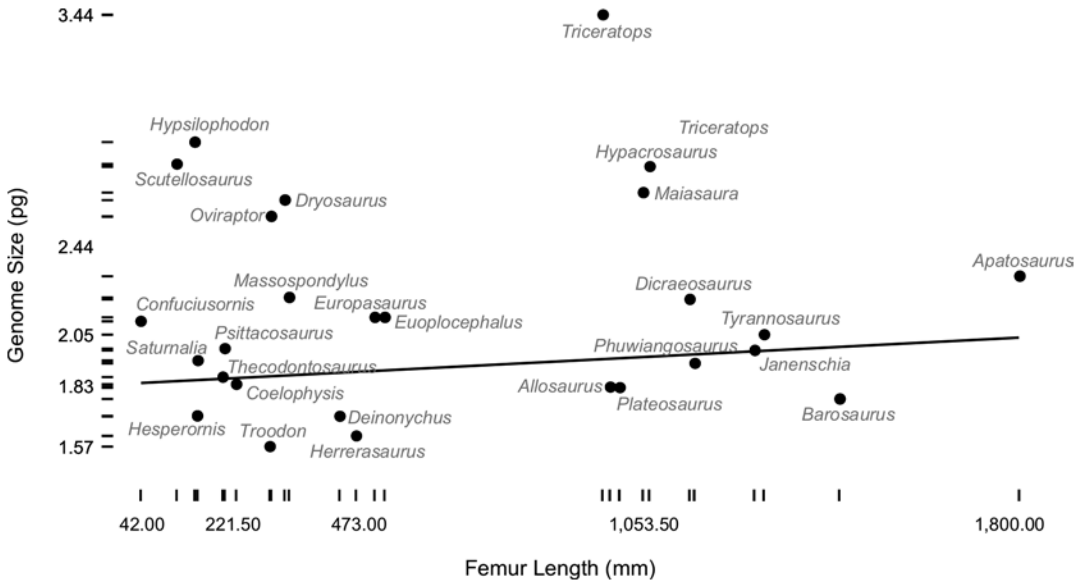

Fig. 13. The relationship between genome size and body size in extinct dinosaurs. The phylogenetic generalized least-square (PGLS) regression line relating genome size to body size in 27 extinct dinosaur species is genome size = 1.84 + 0.0001*femur length, r2 = 0.0005, p-value = 0.282. The axes are labeled with the quartiles of the data (minimum, 25% quartile, median, 75% quartile, and the maximum).

Our results, and palaeogenomics in general, permit the formation and testing of detailed and rigorous hypotheses concerning molecular and cellular information in extinct taxa. For example, because genome size correlates with cell size and cell division rate, and because these associations are thought to be causative, knowing genome size has the potential to clarify aspects of an organism’s biology for which there is no direct fossil evidence. Of specific interest is the observation that genome size is correlated with metabolic rate in tetrapods generally (Gregory, 2002) and in birds particularly (Organ et al., 2007; Organ and Shedlock, 2009). This relationship has prompted speculation that the small genomes in extinct theropods and pterosaurs may be evidence of an elevated metabolic rate (Curry Rogers, 1999; Klein and Sander, 2008). Histological work suggests that sauropods were metabolically active given their inferred rapid growth rates . Yet, such speculation is more difficult to offer here given the estimated genome sizes of sauropods, which lie in the range of many ectothermic lizards and paleognath birds. Regardless, future palaeogenomic work on other extinct tetrapod groups should help clarify when repetitive elements radiated or went extinct within genomes, and whether expansions or contractions in genome size were associated with the acquisition of other traits, such as rapid growth rates or integumentary covering, each of which may be indicative of an elevated metabolism.

In this vein, genome size is an independent line of evidence that may be brought to bear on certain long-debated questions of organismal biology in extinct species and larger-scale patterns of molecular evolution on lineages leading to living species. Yet, there are still many unknowns regarding the relationship of genome size, cell size, and cell physiology. Additional rigorous, phylogenetically based studies of many clades, both extinct and extant, are needed to clarify the issues raised above. Just as fossils can reveal patterns of anatomical character change from extinct ancestors to living descendants, fossils may also help reveal broad patterns of genome evolution over geologic time scales.

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Chapter 4

Saturnalia tupiniquim (Dinosauria: Sauropodomorpha) long bone histology and woven bone osteocyte lacuna density in Sauropodomorpha indicate convergent evolution of fibrolamellar bone in dinosaurs and mammals

Submitted as Stein, K. and Langer, M.C. *Saturnalia tupiniquim* (Dinosauria: Sauropodomorpha) long bone histology and woven bone osteocyte lacuna density in Sauropodomorpha indicate convergent evolution of fibrolamellar bone in dinosaurs and mammals to Paleobiology

Abstract

Sauropods grew to be the largest terrestrial animals to ever roam the earth. The earliest sauropodomorphs started out as relatively small animals, but the first large sauropods evolved soon after. This rapid evolution of sauropodomorph body size and growth rate can be observed in the microstructure of their long bones. We studied the change from cortical zones of reticular fibrolamellar bone with lines of arrested growth in early sauropodomorphs like *Thecodontosaurus* and *Plateosaurus* to an uninterrupted laminar fibrolamellar bone cortex in sauropods. Fibrolamellar bone tissue (FLB) is present in the earliest dinosaurs and dinosaur sister groups, and its presence in *Saturnalia* therefore supports an origin of FLB in an early archosaur ancestor. FLB is also seen in extant mammals and birds, which have high basal metabolic rates and are generally endothermic. Analysis of osteocyte lacunae in the woven bone component of cortical FLB of several sauropodomorphs shows that lacunae were densely spaced, indicative of high growth rates and metabolic rates. Sauropodomorph osteocyte lacuna density furthermore shows a strong negative correlation with body mass, similar to extant mammals. Lacuna density is much higher in sauropodomorphs than in mammals of similar body mass; however, mammals have generally much larger cells. Despite the identical appearance of FLB in mammals and sauropodomorphs, this significant difference in osteoblast proliferation and osteocyte incorporation strategy is most likely the result of the convergent evolutionary history of fibrolamellar bone in these two lineages.

1. Introduction

1.1 Body size evolution in Sauropoda and Sauropodomorpha

Sauropod dinosaurs were the largest terrestrial animals that ever walked the earth. One of several key innovations leading to their enormous size was the evolution of high growth rates, as inferred from bone histology (Sander & Clauss 2008; Sander et al. 2010). Sauropod long bone histology typically consists of fibrolamellar bone (Curry 1999; Sander 2000, Lehman & Woodward 2008, Woodward & Lehman 2009; Klein and Sander 2008; Klein et al. 2009; Sander et al. 2010).

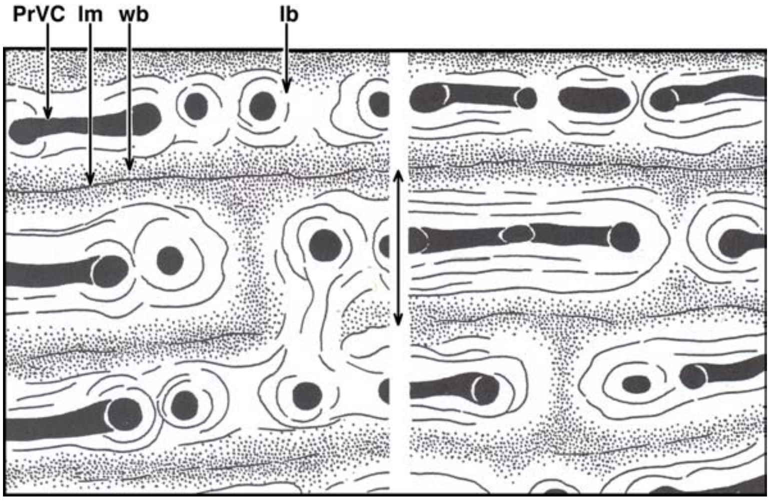

Fig. 14. Laminae of compact laminar fibrolamellar bone. wb (dots): woven (also fibrous) bone matrix of periosteal origin, laid down centrifugally; lb (lines): lamellar or parallel fibred bone matrix of the primary osteons, laid down centripetally; PrVC (black): lumen of primary vascular canal; lm: bright line, a hypermineralized region in woven bone and early site of deposition of a new lamina. The double headed arrow in the center shows the modern definition of one lamina. Adapted from Ricqlès (1975).

Fibrolamellar bone (FLB) (Fig 1) is a tissue that consists of two components, a vascular framework structure of woven bone, and lamellar bone, which later in an animal's ontogeny fills up the vascular spaces, forming primary osteons (bone histological terminology follows Francillon-Vieillot et al. 1990). FLB had already evolved in "prosauropod" ancestors of sauropods (Chinsamy 1993; Sander and Klein 2005; Klein and Sander 2007); however, histology of the most basal sauropodomorphs remains unstudied.

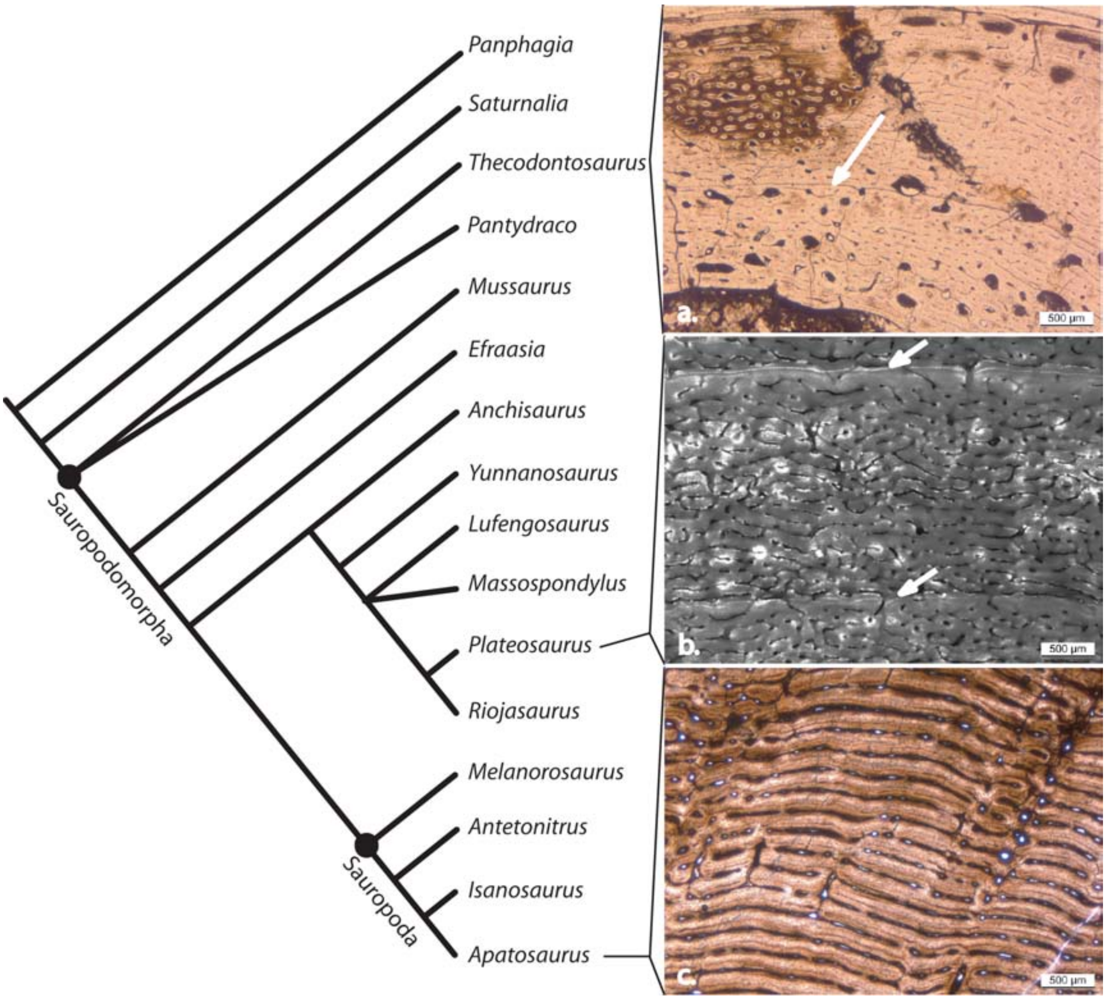

Fig. 15. Phylogenetic relationships and overview of bone histology in Sauropodomorpha. a. Long bone histology of *Thecodontosaurus* (IPB?). The section shows fibrolamellar bone with many longitudinal and some circumferential vascular canals. The arrow indicates a line of arrested growth. This section also shows a thin layer of lamellar endosteal bone separating cortex from medullary cavity. b. micrograph of a *Plateosaurus* femur (IFG 192.1) section, representing prosauropod long bone histology. Fibrolamellar bone with two LAGs indicated by the arrows, and vascularization is more laminar than in *Thecodontosaurus*, but many longitudinal canals are still visible. Picture kindly provided by Nicole Klein. c. *Apatosaurus* (BYU 725-14017) representing Eusauropods and Neosauropods shows a typical laminar fibrolamellar bone tissue in its long bones. LAGs are seldomly visible in sauropod long bones, suggesting they grew continuously. In all micrographs, the periost is located in the top, medullary cavity in the bottom. The phylogenetic hypothesis is not the result of a novel analysis, but a compilation of that of Upchurch et al. (2007); Martinez and Alcober (2009); Yates (2009).

Saturnalia is primarily known from three partial skeletons from the Carnian Santa Maria Formation in southern Brazil (Langer et al. 1999; Langer 2003). These skeletons provide a clear image of the anatomy of the entire animal, and a relatively sound understanding of the anatomy of the earliest sauropodomorphs. *Saturnalia* held the record for most basal known sauropodomorph dinosaur until the discovery of *Panphagia protos* from the Carnian Ischigualasto Formation of Argentina (Martinez and Alcober 2009). Other basal sauropodomorph taxa are *Thecodontosaurus*, *Pantydraco*, and *Efraasia* (Yates 2003; Galton et al. 2007; Smith and Pol 2007; Upchurch et al. 2007; Yates 2007; Sertich and Loewe 2010; Fig 2). *Saturnalia*, like most early sauropodomorphs, was a small, probably facultative quadrupedal animal with a femur length of 15 cm (Langer 2003; Langer et al. 2007; Fig 3.). As sauropodomorphs evolved larger body sizes, they also became obligate quadrupeds. This body size increase takes place early in sauropodomorph evolution, and the basal sauropod *Isanosaurus* from the Upper Triassic of Thailand already attained a size comparable to that of the gigantic forms of the Jurassic (Buffetaut et al. 2002).

Fig. 16. Saturnalia femora MCPV 3846. Arrow indicates plane of sectioning. Scale bar equals 50 mm.

1.2 The nature of fibrolamellar bone

FLB is well known from several amniote groups, including therapsids, dinosaurs, and pterosaurs. It evolved at least twice convergently: in therapsids (Ray et al. 2004; 2009) and in the archosaurs. Following Amprino's Rule (Amprino 1947), FLB indicates generally high growth rates comparable to those of large mammals (Margerie et al. 2002, 2004). FLB is typically seen in animals with high resting metabolic rates like mammals and birds, which suggests that high growth rates in sauropods were made possible by high metabolic rates (Sander 2000; Sander et al. 2004; Sander & Clauss 2008).

It must be noted that high metabolic rates are not synonymous with an endothermic physiology. Endothermy is the ability of some creatures to control their body temperatures through internal means. Classically, endothermy is assumed to be an adaptation to sustained vigorous activity and the necessary increase in basal metabolic rates (Heinrich 1977; Bennet and Ruben 1979; Pough 1980). Farmer (2000, 2001), however, suggested that endothermy may have evolved as a consequence of the selective advantage of extensive parental care, because it enables a parent to control incubation temperature. Sander & Clauss (2008) suggested yet another adaptive value of endothermy, the ability to evolve large body size (via fast growth). Ectothermic animals with a low basal metabolic rate appear to be constrained in body size because they cannot take up energy fast enough from the environment to allow high growth rates (Makarieva et al. 2005; Head et al. 2009) which in turn are necessary to reach a large adult body size because of the increasing odds of surviving in the wild as a juvenile (Dunham et al. 1989).

1.3 The origin of fibrolamellar bone in dinosaurs

Early important bone histological studies that noted FLB in dinosaurs were those by Enlow and Brown (1957) and de Ricqles (1968). Further development of the field has provided a more quantitative approach to determine the growth rates, life history, physiology, and evolution of sauropodomorphs (Chinsamy 1993; Rimblot-Baly et al. 1995; Curry 1999; Sander 2000; Erickson et al. 2001; Sander et al. 2004; Sander et al. 2006; Lehman and Woodward 2008, Klein & Sander 2008, Klein et al. 2009; Sander et al. 2010) and other dinosaurs (Horner et al. 2000; Erickson and Tumanova 2000; Horner et al. 2001; Starck and Chinsamy 2002; Erickson et al. 2004; Horner and Padian 2004; Chinsamy-Turan 2005; Erickson et al. 2006; Erickson et al. 2007; Lehman 2007; Cooper et al. 2008; Lee and Werning 2008; Varicchio et al. 2008; Erickson et al. 2009; Knoll et al. 2009; Redelstorff and Sander 2009).

Most dinosaurs show growth marks, usually zones, annuli or lines of arrested growth (LAGs) that interrupt the FLB of the cortex at regular intervals. These have been interpreted as representing periods of temporary complete, or near-complete, cessation of appositional growth. LAGs represent the external surface of the bone at the time they were formed. LAGs also occur in mammalian FLB, and their presence in dinosaurs thus is not inconsistent with a high basal (or resting) metabolic rate (Sander & Andrassy 2006).

Plateosaurus is a prosauropod from the Norian of Germany and Switzerland, and has a long bone cortex with zones of a fast growing fibrolamellar matrix, interrupted by annuli with slow growing lamellar bone that also contain one or several growth marks (Fig 2b.; Sander and Klein 2005; Klein and Sander 2007). The long bone histology of the prosauropod *Massospondylus carinatus* is similar to *Plateosaurus*, with a fibrolamellar long bone cortex interrupted with several growth marks (Chinsamy 1993). *Thecodontosaurus* bone histology (Fig 2a.; Riqcles et al. 2008; Cherry 2002) also displays a similar pattern, with reticular fibrolamellar bone with LAGs.

More recently, an early appearance of FLB with dense vascularization, and few LAGs was described for the Upper Triassic *Silesaurus opolensis* (Fostowicz-Frelik and Sulej, 2009). *Silesaurus* is a member of the Silesauridae (Fig 4), which contains taxa like *Asilisaurus*, *Pseudolagosuchus*, *Sacisaurus* and *Eucoelophysis* (Nesbitt et al. 2010). Ferigolo and Langer (2007) suggested a basal ornitischian relationship of Silesauridae, but Nesbitt et al. (2010) place them as a dinosaur sistergroup. The members of this group all have a slender and agile body plan, suggesting an active lifestyle. The presence of FLB in *Silesaurus* furthermore suggests that these animals had relatively high growth rates and metabolic rates. Moreover, it also suggests that FLB had already evolved in basal dinosaurs.

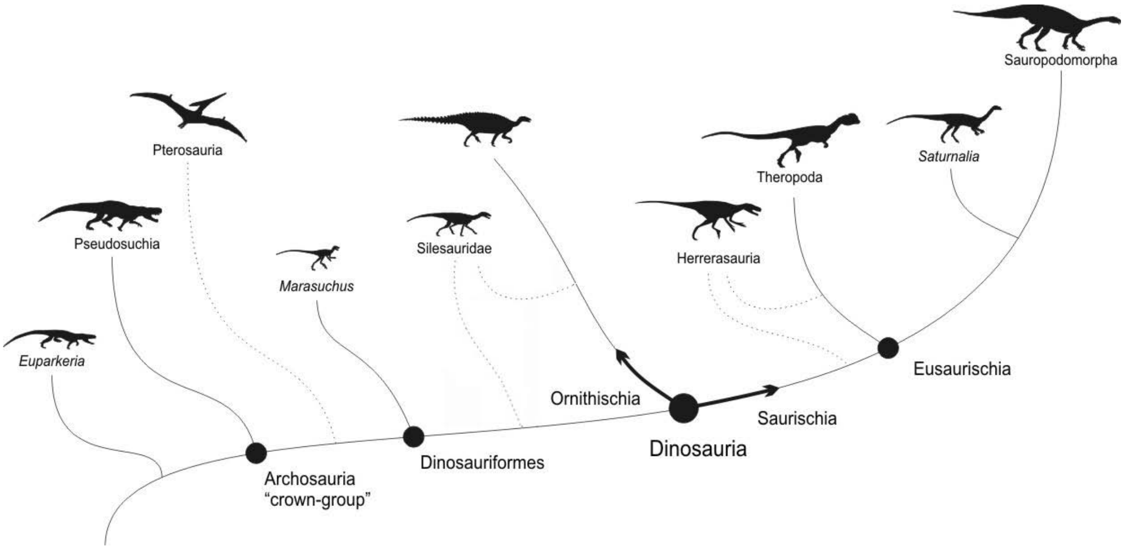

Fig. 17. Archosaur relationships. Note the position of Saturnalia and Silesauridae. Image kindly provided by Max Langer.

The precise origin and evolution of FLB in Archosauromorpha (Archosauria plus stem group representatives such as rhynchosaurs and protorosaurs) has not been studied in great detail. Disagreements about archosauromorph phylogeny over the last decades have made this particularly difficult (Benton and Clark 1988; Benton 1999; Bennett 1996; Gauthier 1986; Juul 1994; Sereno and Arcucci 1990; Sereno 1991). In recent years, however, non-crurotarsan archosauromorph phylogeny is coming to a consensus (Benton 2004; Dilkes and Sues 2009), allowing a phylogenetic approach to comparing bone histologies.

Ricqles et al. (2003, 2008) studied the bone histology of various archosauromorphs, but did not reach a final conclusion. They sampled a wide taxonomic range, albeit with large gaps in the phylogeny. Their major findings are that basal archosauromorphs have lamellar zonal bone (LZB) which is plesiomorphic for amniotes, that at least one basal archosaur

(*Erythrosuchus*) had FLB (as noted by Gross 1934), a basal crurotarsan (*Gracilisuchus*) had also FLB while all other members of the Crurotarsi, including modern crocodiles, have LZB. On the Avemetarsalia branch, at least pterosaurs and dinosaurs have FLB, with coverage of the other groups being poor. This led to three possible hypotheses on the origins of FLB in the Archosauromorpha.

1. FLB is plesiomorphic for Archosauria, but most crurotarsan archosaurs reverted to LZB.

2. LZB is plesiomorphic for Archosauria and FLB evolved three times, in *Erythrosuchus*, in the last common ancestor of pterosaurs and dinosaurs, and in *Gracilisuchus*.

3. The condition varied, and FLB evolved several times in early archosauromorphs.

Unfortunately, because of poor control of sample location on the bony elements, ontogenetic age of the specimen, and due to problems with outdated taxonomic identifications and changing or uncertain phylogenies, Riqcles et al. (2003; 2008) could not falsify either hypothesis. However, new unpublished data suggest fibrolamellar bone may have evolved in the earliest archosauromorphs (Sarah Werning pers. comm.). Therefore, it is more than likely that fibrolamellar bone was already present in the earliest dinosaurs, and by consequence sauropodomorphs. We therefore hypothesized that *Saturnalia* also has fibrolamellar bone in its long bone cortex, probably with growth interruptions.

1.4 Osteocytes and the nature of fibrolamellar bone

Osteocytes are the main cells in bone tissue (Fig 5.). They are osteoblasts that become incorporated into the bone matrix during bone growth (for a review see Franz-Odendaal et al. 2006). Osteocytes are accommodated in lacunae, and their cytoplasmic extensions run through canaliculi (Fig 5.). These canalicular interconnections allow osteocytes to exchange nutrients, waste products, and other chemicals to maintain bone health. Osteocytes may provide us with many insights into bone biology, life history and evolution of extant taxa. In fossil taxa, where the organic material has decayed, osteocytes can still be studied by their lacunae. However, osteocyte lacunae have only been subject of a limited number of studies in extinct vertebrates.

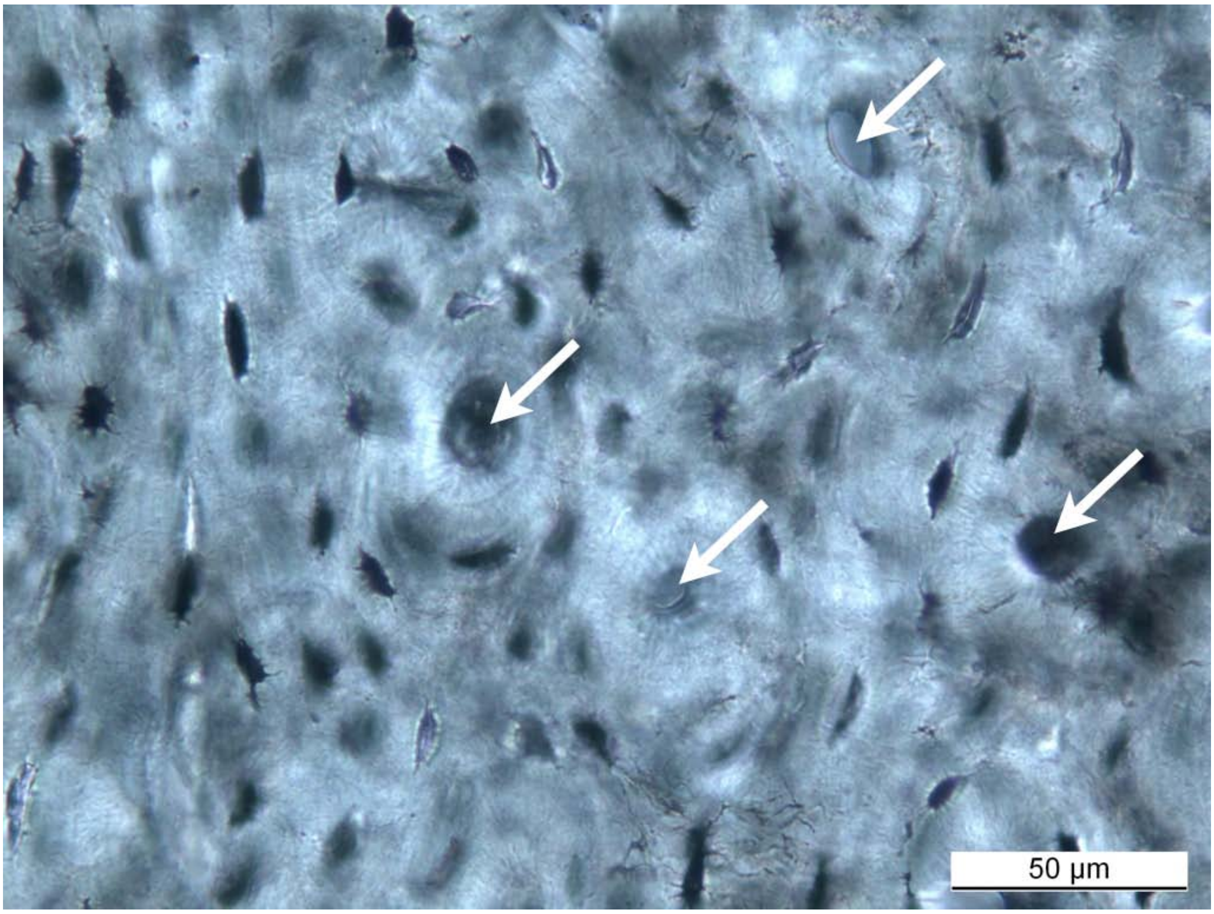

Fig. 18. Osteocyte lacunae in *Cavia porcellus* primary cortical woven bone. Arrows indicate vascular canals. Note the fine capillaries visibly extending from some of the lacunae, these are the canaliculi.

Organ et al. (2007; 2009, see Chapter 3) found that sauropodomorphs, and saurischian dinosaurs (including birds) in general, have much smaller osteocyte lacunae than mammals. Based on a correlation between genome size and cell lacuna size (Organ et al. 2007), Organ et al. (2009) concluded that this is an indication for generally smaller genomes in dinosaurs than in mammals. Thus, because sauropodomorphs belong to a group where small genome size is linked to a high metabolic rate (Gregory 2001; Gregory 2002), they may have had high metabolic rates. However, Organ et al. (2009) refrained from speculating on sauropodomorph metabolic rates, as sauropodomorph genome sizes lie withing the range of many ectothermic lizards and palaeognath birds.

Osteocyte lacuna density (OLD) is another feature that can provide important data about bone cell proliferation, physiology and life history of vertebrate taxa (Franz-Odendaal et al. 2006; Bromage et al. 2009). It has been the focus of several studies in humans (e.g. Mullender et al. 1996a; Vashishth et al. 2000; Parfitt 2002; Qiu et al. 2002), and other mammals (Canè et al. 1982; Mullender et al. 1996b; Hernandez et al. 2004; Skedros et al. 2005; Ma et al. 2008; Bromage et al. 2009). Because lacunar density is a feature that can be measured relatively easily in fossil bone, it is surprising that no comparative data are known for extinct vertebrates.

Bromage et al. (2009) suggested that OLD reflects the rate of osteoblast proliferation, transformation, and incorporation into bone as osteocytes during growth. Lacunar densities should therefore be higher in animals with rapid growth, small body mass, and whose osteoblast proliferation rates would lead to higher osteocyte lacuna densities. Bromage et al. (2009) found an allometric function of the form $OLD=aM^b$ with a negative exponent with OLD the osteocyte density in lamellar bone and M body mass in mammals, indicating a decrease in OLD with increasing body mass. Mullender et al. (1996b) also found a similar relationship for the osteocyte lacuna densities within the cancellous bone tissues of the proximal femur in five mammals.

In summary, high OLD indicates high cell proliferation rates and high local apposition and metabolic rates. On the other hand, cell size may also influence metabolic output. In certain tetrapod clades, smaller osteocytes indicate a higher metabolic rate (Vinogradov and Anatskaya 2006). Therefore, given the presence of fibrolamellar bone, high growth rates and assumed high basal metabolic rates of sauropodomorphs (and dinosaurs in general), we hypothesized that *Saturnalia* and other sauropodomorphs exhibit high OLD, and that OLD will decrease with body mass in Sauropodomorpha. This because small taxa like *Saturnalia* exhibit higher local apposition rates than large sauropods. Although overal increase in absolute body size in sauropods may be larger than in small sauropodomorphs, the local mitotic rates of the osteoblasts will be higher in these smaller taxa, similar to mammals. However, given the comparable growth rates of sauropodomorphs and mammals (Lehmann and Woodward 2008; Case 1978), and generally smaller cell sizes of sauropodomorphs compared to mammals, we also hypothesized that sauropodomorphs have higher OLD than mammals. The aim of this study is thus to understand the difference in the nature of fibrolamellar bone in mammals and sauropodomorphs, and how sauropodomorph bone tissue was organized on a cellular level.

Institutional abbreviations: MCP (Museu de Ciências e Tecnologia PUCRS, Porto Alegre, Brazil), IFG (Institut für Geowissenschaften, Universität Tübingen, Germany), IPB (Institut für Paläontologie, Bonn, Germany), DFMMh/FV (Dinosaurier-Freilichtmuseum

Münchehagen/Verein zur Förderung der Niedersächsischen Paläontologie (e.V.), Germany), SMA (Saurier Museum Aathal, Switzerland), NMB (Naturhistorisches Museum Braunschweig, Germany), BYU (Earth Sciences Museum, Bringham Young University, Provo, Utah), NHUB (Naturkundemuseum of the Humboldt-Universität Berlin, Germany).

2. Materials and Methods

2.1 Saturnalia bone histology

We took a sample of the right femur of the paratype of *Saturnalia*, MCPV 3846 (Fig 3). The femur is not complete, and the sampled region only preserves the caudal part of the bone shaft. This region was chosen nonetheless, as it has the smallest shaft circumference, and hence the most complete cortical growth record. The specimen was sent per airmail from its repository institution in São Paolo to our lab in Bonn, where it was embedded and thin sectioned using standard techniques (e.g. Wells 1989). The specimen was then analysed under polarized light with a Leica DMLP polarising microscope. All images and composite images were made with a Leica DFC firecam and ImageAccess easylab software.

2.2 Osteocyte lacuna density analysis

We took samples from the outer third of the bone cortex of the midshaft femur of six sauropodomorphs (*Saturnalia tupiniquim*; *Thecodontosaurus*; *Europasaurus*; *Apatosaurus*; *Spinophorosaurus, Brachiosaurus, Alamosaurus and Barosaurus*), two extant mammal taxa (*Cavia porcellus*; *Elephas maximus*), an extant amphibian (*Rana temporaria*) and an alligator (*Alligator mississippiensis*) from the thin section collection at the Steinmann Institut in Bonn (sampled specimens are listed in table 1). In all samples except the frogand alligator, which have lamellar-zonal bone, osteocyte density was measured in the woven bone matrix of the FLB. We measured osteocyte lacunar density in woven bone in the outer third of the cortex, because in this location the proportion of woven bone matrix of the FLB in most sauropodomorphs is larger than that of lamellar bone. A sample site without cracks, diagenetic alteration, and if possible, vascular canals, is therefore easier to locate. Hernandez et al. (2004) suggested that osteocyte lacuna density of lamellar cortical bone did not differ from that of periosteal woven bone in the rat. However, this feature may be different in other taxa. We therefore measured the woven bone osteocyte lacuna density in two other mammals, to test whether woven bone data can be compared with lamellar bone lacunar densities in Bromage et al. (2009). We chose to measure OLD in woven bone, as osteocyte lacunae are larger thanin lamellar bone, and hence much more readily identifiable and easier to count. Additionally, some specimens may have very few lamellar bone infilling in the outer cortex, making count a significant number of osteocytes difficult.

We measured osteocyte lacuna density in woven bone of two mammal taxa, a guinea pig (*Cavia porcellus*) and Asian elephant (*Elephas maximus*), and compared them with lamellar bone osteocyte densities of other mammals. Two bradymetabolic tetrapods, the European frog (*Rana temporaria*) and American alligator (*Alligator mississippiensis*) were also included in the analysis. Osteocyte lacuna densities of measured taxa are listed in table 1. Densities were plotted versus body masses in a ln-ln plot (Fig 7.).

	body mass (kg)	body mass source	osteocyte density (#/mm^3)	osteocyte density source	c-value (pg)	C-value source	old*C-value
Rattus norvegicus **(adult rat)**	0.3	A.	58000	a.	3.36	D.	194880
Phanourios minutus **(adult pygmy hippo)**	200	A.	23641	a.	-	-	-
Hippopotamus amphibius **(adult hippo)**	2000	A.	16667	A.	-	-	-
Cavia porcellus **(adult Guinea pig)**	0.7	original data	36190	original data	3.84	D.	138788,65
Elephas maximus **(adult elephant female)**	3000	original data	19264	original data	4.03	D.	77633.92
Galago moholi **(adult)**	0.244	A.	51724	A.	-	-	-
Cheirogales major **(adult)**	0.4	A.	31526	A.	-	-	-
Otolemur crassicaudatus **(adult)**	1.15	A.	44353	A.	3.98	D.	176524.94
Chlorocebus aethiops **(adult female)**	3.515	A.	32012	A.	4.00	D.	128112.02
Macaca mulatta **(adult macaque)**	3	A.	22222	A.	3.37	D.	74777.03
Pan troglodytes **(ca. 6y female)**	33.7	A.	18706	A.	3.68	D.	68744.55
Homo sapiens **(adult female)**	62	A.	20444	A.	3.50	D.	71554
Rana temporaria **(adult common European frog)**	0.039	original data	13828	original data	4.26	D.	58948.76
Alligator missisippiensis **(adult SMNS10481 femur)**	100	original data	9064	original data	2.49	D.	22569.36
Saturnalia tupiniquim	20	original data	53432	original data	1.94	E.	103658.08

(MCPV3846 femur)							
Thecodontosaurus antiquus **(IPB?tibia, adult)**	25	B.	47611	original data	1.87	E.	89032.57
Europasaurus holgeri **(DFMMh/FV415 femur, adult)**	690	original data	39386	original data	2.13	E.	83892.18
Apatosaurus **(SMAJaques, 164cm femur, adult)**	8000	C.	33202	original data	2.31	E.	76696.62
Spinophorosaurus **(NMB1698-R, 115cm humerus, adult)**	11000	U. Joger pers. comm.	27392	original data	-	-	-
Brachiosaurus **(BYU725-17336, 175cm humerus, adult)**	19000	C.	21923	original data	-	-	-
Brachiosaurus **(NHUBdd452, 135cm femur, subadult)**	10000	C.	35647	original data	-	-	-
Alamosaurus **(TMM43090-1, 130cm humerus, adult)**	16000	C.	26246	original data	-	-	-
Barosaurus **(XVI 5, 79cm femur, juvenile)**	1500	C.	45480	original data	1.77	E.	80499.60

Table 1: Body mass, osteocyte lacuna density and genome size data for selected mammal and sauropodomorph taxa. Body masses of *Saturnalia* and *Europasaurus* were estimated with the method of Alexander (1989). Sources: A. Bromage et al. 2009; B. Seebacher 2001; C. Paul 2010; D. Gregory 2010; E. Organ et al. 2009.

The method for measuring lacunar densities used here is similar to that of Bromage et al. (2009), albeit with a less sophisticated image processing technique. Using the Leica DMLP microscope at 40x magnification, a 257-µm wide by 192 µm high XY field of view was located in each specimen. Once an XY field was selected, a z-stack with an image spacing of about 5 µm was aquired. The through series z-stack of images from each 5 µm focal plane was acquired using the Leica FireCam and Imageaccess software, until the final focus plane had been reached. Each focal plane image was printed on paper, lacunae in each image were identified, and drawn on a sheet of overhead transperency film. For each sample, the identified lacunae on the transparent sheet were then manually counted.

Thickness of the thin sections was determined with a standard microscopic procedure. The sample was brought in focus on the upper surface of the epoxy resin. The stage was then lowered until the lower scratched surface of the epoxy resin was in focus. The difference in stage height setting, as read off the fine focus dial, was multiplied with the refractive index of the resin. This thickness was then multiplied with the surface area of the sample, corrected for any blood vessels, to obtain the total volume of bone. All measurements were then standardised by extrapolation of the number of lacunae per measured volume of bone to a 1 mm^3 unit value.

Femur length would have been a better proxy for body size in this case, as OLD represents local apposition rates, which is a linear measurement. However, because Bromage et al. (2009) only provided body masses for their mammal samples, and body masses of the sampled specimens in this study were readily available from the literature (listed in Table 1), body mass was chosen.

2.3 Statistical evaluation

In order to analyse a potential allometric relationship according to $y = a\ M^b$, data on estimated body mass, OLD and osteocyte density were ln-transformed and subsequently analysed by linear regression analysis using PASW 18.1 (SPSS Inc., Chicago, IL). Results were re-transformed to the allometric form. Differences between mammals and Sauropodomorpha were evaluated by comparing the 95% confidence intervals of the estimated regression parameters.

3. Results

3.1 Saturnalia bone histology

Because in the sectioned sample of femur MCPV 3846 (Fig 6), only the caudal half of the diaphysial cortex is preserved, any variations in histology on the cranial side of the section and the size of the medullary cavity therefore remain unknown. However, a thin lining of endosteal bone creates a sharp margin (cementing line) at the transition of the cortex and the medullary cavity (Fig 6a,b). It consists of typical lamellar bone (Fig 6e), and suggests that the medullary cavity was hollow in this region of the bone. The endosteal bone tapers towards the lateral side, indicating it probably did not line the entire medullary cavity. It is thickest on the medial side, where it truncates a wedge of convolute tissue of primary origin (Fig 6e). This convolute tissue is coarse cancellous bone, and has an endosteal origin. The osteocyte lacunae in this tissue are exceptionally plump and densely packed. The compacta shows a fibrolamellar bone microstructure, and is densely vascularized (Fig 6a,b,d). The vascular canals are mainly organised in a reticular fashion, with more circumferentially oriented canals on the mediocaudal side, and more longitudinal canals towards the lateral side of the section (Fig 6f). Two annuli with lamellar bone and few longitudinal vascular canals can be seen in the inner cortex (Fig 6a,b,c). Both annuli show one major and at least 3 less pronounced lines of arrested growth. The second annulus is less pronounced, and much thinner than the first. No signs of resorption or secondary remodelling are present in the cortical bone. The sample of *Saturnalia* studied by us lacks an external fundamental system (EFS, outer circumferential lamellae *sensu* Ham 1953). The absence of an EFS and some newly formed vascular canals at the periosteal edge of the cortex suggest that the sampled individual was still actively growing.

3.2 Osteocyte lacuna density

Woven bone OLD of the Guinea pig and the elephant fall on the regression of lamellar bone OLD for mammals (Fig 7a). We therefore assume that OLD in lamellar and woven bone in the outer long bone cortex do not differ significantly, as suggested by Hernandez et al. (2004). Lacuna density in the lamellar-zonal bone of the frog is expectedly low. In Sauropodomorpha, OLD correlates with body mass according to

$$\text{OLD} = 72330\ (95\%\text{CI } 48874\text{-}107044)\ \text{BM}^{-0.096\ (95\%\text{CI } -0.146\ -\ -0.046)}$$

$$(R^2=0.712\ p=0.003)$$

A comparison to the regression for mammals with

$$\text{OLD} = 36461\ (95\%\text{CI } 30242\text{-}43958)\ \text{BM}^{-0.105\ (95\%\text{CI } -0.153\ -\ -0.058)}$$

$$(R^2=0.710\ p=0.001)$$

reveals that the salings exponents are very similar between both groups with widely overlapping confidence intervals. However, the confidence intervals for the factor a are significantly different, and therefore, the hypothesis that the scaling is identical in sauropodomorphs and mammals can be rejected.

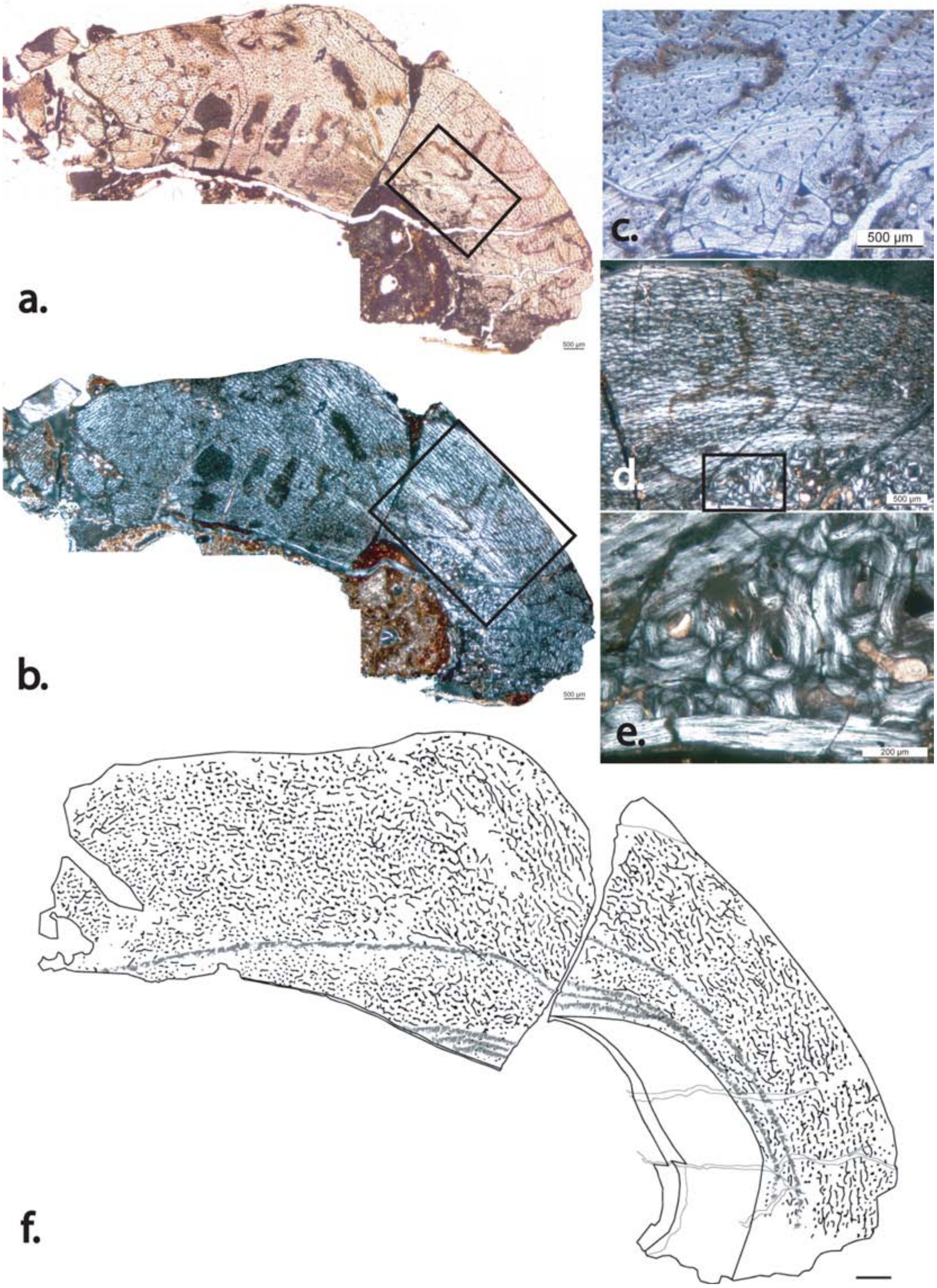

Fig. 19. Long bone histology of *Saturnalia* MCPV 3846. a. composite image under normal polarized light view. b. Composite image under crossed polars view. The cortex shows a fibrolamellar tissue and reticular vascularization. Note the presence of two annuli, and absence of an EFS, or an EFS like feature. c. Magnification of boxed area in a. Note the lines of arrested growth in the annuli. d. Magnification of boxed area in b. e. Magnification of boxed area in d. Note the cementing line between the coarse cancellous bone and endosteal bone. These tissues were probably deposited during medullary cavity drift. f. Interpretative drawing of the section figured in a. and b., with vascular canals, annuli coarse cancellous bone and endosteal bone as most prominent features.

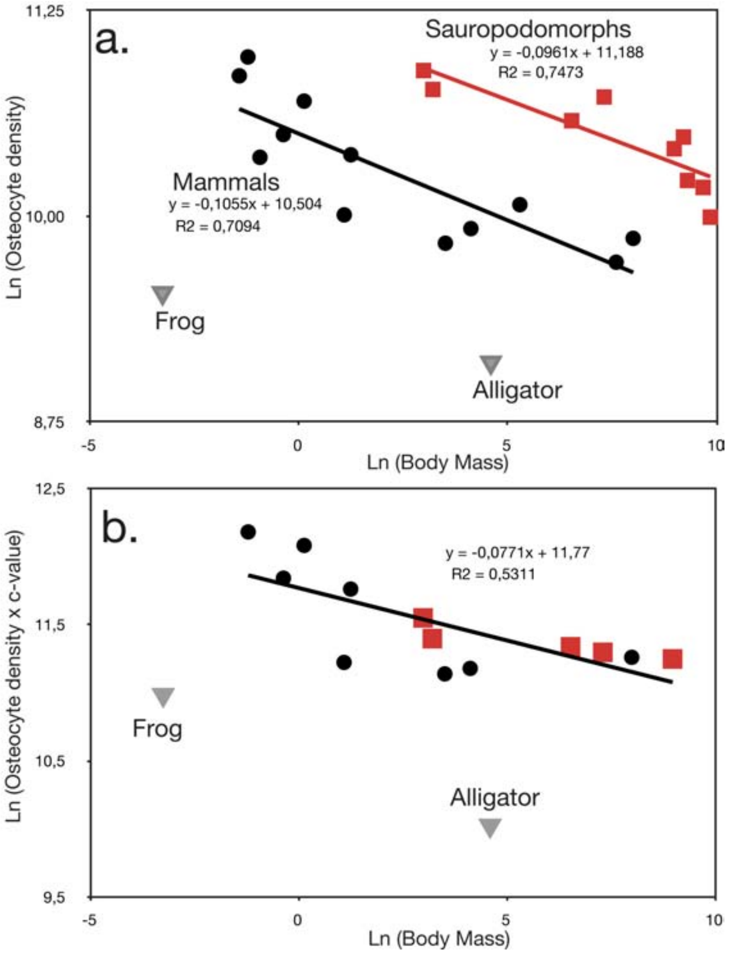

Fig. 20. a. Ln-ln plot of body mass versus osteocyte density in mammals and sauropodomorphs. A frog sample was added as a poikilotherm representative. R2 values indicate good correlation in both groups. Sauropodomorph osteocyte lacuna density is 125% higher than that of mammals of the same weight. b. Ln-ln plot of body mass versus osteocyte lacuna density (OLD) x genome size (c-value). Genome size was used as a cell size proxy, because of the lack of mammal osteocyte lacuna size data (for data sources see table 1). Inclusion of cell size proxy reveals a common trend among mammals and sauropodomorphs. The poikilotherm frog and alligator do not follow this trend.

The discrete character of the regression lines probably reflects another variable, related to cell size (cf. Organ et al. 2009). This suggestion is based on the idea that not simply the number of cells per unit volume of bone reflects the tissue’s growth rate, but rather the total of matrix-producing volume of cell matter per unit volume of bone. Many small cells may give a similar or even more productive result as fewer large cells (Hawkeye et al. 1991; Kuramoto 1981).

To evaluate this hypothesis, data on osteocyte lacunae size are required. Although lacuna size measurements are readily available for selected sauropodomorph taxa (Organ et al. 2009, Chapter 3), Bromage et al. (2009) unfortunately did not provide size measurements for the lacuna of the taxa in their analysis. We therefore used genome size data as a proxy for cell size (Organ et al. 2009 for Sauropodomorpha; Gregory 2010 for mammals, see table 1). We multiplied the available genome sizes (in pg) with the osteocyte densities, which gave us a proxy for the total volume of lacunae in each sample. These volume measures were plotted against body mass in a log-log plot (Fig 7b). Regression using all data results in a scaling of

$$\text{OLD x C-value} = 129185\ (95\%\text{CI } 102847\text{-}162430)\ \text{BM}^{-0.077\ (95\%\text{CI } -0.125\ -\ -0.029)}$$

$$(R^2=0.529\ p=0.005)$$

Although these data are too preliminary to corroborate the assumption that Sauropodomorpha and mammals are similar in their scaling of osteocyte volume (Fig 7b), they do suggest that total volume of bone producing cells is a better measure for local bone apposition rate than either osteocyte size or osteocyte density.

4. Discussion

4.1 Saturnalia and sauropodomorph long bone histology

Saturnalia bone histology (Fig 6) demonstrates the oldest and phylogenetically most basal record of FLB in Sauropodomorpha and one of the oldest records of FLB in dinosaurs in general. This suggests that FLB is plesiomorphic for Saurischia and perhaps even for dinosaurs in general. Moreover, other early dinosaurs, like *Herrerasaurus* and *Lesothosaurus*

(Ricqles et al. 2003; Ricqles et al. 2008) also show similar FLB tissues. The presence of FLB in silesaurids furthermore suggests a plesiomorphic condition of FLB in Dinosauria (Fostowicz-Frelik et al. 2009, Sarah Werning Pers. Comm.). FLB was one of the key innovations in sauropod gigantism (Sander and Clauss 2008, Sander et al. 2010), and therefore a preadaptation for the growth rate acceleration in Sauropoda. The annuli in *Saturnalia* indicate that, like prosauropods, it underwent growth interruptions, which were most likely seasonal. Such growth interruptions were lost in sauropod long bones because of their continuous growth probably resulting from an increase in bone apposition rate (Sander 2000, Sander et al. 2004, Klein and Sander 2008).

Endosteal bone is not uncommon in basal sauropodomorphs, other dinosaurs, and dinosauromorphs (Klein and Sander 2007; Chinsamy 1993; Erickson et al. 2000; Fostowicz-Frelik and Sulej 2009). Reid (1997) found that it is formed in marrow-filled limb bones after active growth has stopped, or is formed and resorbed during pauses in medullary cavity expansion. It may also result from drift to one side of the bone (Francillon-Vieillot 1990, Reid 1997). A different kind of endosteal bone is homologous to avian medullary bone, and hence an indication of sexual maturity, and female sex (Schweizer et al. 2005; Lee and Werning 2008). However, the lamellar organisation of the endosteal bone in *Saturnalia* suggests it was deposited rather slow, making a homology with medullary bone rather unlikely. Additionally, some authors suggested this tissue can be pathological in nature and cautioned for overinterpretations (Chinsamy and Tumarkin-Deriatzan 2009). The location of the endosteal wedge on the medial side, together with coarse cancellous bone, makes a deposition during medullary cavity drift (cf. Reid 1997) the most likely interpretation.

The well vascularized cortical bone of *Saturnalia* suggests that it was deposited at a high rate. Cortical bone in *Saturnalia* shows a reticular vascular orientation, with some circumferential anastomoses. In larger sauropodomorph taxa, vascularization becomes more laminar (circumferential orientation), with a culmination of this trend in Neosauropoda (Sander 2000; Klein and Sander 2007). Neosauropod fibrolamellar bone tissue is highly organized into laminae (laminar bone) (Sander 2000; Klein and Sander 2008). In the ontogeny of neosauropods, a similar phenomenon takes place: juveniles show a reticular vascularization, which becomes more laminar as they grow large (Klein and Sander 2008). This apparent correlation between body size and vascular orientation suggests that vascular canal orientation is determined by a trade-off between high growth rates and biomechanical requirements (cf. Margerie et al. 2004).

4.2 Osteocyte lacuna density correlates with body mass in Sauropodomorpha

The observation that for a given body mass, sauropodomorphs have one fourth more lacuna per unit volume of bone than mammals, requires further explanation. Assuming that osteocyte density reflects local bone apposition rates (Bromage et al. 2009), this suggests that sauropodomorphs had much higher bone apposition and somatic growth rates than mammals. Known data of sauropodomorph and mammal growth rates (Case 1978; Case 1979; Erickson et al. 2001; Lehman and Woodward 2008) make this highly unlikely. The fibrolamellar bone in sauropodomorph and mammal long bone cortices shows an almost identical microstructure, but on the cellular level, these tissues show a marked difference. Although fibrolamellar bone in both clades indicates high growth rates, two different strategies were used to attain these high rates. Sauropodomorphs had many small osteocytes, whereas mammals generally have larger, but fewer osteocytes. These diverging strategies support the view that fibrolamellar bone has evolved convergently in these lineages. Unfortunately, no OLD data exist for modern birds. Therefore, this database needs to be extended, and OLD needs to be measured for theropods, including birds to see if the mammal-dinosaur dichotomy holds up.

The importance of osteocyte size for bone apposition rate may explain why there is no significant difference between densities in lamellar bone and woven bone in mammals (cf. Hernandez et al. 2004). Lacunae in lamellar bone are typically more flattened than the plump lacunae in woven bone (Francillon-Viellot et al. 1990). This observation has previously been interpreted as a result of fast growth in woven bone, and slow growth in lamellar bone (Amprino 1947; Francillon-Viellot et al. 1990), even though cell density in these tissues apparently does not differ much (Hernandez et al. 2004; this study). A more extensive database, including extant birds and reptiles with known basal metabolic rates is needed to test the significance of the relationship between osteocyte lacuna size and body mass.

More work is also needed to test the hypotheses that osteocyte lacuna densities in woven and lamellar bone do not differ significantly, and that total osteocyte volume per unit volume of bone is correlated with differences in bone deposition rates in these tissues.

5. Conclusion

Fibrolamellar bone in *Saturnalia* indicates that it had a high growth rate and thus that this high growth rate had already evolved at the base of Dinosauria. Furthermore, it supports the hypothesis that fibrolamellar bone had evolved in a common archosaur ancestor of dinosaurs and silesaurs, as suggested by other authors (Ricqles et al. 2008; Fostowicz-Frelik and Sulej 2009).

Our results suggest that there is an apparent scaling of body mass as a function of osteocyte lacuna density in sauropodomorphs, as was previously suggested for mammals. The discrete gap between sauropodomorph and mammal osteocyte lacuna density (mammals of comparable body mass having lower densities) is probably an effect of the difference in lacuna size (mammals having generally larger lacuna than dinosaurs). Although cortical long bone tissue in large mammals and dinosaurs does not differ significantly in structure, our data suggest that on the cellular level, sauropodomorphs used a different strategy to attain high growth rates. Instead of having large bone cells like mammals, sauropodomorphs had a much higher osteoblast density in the periosteal epithelium. These findings support the view that fibrolamellar bone tissue in mammals and sauropodomorphs evolved convergently.

Clearly, the pattern observed in the OLD data presented here needs further testing. More osteocyte lacunae size data need to be collected for extant mammals, and more dinosaur OLD data, including theropods and birds, are needed.

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Chapter 5

Small body size and extreme cortical bone remodeling indicate phyletic dwarfism in *Magyarosaurus dacus* (Sauropoda: Titanosauria).

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Abstract

Sauropods were the largest terrestrial tetrapods (> 10^5 kg) in Earth's history and grew at rates that rival those of extant mammals. *Magyarosaurus dacus*, a titanosaurian sauropod from the Upper Cretaceous (Maastrichtian) of Romania, is known exclusively from small individuals (<10^3 kg) and conflicts with the idea that all sauropods were massive. The diminutive *M. dacus* was a classical example of island dwarfism (phyletic nanism) in dinosaurs, but a recent study suggested that the small Romanian titanosaurs actually represent juveniles of a larger-bodied taxon. Here we present strong histological evidence that *M. dacus* was indeed a dwarf (phyletic nanoid). Bone histological analysis of an ontogenetic series of *Magyarosaurus* limb bones indicates that even the smallest *Magyarosaurus* specimens exhibit a bone microstructure identical to fully mature or old individuals of other sauropod taxa. Comparison of histologies with large-bodied sauropods suggests that *Magyarosaurus* had an extremely reduced growth rate, but had retained high basal metabolic rates typical for sauropods. The uniquely decreased growth rate and diminutive body size in *Magyarosaurus* were adaptations to life on a Cretaceous island and show that sauropod dinosaurs were not exempt from general ecological principles limiting body size.

1. Introduction

1.1 Body size in sauropods

Sauropod dinosaurs were the largest animals that ever roamed the surface of the Earth (Mazetta et al. 2004, Upchurch et al. 2004). Gigantic size was acquired early in the evolutionary history of the group, in the Late Triassic (Buffetaut et al. 2002). Recent studies of bone histology have shown that sauropods attained their gargantuan sizes by an evolutionary increase of their growth rate to levels comparable to those of extant endothermic mammals (Erickson et al. 2001, Sander et al. 2004). However, not all sauropods were multi-tonne animals. Some titanosaurs are known to have had relatively small body sizes by sauropod standards; e.g. the South American *Neuquensaurus australis* reached a body length of about 7-9 m (Salgado et al. 2005, Wilson 2006), and its body mass is estimated at 3500 kg. The recently described basal macronarian *Europasaurus holgeri* from the Late Jurassic of Germany (Sander et al. 2006) was even smaller, with a total estimated adult body length of about 6.2 m and a body mass of 800 kg.

Another small-bodied titanosaurian sauropod, *Magyarosaurus dacus*, is known from the Upper Cretaceous (Maastrichtian) continental formations of the Haţeg Basin of Romania (Weishampel et al. 1991, Grigorescu 1992). These strata contain an array of relatively small-bodied dinosaurs taxa, including the basal hadrosaurid *Telmatosaurus* (Weishampel et al. 1993), and two species of the non-iguanodontian euornithopod *Zalmoxes* (Weishampel et al. 2003). In a famous early evolutionary hypothesis involving dinosaurs, the small body size of these taxa prompted the brilliant Hungarian paleontologist Franz Baron Nopcsa (Appendix 1) to hypothesize that, like Mediterranean dwarf proboscideans (Bate 1905), the Haţeg dinosaurs evolved their diminutive body size on a paleo-island (Nopcsa 1914, Nopcsa 1923). Later, however, rare larger titanosaur bones were recovered from the Haţeg Basin as well and described (Huene 1932) as “*M.*” *hungaricus*.

At present all titanosaur bones from the Hațeg basin are tacitly grouped together as *M. dacus* (Weishampel et al. 1991, Leloeuff 1993). Morphological work by ZC suggests that the larger taxon is different from *M. dacus*, we will hence use the name *M. dacus* to the exclusion of these large specimens. A full redescription, however, is beyond the scope of this paper. Today, *M. dacus* is known form numerous but mostly isolated bones of different-sized individuals, representing a growth series (Fig.1). *Magyarosaurus* has only been incorporated in one phylogenetic analysis (Curry Rogers 2005) in which the position of *Magyarosaurus* is resolved relatively high within the Titanosauria, inside the lithostrotian *Rapetosaurus* clade. This suggests that *Magyarosaurus* is closely related to taxa such as *Rapetosaurus*, *Nemegtosaurus*, *Malawisaurus*, and *Trigonosaurus*. Neither of these taxa show any significant size reduction compared to members of less derived outgroups. Small body size in *M. dacus* would thus represent an autapomorphic feature.

1.2 Morphological and morphometric evidence for a nanoid fauna

Modern work on this classical dinosaur fauna suggests that phylogenetic size reduction (nanism *sensu* Gould and McFadden 2004) through paedomorphosis (Alberch et al. 1979) had occurred in *Telmatosaurus* (Weishampel et al. 1993) and *Zalmoxes robustus* (Weishampel et al. 2003), based on patterns of heterochronic shifts in the morphology and morphometry in these taxa. Similarly, morphometric analysis of a wide range of sauropod humeri indicated that *M. dacus* bones were more similar to the bones of subadult than adult representatives of other, more typical sauropod taxa. These results were considered consistent with the interpretation of *M. dacus* as a heterochronic dwarf (Jianu and Weishampel 1999).

1.3 Alternative hypothesis: small size reflects juvenile status

The co-occurrence of the rare large-bodied titanosaurian elements (*"M." hungaricus* Huene 1932) with *M. dacus* (Leloeuff 2005) and uncertainty about the paleogeographic setting of the Haţeg Basin (Jianu and Boekschoten 1999), has drawn the insular nanism interpretation for *Magyarosaurus* into question. This led to the suggestion that the small titanosaurian remains collected in the Haţeg Basin are not dwarfs at all, but represent merely juveniles of a sauropod with a more typical, massive adult body size (Leloeuff 2005) such as "*M.*" *hungaricus*. Historically, the ontogenetic status of dinosaurs has been difficult to resolve based on bone morphology alone because, unlike mammalian long bones, dinosaur long bones lack morphological indicators of full size having been attained. However, fossil bone histology has evolved into a powerful tool for detecting the ontogenetic status of non-mammalian tetrapods (for reviews see Erickson 2005, Chinsamy-Turan 2005, for recent applications see Xu et al. 2006, 2007, 2009). Because sauropod dinosaurs are one of the two histologically best sampled clades of dinosaurs (the other being Theropoda) we employ long bone histology to resolve the controversy surrounding *Magyarosaurus dacus* and test the competing hypotheses of insular nanism vs. juveniles of a large-bodied species. We sampled a growth series of the small *M. dacus* as well as one of the two long bones of "*M.*" *hungaricus,* available for study (Table 1).

1.4 Size and age in dinosaurs

Sauropods as well as theropods (and ornithischian dinosaurs, where sample size is sufficient, e.g. Erickson and Tumanova 2000, Erickson et al. 2009, Lee and Werning 2008) follow a narrow growth trajectory, i.e. they lack developmental plasticity. In sauropods, this is documented by a close correlation between histologic ontogenetic stage (HOS) and body size (Curry 1999, Sander 1999, Sander 2000, Klein and Sander 2008, Lehman and Woodward

2008, Klein et al. 2009, Woodward and Lehman 2009). In theropods, that commonly show good quantifiable growth records, growth curves vary little between individuals (Erickson et al. 2004, Erickson et al. 2006, Erickson et al. 2007, Bybee et al. 2006, Horner and Padian 2004, Cooper et al. 2008, Varicchio et al. 2008). This indicates that dinosaurs, just like mammals, showed little intraspecific variation in asymptotic body size. Hence large differences in adult size, in otherwise morphologically similar fossils, suggests that they represent different biological species.

2. Materials and Methods

2.1 Materials

Since Nopcsa's time much new material has been recovered, and *M. dacus* is now known from numerous small-sized long bones and vertebrae. We sampled limb bone material (humeri, ulnae, femora, tibiae, fibulae, Table 1, Fig. 1) from the collections at the Faculty of Geology and Geophysics of the University of Bucharest, Romania (FGGUB) and the Geological Survey of Hungary in Budapest (MAFI). 21 specimens were sampled, representing 19 *M. dacus* individuals, and one *"M." hungaricus*. The humeral growth series of the diminutive *M. dacus* covers a size range from approximately 22 cm to 49 cm in humerus length, while the large specimens are twice this large, with a sampled "*M.*" *hungaricus* humerus having an estimated length of 91 cm. For comparative purposes, we also sampled 5 individuals of *Alamosaurus sanjuanensis* to augment previous data (Woodward and Lehman 2009). We sampled the specimens with a histological coring technique (Sander 2000, Stein and Sander 2009). Samples were processed into thin sections, which were then studied histologically under a Leica DMLP polarized light microscope. Images were acquired with a Leica DFC420 digital camera and processed with Imagic Imageaccess software.

Specimen	Collection	Locality	Taxon	Bone type	Side	Length (mm)	Minimal shaft circumference (mm)	Percentage maximum size	Standardized length (to humerus)	Standardized percentage maximum size	HOS
R.1220	FGGUB	Groapa	*Magyarosaurus*	femur	r	(346)	176	64	266	54.5	13
R.1511	FGGUB	Groapa	*Magyarosaurus*	femur	l	(466)	179	86	358	73	13
R.1046	FGGUB	Ciula	*Magyarosaurus*	femur	l	525	193	97	403.5	82.5	14
R.1992	FGGUB	Ciula	*Magyarosaurus*	femur	r	(540)	195	100	414.5	85	14
Ob.3092	MAFI	Vălioara	*Magyarosaurus*	humerus	l	(222)	115	46	222.5	46	12
R.1246	FGGUB	Groapa	*Magyarosaurus*	humerus	r	(320)	122	65.5	320	65.5	14
R.1195	FGGUB	Scoaba	Titanosauria indet. (?*Magyarosaurus)*	humerus	l	(346)	150	71	346	71	13
Ob.3089	MAFI	Vălioara	*Magyarosaurus*	humerus	l	(365)	136	75	365	75	14
v.13492	MAFI	Vălioara	*Magyarosaurus*	humerus	r	372	140	76	372	76	13
Ob.3128	MAFI	Vălioara	*Magyarosaurus*	humerus	l	(432)	151	88	432	88	14
R.1047	FGGUB	Ciula	*Magyarosaurus*	humerus	r	403	183	82.5	403	82.5	13
R.1048	FGGUB	Sînpetru	*Magyarosaurus*	humerus	l	(488)	194	100	488	100	14
Ob.3104	MAFI	Vălioara - Budurone	*“M.” hungaricus*	humerus	r	(914)	365		914	-	12
R.1252	FGGUB	Groapa	*Magyarosaurus*	tibia	l	(354)	105	79	-	-	12
Ob.4212	MAFI	Vălioara	*Magyarosaurus*	tibia	l	(323)	109	72	-	-	12.5
R.1380	FGGUB	Cărare	*Magyarosaurus*	tibia	l	(402)	134	89	-	-	13
R.1045	FGGUB	unknown	*Magyarosaurus*	tibia	r	450	181	100	-	-	14
Ob.3087	MAFI	Vălioara	*Magyarosaurus*	tibia	l	(858)	260		-	-	14
Ob.3086a	MAFI	Vălioara	*Magyarosaurus*	fibula	l	(388)	100		-	-	14
Ob.3086b	MAFI	Vălioara	*Magyarosaurus*	fibula	l	(384)	101		-	-	14
R.1598	FGGUB	Groapa	*Magyarosaurus*	ulna	l	(219)	95	65	-	-	14
Ob.3099	MAFI	Vălioara	*Magyarosaurus*	ulna	r	337	128	100	-	-	14

Table 1: List of sampled titanosaur specimens with dimensions (brackets indicate total estimated length), provenance, relative size, and histologic ontogenetic stage (HOS).

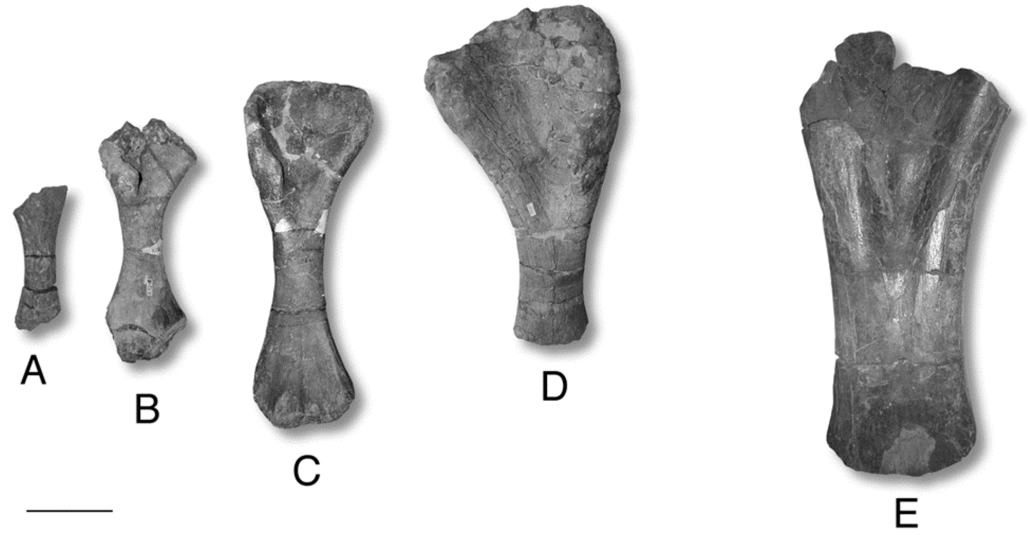

Fig. 1. Photographs of some of the sampled titanosaur bones from the Maastrichtian of Romania. (A-D) *Magyarosaurus dacus* humeri, specimens (A) MAFI Ob. 3092 (the smallest recorded body size, 45% maximum size) (B) FGGUB R.1246 (65% max. size), (C) MAFI v.13492 (76% max. size), (D) FGGUB R.1048 (the largest known specimen). (E) "*Magyarosaurus*" *hungaricus*, MAFI Ob.3104. Scale bar equals 100 mm.

2.2 Mass estimates

Most of the bones were found in isolation and come from a number of different localities within the Hațeg Basin. However, in a few cases associated material allowed the sampling of multiple appendicular elements from the same skeleton (FGGUB R.1046, FGGUB R.1047, FGGUB R.1992). The length of the fragmentary femora was estimated from FGGUB R.1046; the length of fragmentary humeri from FGGUB R.1047; that of the tibiae from FGGUB R.1045; all ulnae and fibulae are virtually complete. We used a bone size estimation method based on identification of morphological landmarks and estimation of the preserved percentage of total length. Size standardization was performed for femora relative to humeral length. The humerus to femur ratio (0.768) is calculated from associated specimens

FGGUB R.1046 and FGGUB R.1047. Unlike in most other studies, the humerus was chosen because it represents the largest subset of our samples and histology is better preserved than in femora. Note, however, that humerus length was scaled to femur length in the HOS diagram (see results, Fig. 4).

The masses of *Neuquensaurus* and *Magyarosaurus* were estimated using an equation for calculating large quadrupedal animal masses based on humerus and femur circumference (Anderson et al. 1985, McNeil Alexander 1989). Humerus and femur data for *Neuquensaurus* were obtained from the literature (Wilson 2006). For *M. dacus*, measurements were directly taken from an associated humerus (FGGUB R.1047) and femur (FGGUB R.1046) (Table 1).

2.3 Aging sauropod long bones using histologic ontogenetic stages

Hundreds of individuals of different ontogenetic stages from close to 20 taxa accross the entire sauropod phylogeny have been sampled so far (for a review see Sander et al. In press). This breadth of sampling has led to the identification of histologic indicators of ontogenetic stage (Curry 1999, Sander 1999, Sander 2000), formalized in the histologic ontogenetic stage (HOS) scheme (Klein and Sander 2008). This scheme allows qualitative ontogenetic comparisons between humeri and femora of different sauropod taxa (Klein et al. 2009, Woodward and Lehman 2009). The previously employed HOS scale ranges from HOS 1, representing embryonic bone, to HOS 13, representing individuals with a completely or almost completely remodeled long bone cortex (Klein and Sander 2008). Histologic ontogenetic stages 12 and 13 are only seen in very old and large sauropod individuals that had lived for many years after reaching asymptotic body size, such as in the 1.58 and 1.76 m femora (BYU 601-17328, OMNH 01991) of *Apatosaurus* (Fig. 3). Some sauropod individuals, however, are characterized by a completely remodeled cortex, displaying successive crosscutting relations of secondary osteons in the outer bone cortex (with the inner and middle cortex displaying this feature anyway, since remodeling progresses from the medullary region outwards (Curry 1999, Sander 1999, Sander 2000, Klein and Sander 2008)). This feature is only seen in the largest and oldest sauropod individuals, such as in a 1.8 m-femur (OMNH 4020) of *Apatosaurus.*

The following tissue types have been recognized previously, the ontogenetic succession of these resulted in a scheme of 13 histologic ontogenetic stages (Klein and Sander 2008, Table 2):

Type A bone tissue is embryonic tissue, with non-laminar organization of vascular canals.

Type B bone tissue is fibrolamellar bone that is dominated by woven or fibrous bone. The vascularization is not laminar but mainly longitudinal, and the density of the vascular canals is very high. The vascular canals are large and essentially circular in cross section, but with an irregular margin, similar to small erosion cavities of the remodeling zone. Type B bone tissue normally has no true primary osteons developed and only a thin sheath of lamellar bone lines the vascular canals, indicating that primary osteon formation has started. No secondary osteons or growth marks are developed in the type B bone tissue.

Type C bone tissue consists of a primarily laminar fibrolamellar bone with a still very high vascular canal density. The type C bone tissue usually also starts with longitudinal vascular canals that later grade into vascular canals with a more circumferential appearance. However, longitudinal vascular canals in type C bone tissue are easy to distinguish from those in type B bone tissue by a more regularly round margin compared to the irregular longitudinal vascular canals in type B bone tissue and an already laminar organization even of the longitudinal vascular canals.

The transition from type C bone tissue to **type D bone tissue** is also gradual and not abrupt. The change is again indicated by the increase in lamellar bone in the primary osteons, and the vascular canals in the type D bone tissue have a thick lining of lamellar bone. However, vascularization is still high in type D bone tissue: the vascular canals are large, but smaller compared to that seen in type C bone tissue. Vascularization is primarily laminar, but a few areas have vermiform or more reticular organization. In the type D bone tissue, the formation of secondary osteons starts. Thus, incompletely filled ("young") large secondary osteons are developed mainly between larger erosion cavities of the remodeling zone and are closely associated with the medullary cavity. Growth marks are rather rare in this tissue type.

Type E bone tissue represents a still relatively fast growing tissue. The transition between type D bone tissue and type E bone tissue again is gradual. These bone tissue types, in fact, only differ in organization and degree of vascular density. The vascular spaces in type E bone tissue are still present but are smaller in comparison to type D bone tissue because the thickness of the layer of lamellar bone lining in the vascular canals increases, resulting in very distinctive primary osteons. The secondary osteons between the erosion cavities in the inner cortex are more densely spaced in type E bone tissue. Additionally, the spread of secondary osteons into the primary cortex has started by now, resulting in scattered secondary osteons that may extend in some specimens up to the middle of the primary cortex. Growth marks may occur, but remain rare and are not typical for type E bone tissue.

Type F bone tissue is characterized by a clear decrease in vascularization, finally resulting in a near complete infilling of the primary vascular canals by lamellar bone. In some specimens a change of bone tissue type from fibrolamellar to lamellar-zonal bone and the deposition of an EFS is initiated. The EFS indicates that a growth plateau has been reached.

The vascular canals of primary osteons are more or less completely filled by lamellar bone tissue . Remodeling by secondary osteons has increased significantly, and dense secondary osteons are deposited at least up to the middle to inner two thirds of the primary cortex. In type F bone tissue, growth marks are usually present, including the closely spaced LAGs of the EFS. However, some specimens do not show growth marks or an EFS in the type F bone tissue.

Type G bone tissue is characterized by an almost complete or complete remodeling of the primary cortex by secondary osteons.

Bone tissue type	HOS
Cortex consists of type A bone tissue.	stage 1
Cortex consists primarily of type A bone tissue with type B bone tissue laid down in the outer cortex.	stage 2
Cortex consists primarily of type B bone tissue while in the inner cortex remains of type A bone tissue can be preserved.	stage 3
Cortex consists primarily of type B bone tissue with type C bone tissue laid down in the outer cortex.	stage 4
Cortex consists primarily of type C bone tissue, while in the inner cortex remains of type B bone tissue can be preserved.	stage 5
Cortex consists primarily of type C bone tissue with type D bone tissue laid down in the outer cortex.	stage 6
Cortex consists primarily of type D bone tissue, while in the inner cortex remains of type C bone tissue can be preserved.	stage 7
Cortex consists primarily of type D bone tissue with type E bone tissue laid down in the outer cortex.	stage 8
Cortex consists primarily of type E bone tissue, while in the inner cortex remains of type D bone tissue can be preserved.	stage 9
Cortex consists primarily of type E bone tissue with type F bone tissue laid down in the outer cortex.	stage 10
Cortex consists primarily of type F bone tissue while in the inner cortex remains of type E bone tissue can be preserved.	stage 11
Cortex consists primarily of type F bone tissue while in the outer cortex an EFS is deposited.	stage 12
Cortex consists of type G bone tissue which means it is nearly completely remodeled by secondary osteons.	stage 13
Cortex consists of type H bone tissue, which means that multiple generations of secondary osteons with interstitial laminae are visible in the outer cortex.	stage 14

Table 2: Fourteen histologic ontogenetic stages (HOSs) in sauropod long bones. Thirteen HOSs have been recognized in sauropod long bones by previous study (Klein and Sander 2008), and a fourteenth HOS is added here. There are more HOSs than ontogenetic bone tissue types (type A – H) because in any one growth series there are specimens that preserve

more than one bone tissue type in sequence. The preservation of successive bone tissue types depends on remodeling and resorption activity because strong resorption will result in a relatively thin cortex and strong remodeling activity will obliterate the primary growth record. This is why the HOSs that are based on the transition from one bone tissue type to the next are set up based on this transition occurring in the outer cortex which is least affected by variability in resorption and remodeling.

2.4 A new histologic ontogenetic stage for sauropods

Degrees of remodeling in large sauropod individuals have not been distinguished before (Klein and Sander 2008), but we feel it is necessary to make this distinction for comparative histological purposes, as is done in forensic science (Kerley 1965, Kerley and Ubelakker 1978, Thomas et al. 2000). Therefore, we define a tissue type with a completely remodeled cortex and at least two generations of crosscutting secondary osteons in the outer cortex as tissue type H, representing HOS 14. Although it is tempting to define additional HOSs for every generation of secondary osteons, this is problematic. Eventually, as remodeling continues, it will have obscured earlier generations of secondary osteons, making it impossible to detect the precise number of generations of secondary osteons. The introduction of HOS 14 thus serves to refine the histologic ontogenetic staging of sauropods (Table 2).

3. Results

3.1 Ancestral character state optimisation for body size

We have optimized the ancestral character state for size on the only current phylogeny including *Magyarosaurus dacus* (Curry Rogers 2005). We took femur length as a proxy for body size, as it is readily measurable with relatively high accuracy, or easily obtained from the literature. Mass estimates vary greatly even for the same skeleton depending on method and inherently have large errors. We also added *Europasaurus* to the optimization as a basal macronarian (Sander et al. 2006). Femur length of *Magyarosaurus* was measured on specimen FGGUB R.1046 and femur lengths of other taxa were taken from literature (Sander et al. 2006, Mazzetta et al. 2004, Smith et al. 2001, Carrano 2005, Gomani 2005, Curry Rogers 2009, see Table 3). These lengths were mapped as a continuous character on the phylogeny in TNT (Goloboff et al. 2008). The resulting optimisation (Fig. 2) illustrates the marked size decrease of *Europasaurus* and *Magyarosaurus*, compared to its close relatives. Even though *Magyarosaurus* is part of a clade of generally smaller titanosaurs, its femur length is significantly smaller than that of its sister taxa, indicating an autapomorphic size decrease.

Taxon	**Fem length (mm)**	**Specimen**	**Data source**	**Length class cutoff**	**Length class**
Argentinosaurus	2557	MLP-DP 46-VIII-21-3	Mazetta et al (2004)	0,22	5
Apatosaurus	2500	YPM 1860	Carrano (2005)	0,60	5
Antarctosaurus	2350	MLP 23- 316	Mazetta et al (2004)	**1,26**	5
Paralititan	2054	est by Smith et al. (2001)	Smith et al. (2001)	0,26	4
Brachiosaurus	2000	FMNH P25107	Carrano (2005)	0,45	4
Argyrosaurus	1910	PVL 4628	Carrano (2005)	0,58	4
Camarasaurus	1800	AMNH 5761a	Carrano (2005)	0,86	4
Diplodocus	1645	YPM 1920	Carrano (2005)	0,21	4
Alamosaurus	1610	TMM-HW16	Carrano (2005)	0,37	4
Andesaurus	1550	MUCPv-132	Carrano (2005)	0,32	4
Rapetosaurus	1500	est by Curry Rogers et al (2009)	Curry Rogers et al (2009)	0,70	4
Opisthocoelicaudia	1395	ZPAL MgD-I/48	Carrano (2005)	0,47	4
Janenschia	1330	HMN IX	Carrano (2005)	0,30	4
Aegyptosaurus	1290	BSP 1912 VIII 61	Carrano (2005)	0,31	4
Phuwiangosaurus	1250	P.W. 1-1/1-21	Carrano (2005)	0,24	4
Dicraeosaurus	1220	HMN m	Carrano (2005)	**1,02**	4

Epacthosaurus	1095	UNPSJB-PV 920	Carrano (2005)	**1,28**	3
Euhelopus	955	PMU R234	Carrano (2005)	0,05	2
Malawisaurus	950	Mal-201	Gomani (2005)	0,79	2
Saltasaurus	875	PVL 4017-80	Carrano (2005)	0,83	2
Ampelosaurus	802	MDE uncat. 1	Carrano (2005)	0,04	2
Neuquensaurus	799	MLP-Cs 1094	Carrano (2005)	0,39	2
Rocasaurus	768	MPCA-Pv 56	Carrano (2005)	**1,07**	2
Lirainosaurus	686	MCNA 7468	Carrano (2005)	**2,13**	1
Magyarosaurus	540	FGGUB R.1046	this study	0,56	0
Europasaurus	510	DFMMh/FV415	Sander et al. (2006)	-	0
Agustinia	Unknown				
Aeolosaurus	Unknown				
Trigonosaurus	Unknown				
Jabalpur indet.	Unknown				
Jainosaurus	Unknown				
Malagasy Taxon B	Unknown				
Nemegtosaurus	Unknown				
Quaesitosaurus	Unknown				
Santa Rosa indet.	Unknown				
Isisaurus	Unknown				

Table 3: The femur lengths used in the ancestral character state optimization as a proxy for body size for the taxa included in the phylogenetic tree used in the current analysis (Curry Rogers 2005). The six femur length classes were devised on the basis that the greatest gaps in the length distribution should be used as class boundaries. We used the following procedure to identify these class boundaries: taxa were first ranked by increasing femur length, with the largest femur length available for each taxon being entered, whenever possible. In a pairwise comparison and starting with the largest femur, i.e., that of *Argentinosaurus*, the length of the smaller femur was subtracted from the larger. The difference was then divided by the length of the larger of the two and multiplied by 10. The resulting cutoff values for the successive pairs vary from 0.11 to 2.06, and a new size class was established for cutoff values >1 (printed in bold).

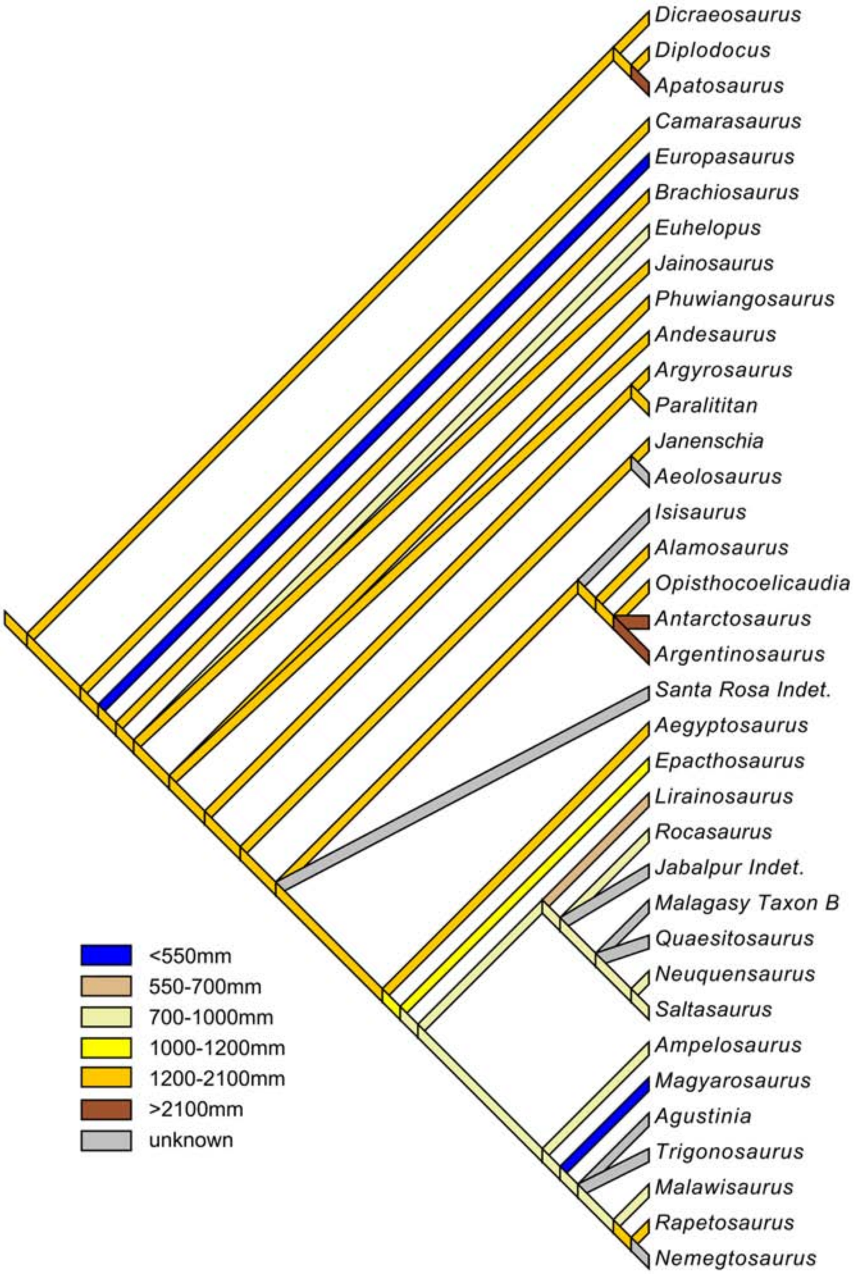

Fig. 2: Femur length as a proxy for body size, mapped on the only phylogeny that includes *Magyarosaurus* (Curry Rogers 2005). *Europasaurus* was inserted as a basal macronarian (Sander et al. 2006). Both *Magyarosaurus* and *Europasaurus* unequivocally illustrate the phenomenon of autapomorphic size decrease. Even though *Magyarosaurus* is part of a generally smaller clade of derived titanosaurs, it is still significantly smaller than its sister taxa. For length class determination see Table 3. Character optimization analysis was performed in TNT (Goloboff et al. 2008).

3.2 M. dacus long bone histology

Like those of other sauropods (Sander 2000, Klein and Sander 2008, Woodward and Lehman 2009, Sander et al. in press), *M. dacus* long bones are characterized by a small medullary cavity and relatively thick cortex (Fig. 3A). The medullary cavity merges into the cortex via cancellous bone that surrounds large erosion cavities. The cancellous bone is secondary in origin, and the erosion cavities become smaller as they grade into the innermost cortex. The cortical bone histology, however, represents a radical departure from that seen in any other sauropod, with the exception of the very largest and oldest of normal-sized sauropods. In all but the smallest individuals of *M. dacus* and irrespective of type of skeletal element, the primary bone of the cortex is completely replaced by dense secondary osteons or Haversian bone (Fig. 3A, B, E, F). The smallest individual (MAFI Ob.3092, less than 46 % the length of FGGUB R.1048, the largest *M. dacus* humerus and 24 % the length of the "*M.*" *hungaricus* humerus) retains primary bone in the outer cortex that, however, is also disrupted by numerous secondary osteons (Fig. 3C, D). This primary bone is of the laminar fibrolamellar type with circumferential vascular canals and primary osteons. Unlike in typical laminar fibrolamellar bone of large mammals and other dinosaurs, the bone matrix between the vascular canals in *M. dacus* consists mostly of parallel-fibered and lamellar bone, with a minimal amount of woven bone. This well-organized primary bone matrix suggests that primary bone deposition rates were relatively slow (Francillon-Vieillot et al. 1990, Castanet et al. 2000, Margerie et al. 2002), though the bone retained the extensive network of vascular canals typical of fibrolamellar bone seen in other sauropods and fast-growing extant vertebrates (Castanet et al. 2000, Margerie et al. 2002, Margerie et al. 2004).

An external fundamental system (EFS, outer circumferential lamellae *sensu* Ham 1953) was not observed in any of the *Magyarosaurus dacus* individuals in this study. In the smallest individuals, those that retain some primary bone in their outermost cortex, an EFS could have been observed, if present. In the larger, completely remodeled ones, an EFS, if present, would have been obscured by this remodeling. An additional agent of destruction of an EFS is preparation. Some specimens showing secondary osteons truncated by the outer bone surface (Fig. 3E, F) suggest that bone has been removed by rough preparation methods, possibly leading to the loss of the µm-thin EFS.

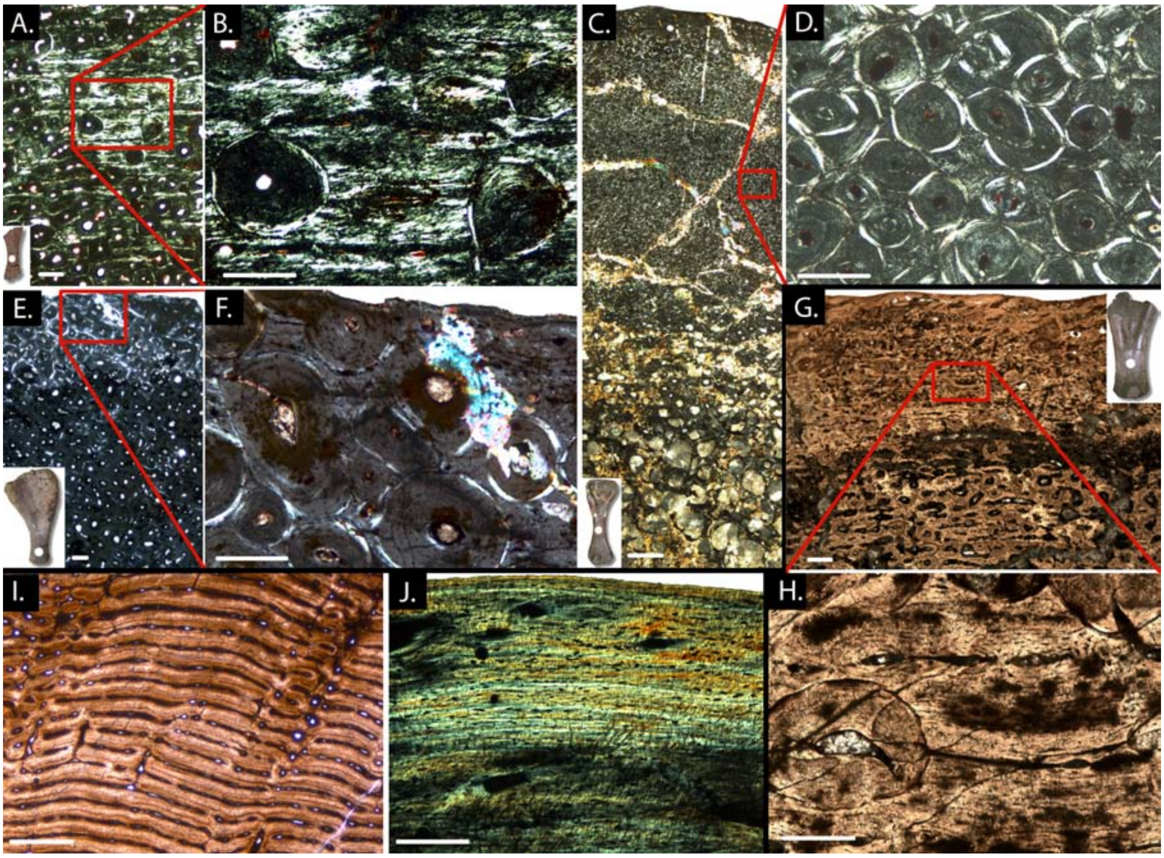

Fig. 3. Micrographs of long bone histology. (A-E) Long bone histology of *Magyarosaurus dacus* under crossed polarizers. (A) Micrograph of a mid-shaft section of the smallest available specimen of *Magyarosaurus dacus* (MAFI Ob.3092, 46% max size). (B) Close-up of (A): largely interstitial laminar primary bone in the outermost cortex. The vascular canals are oriented circumferentially as in laminar fibrolamellar bone, but the bone matrix between the vascular canals consists largely of parallel-fibered and lamellar bone, with only a minute fraction of fibrous (or woven) bone tissue. (C) Micrograph of a midshaft section of MAFI v.13492 (76% max. size). The cortex is completely remodeled, in some areas several generations of secondary osteons can be seen crosscutting each other. (D)

Closeup of (C): cortex dominated by several generations of secondary remodeling. (E) Micrograph of a mid-shaft section of the largest available *M. dacus* humerus (FGGUB R.1048). (F) Close-up of (E): Note the secondary osteons of the third generation, and truncated secondary osteons at the outer bone surface. (G-H) Long bone histology of *'M.' hungaricus* under polarised light. (G) Micrograph of a midshaft section of *'M.' hungaricus* (MAFI Ob.3104). The specimen is strongly remodeled, but the interstitial primary tissue is of the highly vascularized laminar fibrolamellar kind, with well developed primary osteons in the middle cortex, and poorly developed primary osteons with no lamellar bone infilling in the outermost cortex. Note that secondary osteons of the first generation are less well developed than in the largest *M. dacus* specimens. (H) Close-up of (G): Secondary osteons crosscutting well developed primary osteons in the middle cortex. (I) Laminar fibrolamellar bone of *Apatosaurus* (BYU 72517014). (J) Alligator (SMNS 10481) long bone histology showing lamellar-zonal bone. (Scale bars: A, B, D-H, 200 µm; C, 1000 µm; I, J, 500 µm).

3.3 Histologic Ontogenetic Stages in the M. dacus sample

We emphasize again that in its extreme degree of cortical remodeling even in very small individuals, the long bone histology of *Magyarosaurus* is unique among sauropods. In some larger individuals, three to four generations of secondary osteons can be observed (FGGUB R.1048, Fig. 3E, F). However, the *Magyarosaurus dacus* sample is still amenable to relative age determination of individuals using histologic ontogentic stages (HOS; Klein and Sander 2008). The smallest individual, represented by specimen MAFI Ob.3092, records HOS 12 (Fig. 4). The bone microstructure of specimens FGGUB R.1220, FGGUB R.1511, FGGUB R.1246, FGGUB R.1195, MAFI v.13492, and FGGUB R.1047 corresponds to HOS 13, where the cortex is completely or almost completely remodeled. In a number of femora and humeri (FGGUB R.1046, FGGUB R.1992, MAFI Ob.3089, MAFI Ob.3128, FGGUB R.1048), at least one additional generation of secondary osteons, crosscutting secondary osteons of the first or subsequent generations are present in the outer cortex. The microstructure of these specimens corresponds to tissue type H, and is thus assigned to HOS 14 (Klein and Sander 2008, Fig. 4, see Materials and Methods).

Titanosaur long bone histology has only received limited study so far. However, specimens of the basal titanosaur *Phuwiangosaurus* and the advanced titanosaur *Alamosaurus* that are the size of the smallest *M. dacus* show HOS 3-5 (Klein and Sander 2008, Klein et al. 2009, Woodward and Lehman 2009; Fig. 4). HOS 14 has not been observed in *Alamosaurus* and *Phuwiangosaurus*, but fully remodeled specimens (HOS 13) have femur lengths of 1400 mm, nearly 2.5 times the size of the largest *M. dacus* (Fig. 4).

3.4 Long bone histology and HOS of "M." hungaricus

The histology of the large titanosaurian bone (MAFI Ob.3104) is different from the *Magyarosaurus dacus* bones (Fig. 3G, H). The inner and outer cortex are dominated by secondary osteons, but with laminar primary bone still present in the outermost cortex. The primary bone is of the fibrolamellar kind, with a thick lining of lamellar bone in the vascular canals. These vascular canals, however, are not as narrow as in the *M. dacus* bones. Erosion cavities, but also mature secondary osteons, are visible in the outermost primary cortex. The outer bone surface is intact in MAFI Ob.3104, but there is no EFS, indicating that the animal was not fully grown. The bone microstructure of MAFI Ob.3104 corresponds to bone tissue type F (Klein and Sander 2008), and is assigned to HOS 11 (Fig. 4). This is a lower stage than in the smallest bones in the *M. dacus* sample. However, this MAFI Ob.3104 is over four times larger than the *M. dacus* specimens showing a later HOS (Fig. 1, Fig. 3, Table 1).

The histological sample of *'M.' hungaricus* shows an identical bone microstucture to *Phuwiangosaurus*, and a similar histology to the advanced titanosaur *Alamosaurus* (Klein and Sander 2008, Klein et al. 2009, Woodward and Lehman 2009, KS personal observation,

Fig. 4). *'M.' hungaricus* thus displays a typical titanosaur long bone microstructure. A general observation of these titanosaur taxa compared to more basal neosauropods (e.g. *Apatosaurus*), are their accelerated remodeling rates (KS, MPS Personal observations, Klein et al. 2009, Woodward and Lehman 2009), which may be a result of continued peramorphic processes in Sauropodomorpha (Sander 2000, Woodward and Lehman 2009, McNamara 1997).

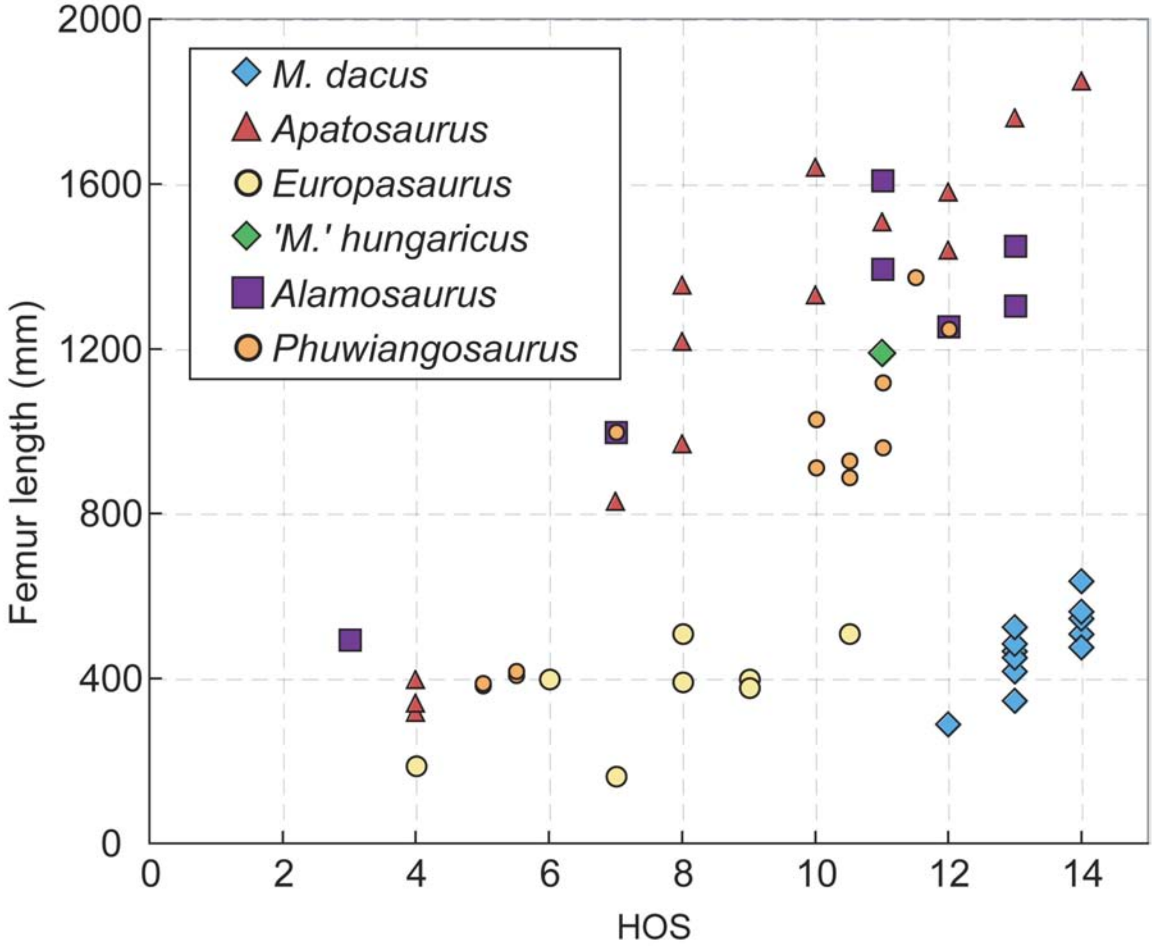

Fig. 4. Plot of histologic ontogenetic stage (HOS, 35) vs. body size as expressed by femur length in *Magyarosaurus dacus*, compared to *Europasaurus, Apatosaurus, Alamosaurus* and *Phuwiangosaurus*. The samples of *Magyarosaurus dacus* derive from humeri that were normalized to femur length. The single '*M.*' *hungaricus* sample is also included. Data for *Alamosaurus* were obtained from (Woodward and Lehman 2009), supported by own data. Data for *Phuwiangosaurus* were obtained from (Klein et al. 2009).

3.5 Interpretation

Both, the comparison of bone tissue types and of HOS (Fig. 4) indicates that the small (*M. dacus*) and large titanosaur bones (*"M." hungaricus*) cannot be placed on the same growth trajectory. This suggests that two distinct titanosaur taxa are present in the Hațeg Basin, with the great majority of bones belonging to a growth series of the diminutive *M. dacus.* We reject therefore the hypothesis (Leloeuff 2005) that the small titanosaur bones from the Hațeg Basin are merely juveniles of the large-bodied sauropod taxon, and conclude that *M. dacus* is a dwarf taxon.

4. Discussion

4.1 Potential problems: lack of EFS

The lack of an EFS in any of the studied long bones represents a weakness in our argument for *M. dacus* having been a dwarf taxon. An EFS would most convincingly indicate that growth had terminated (Erickson 2005, Chinsamy-Turan 2005). However, we see the evidence as conclusive that the *M. dacus* sample does not represent juveniles of the larger *"M." hungaricus*. First, as noted earlier, the advanced remodeling reaching the outer bone surface in the larger specimens of *M. dacus* would have obliterated any EFS, the lack of which thus cannot be cited as evidence for the *M. dacus* specimens being juveniles. Second, "*M.*" *hungaricus* having been the adult of *M. dacus* would mean that an earlier HOS is present in specimens differing four-fold in size. Such an extreme variability in size at a given HOS is not seen in any other sauropod (Sander 2000, Klein and Sander 2008, Klein et al. 2009, Woodward and Lehman 2009) and runs counter to the general observation of a close correlation between body size and histology in dinosaurs in general (Curry 1999, Sander

1999, Sander 2000, Klein and Sander 2008, Lehman and Woodward 2008). The only known exception to this pattern appears to be the Triassic basal sauropodomorph *Plateosaurus* (Sander and Klein 2005), but this taxon is much more basal in the saurischian phylogeny than *Magyarosaurus*. Third, the completely remodeled cortex of the larger *M. dacus* specimens is wholly inconsistent with a juvenile status, not only in comparison with other sauropods (as seen in the HOS comparisons) but also with amniotes in general. Even in slow-growing mammals such a humans, complete remodeling of the long bones is a sign that full size has been reached (Chinsamy-Turan 2005, Francillon-Vieillot et al. 1990, Kerley 1965, Kerley and Ubelakker 1978, Castanet et al. 1993, Thomas et al. 2000).

4.2 Co-occurence of large and small titanosaurs on an island

The very rare fossils of the larger titanosaur *'M.' hungaricus* in the Hațeg fauna are an interesting exception to the general dwarfing of other dinosaurs on Hațeg island. The presence of a few individuals of a larger titanosaurian species might relate to a time of lower sea level, for example, when the effective island size increased and allowed the survival of a larger-sized subsequent immigrant population, or they represent the remains of stray animals from nearby larger land masses. A similar example comes from the Pliocene-Pleistocene from Sulawesi, where the presence of the large-sized *Stegodon* among smaller proboscideans was explained as the result of a late immigration event (De Vos et al. 2007). Alternatively, the large bones may represent an early immigrant population before it reduced in size or went extinct. Nanism is known to occur very rapidly (Millien 2006), at a time scale of 10^3 years, which is well below the time resolution in terrestrial sedimentary deposits, potentially making early colonists and later dwarfs seem comtemporaneous.

However, determining the most likely scenario is beyond the scope of this contribution, and will ultimately rely on future paleobiogeographic and phylogenetic work on the Haţeg dinosaur assemblage.

4.3 Significance of the unique long bone histology of M. dacus

The nanoid status of *M. dacus* is unique among titanosaurs, all of which have body masses an order of magnitude greater (Mazetta et al. 2004, Seebacher 2001). The only other island nanoid sauropod known is *Europasaurus* from the Upper Jurassic of Germany (Sander et al. 2006). At 900 kg, *M. dacus* had a similar adult body mass as *Europasaurus*, but the two taxa show distinctive histologies and ontogenetic growth trajectories (Fig. 4). *Europasaurus* does not have as intensely remodeled bone cortices as *M. dacus*, even in the largest known individual, which shows a clear EFS (Sander et al. 2006). The fully grown *Europasaurus* individuals are HOS 10.5, and the smallest ones (34% maximum size) are only HOS 4. *Europasaurus*, like large-bodied sauropods, also shows fibrolamellar bone in its long bone cortex (Fig. 3I), and only late in its ontogeny, growth marks and Haversian remodeling started to appear (Sander et al. 2006). The primary bone in the smallest individual of *M. dacus* (46% maximum size) shows a large proportion of parallel-fibred bone, and our sample of *M. dacus* exhibits HOS ranging from 12 – 14. These observations suggest a reduced growth rate of *M. dacus*, not only in comparison with large sauropods but also with *Europasaurus* (Fig. 4).

4.4 Implications for metabolic rate

The highly vascularized fibrolamellar tissue in the long bones of *M. dacus*, albeit with a strong lamellar component, suggests that the high metabolic rate of sauropods (Sander et al. 2004, Sander and Klein 2005, Sander and Clauss 2008) has been retained in

Magyarosaurus because the phyletic nanism did not result in the reversal to a bone histology seen in similar-sized ectothermic vertebrates (Erickson and Brochu 1999). In ectotherms like crocodiles (Fig. 3J) and large pseudosuchians (Riqclès et al. 2003), lamellar-zonal bone predominates, and they lack strongly vascularized primary bone and Haversian bone of the kind observed in *Magyarosaurus*. That this is an evolutionary option for endothermic amniotes in a resource-limited habitat is shown by the Neogene dwarf goat *Myotragus* from the Balearic islands which shows typical lamellar-zonal bone (Köhler and Moya Sola 2009, KS, MPS Personal observations). Instead, *Magyarosaurus* reduced both adult body size and overall ontogenetic growth rate, presumably in order to adapt to island dwelling with its resource limitations.

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Chapter 6

Dinosaurs and the island rule: the dwarfed dinosaurs from Haţeg Island

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Abstract

Islands are fascinating natural laboratories of evolution. One much debated theme among evolutionary ecologists is whether there is an 'island rule' or not, the observation that large animals tend to become smaller and small animals larger. Franz Nopcsa was the first, in 1914, to suggest that the dinosaurs from the latest Cretaceous of Haţeg, Romania were an island fauna, based on its low diversity and apparently unbalanced composition, and the basal position ("primitiveness") of many of the included taxa within their respective clades. In turn, the small size of the taxa compared to their relatives from other landmasses in conjunction with the proposed island setting were used to support the presence of the island rule and size reduction (dwarfing; nanism) among the Haţeg dinosaurs. In Nopcsa's day, palaeontologists had seen the same phenomenon many times in the Pliocene, Pleistocene, and Holocene mammals of the Mediterranean islands. Although often quoted as a key example of the island rule, the supposedly dwarfed Haţeg dinosaurs have never been investigated thoroughly. Here we review a wealth of new data, from tectonics and regional geology to limb proportions and dinosaur bone histology, which support Nopcsa's original claim of insularity of the Haţeg fauna. Current evolutionary studies confirm that the island rule applies in many, if not all, modern cases, as well as to the Mediterranean island mammals. Geological evidence confirms that Haţeg was probably an island in the Late Cretaceous, and phylogenetic, ecological, and bone histological evidence shows that at least two of the Haţeg dinosaurs, the sauropod *Magyarsaurus* and the ornithopod *Telmatosaurus*, as well as possibly the ornithopod *Zalmoxes*, were dwarfs by progenesis, a form of paedomorphosis.

1. Introduction

The Haţeg dinosaurian faunas from the latest Cretaceous of Romania have achieved widespread recognition as dwarfed island faunas. The suggestion was made first by Baron Franz Nopcsa (1877-1933) at a meeting in Vienna in November 1912 (published as Nopcsa, 1914b). Nopcsa (1914b) wrote that “while the turtles, crocodilians and similar animals of the Late Cretaceous reached their normal size, the dinosaurs almost always remain below their normal size.” He observed that most of the Transylvanian dinosaurs hardly reached 4 m in length and, for the largest (what was to become *Magyarosaurus dacus*), it was a puny 6 m long compared to a more representative 15-20 m for other sauropods. During the discussion following his paper, Othenio Abel (1875-1945) agreed, and pointed to dwarfing of Mediterranean Pleistocene elephants, hippopotamus, and deer, as well as to island giantism among smaller animals. Nopcsa and Abel referred to earlier work by Forsyth Major on Malta and the then current discoveries by Miss Dorothea Bate on Cyprus and Crete.

The idea of dwarfing in the Haţeg dinosaurs has been suggested many times, on the basis of morphometrics (Weishampel *et al.*, 1991, 1993, 2003; Jianu & Weishampel, 1999; Grigorescu, 2005), and yet additional testing is required. In sequence, these points must be established: the ‘island rule’ is established from observations of modern and Pleistocene examples, the Haţeg fauna lived on an island, the dinosaurs (or some of them) are on average smaller than their nearest relatives from elsewhere, and the putative dwarfed dinosaurs really are small-sized adults, and not juveniles. We shall explore these points in this paper.

Museum abbreviations used in this paper are: BMNH, Natural History Museum, London; FGGUB, Faculty of Geology and Geophysics, University of Bucharest, Bucharest, Romania; MAFI, Magyar Állami Földtani Intézet, Budapest, Hungary.

2. Nopcsa’s contribution

The famous story is told that in 1895 Nopcsa's younger sister Ilona discovered dinosaur bones at the family estate at Săcel in Transylvania (also called Szacsal, near Szentpéterfalva or Sânpetru), then part of the Austro-Hungarian Empire, now in western Romania. Nopcsa was enrolled at the University of Vienna and he undertook to study the fossilized bones. He advanced quickly in his studies, and at the age of twenty Nopcsa (1897) first reported dinosaur remains from Haţeg. He continued to do field work in the area, to collect new fossils, and to publish on them until the outbreak of the First World War. The family estate was seized by the Romanian government in 1920, and Nopcsa never came back, although he continued to publish on the Haţeg faunas until his untimely suicide.

Grigorescu (2005) outlined Nopcsa’s key contributions to knowledge of the Haţeg fauna as:

(1) Systematic palaeontology. Nopcsa described nine species of dinosaurs and other fossil reptiles, of which six are still regarded as valid taxa.

(2) Chronostratigraphy and mapping. Nopcsa dated the Haţeg deposits as terminal Cretaceous in age, and he produced the first detailed geological map of the region. Through this work, he correlated several continental deposits in different neighbouring basins as the Sânpetru Formation.

(3) Evolution. Nopcsa recognized that most of the Haţeg taxa were primitive, and they showed, he felt, most similarity with Late Jurassic and Early Cretaceous forms from elsewhere.

(4) Palaeobiology. Nopcsa interpreted the apparently primitive nature of the Haţeg reptiles as a result of isolation on an island. He also noted that most of the Haţeg species were smaller than their relatives elsewhere, and he attributed this to the

phenomenon of 'island dwarfing'.

After Nopcsa's death, only limited work was done in the Haţeg area for the next seventy years. The age of the deposits was confirmed as Maastrichtian (they had been dated as Danian, when the Danian was still considered to be the final stage of the Cretaceous, rather than the first of the Paleogene). Grigorescu (2005) recounted how he became interested in the area again, because he used the area around Sânpetru village for teaching field geology to his students, and because of intense interest from foreign palaeontologists in these Romanian localities and fossils, stemming from the classic work by Nopcsa. They found bones so frequently that Grigorescu decided to initiate more formal studies, and these began with an excavation in 1977. Further excavations by a team from the Deva Museum also began in 1977. These field campaigns led to improvements in knowledge of the stratigraphy of the beds, and major expansions of the fauna to include dinosaur eggs (Grigorescu *et al.*, 1990; Smith *et al.*, 2002), a wide range of microvertebrates (Grigorescu *et al.*, 1999), including lissamphibians and squamates (Folie & Codrea, 2005), pterosaurs (Buffetaut *et al.*, 2002), and mammals (Grigorescu *et al.*, 1985; Csiki & Grigorescu, 2000), as well as new dinosaurs (Csiki & Grigorescu, 2002).

3. Size change on islands

3.1 Principles, and debate over validity of the island rule

Nopcsa's (1914b, 1923) proposal of insular dwarfing was not an original concept, and it derived from former and then-current discussions of the Pleistocene mammals of the Mediterranean islands. It is interesting then that modern reviews of the subject (e.g. Foster, 1964; Van Valen, 1973; Case, 1978; Lomolino, 1985, 2005, 2006; Damuth, 1993; Raia & Meiri, 2006; Welch, 2008) make little reference to the Victorian and early twentieth century publications by Forsyth Major, Bate, Nopcsa and others on Pleistocene mammals and Cretaceous dinosaurs. The principle of size change in mammals on islands was established by Foster (1964), who noted that small taxa generally become larger and large animals smaller. This phenomenon was termed the *island rule* by Van Valen (1973), the commonest term, although it has been called 'Foster's island rule' (e.g. Palombo, 2007), or simply 'Foster's rule' (e.g. Welch, 2008). The island rule has been identified among mammals (Foster, 1964), birds (Clegg & Owens, 2002), and snakes (Boback & Guyer, 2003), but its occurrence in lizards and other groups is equivocal (Case, 1978; Lomolino, 2005). Meiri (2007) was clear that lizards do not follow the island rule, and indeed Meiri *et al.* (2004, 2006, 2008) have argued that there are so many exceptions that the term 'island rule' should be abandoned.

It is important to establish some aspects of terminology. The phenomenon of small size on islands has been termed 'island dwarfing' or 'insular dwarfism', and other variants, whereas large size is often termed 'gigantism'. Gould & MacFadden (2004) argued that the terms dwarf, dwarfing, dwarfism, and gigantism generally refer to medical abnormalities within species, and yet the terms have been widely extended to indicate size changes between races or species. They recommend the term *nanism* for phyletic size reduction and *giantism* for phyletic increase in size. We follow their use of 'giantism', but we continue to use the term 'dwarfing' because it is so widely used in the context of discussions of the island rule.

Foster (1964) reported the common occurrence of giantism among smaller island taxa (rodents) and dwarfing among others (lagomorphs, carnivores, artiodactyls), with mixed results indicated by the rather small samples of marsupials and insectivores. Case (1978) agreed broadly with these data, except for the carnivores: he suggests that these show a mixture of responses when a wider census of island taxa is taken. In his overview, Case (1978) found that lagomorphs, bats, artiodactyls, elephants, foxes, raccoons, snakes, and teiid and lacertid lizards often show reduced size on islands, whereas cricetid rodents, iguanid lizards, tortoises, and bears often show larger size on islands.

More recent comprehensive overviews have offered conflicting viewpoints, with Lomolino (1985, 2005) presenting a strong case for the island rule among mammals and other groups, and Meiri *et al.* (2004, 2006, 2008) expressing considerable uncertainty. Lomolino (1985, 2005) confirmed Foster's earlier findings (Table 1), with significant tendencies for small mammals (lagomorphs, rodents) to become larger on islands (size ratio > 1.00), and for large mammals (carnivores, artiodactyls) to become smaller (size ratio < 1.00). Marsupials and insectivores showed no clear pattern, but mammals as a whole showed a highly significant correlation ($p < 0.0001$).

When Meiri *et al.* (2008) re-analysed the data, they found significant support for the island rule in only limited cases: artiodactyls and carnivores (in particular herpestids and viverrids) tend to become smaller on island, and rodents (especially murids) tend to become larger, but there were no significant trends for other groups, nor for mammals as a whole. Meiri *et al.* (2008) also found that there was a substantial phylogenetic signal in all subsets of the data; such a signal could lead to pseudoreplication and apparently more significant correlations if, for example, several closely related taxa showed parallel responses. So, they argue, an apparently significant relationship could arise if rodents, say (rather than all small mammals) become larger on islands whereas artiodactyls (but not all large mammals) become smaller. When the phylogenetic signal is removed (Table 1, last two columns), surprisingly the all-mammal sample shows a significant (but weak) negative relationship, but the constituent clades do not. So, overall, after phylogenetic correction, it remains true, as

Foster (1964) had said, that small mammals tend to become larger, and large mammals smaller on islands. Meiri *et al.* (2008) argue that this weak relationship could rest entirely on those clades that do show size changes in line with the island rule: giantism in murid rodents, and dwarfing in artiodactyls, heteromyids, and some carnivores.

Why did two analyses of essentially the same data set lead to such opposite interpretations? First, both authors were assessing different proposals, or null models, as Welch (2008) pointed out:

(1) Lomolino (1985, 2005) and others were testing whether or not there is a size difference between pairs of island and mainland species, with assumptions that size changes on islands do not depend on the ancestral state, and that the mainland relatives remain close to the ancestral state.

(2) Meiri *et al.* (2008) were testing whether there is a heritable tendency to change body size in a particular way after island colonization, assuming that this tendency evolves independently of current body size.

Both models may lead to biased results. In the first, any size evolution of the mainland population could suggest that the island population has changed in size, when it need not have. Further, the use of a regression as the basis of the test (Lomolino, 1985, 2005) may be inappropriate because this is a parametric approach and assumes equal variances for all data points, which is unlikely, not least because the island colonizations are scattered widely through time and so each pairwise comparison involves different amounts of evolutionary change (Welch, 2008). A nonparametric test would be more appropriate (Meiri *et al.*, 2004; Bromham & Cardillo, 2007). The second test is also problematic: Meiri *et al.* (2008) argued that previous studies involved pseudo-replication because phylogeny was not taken into account. However, this is only a requirement in the context of null model (2), and is not true of null model (1), in which no example of body size evolution was counted more than once because all island-mainland pairs of taxa were phylogenetically independent. Welch (2008) showed that many tests used so far in the context of model (1) may falsely detect the island rule when island and mainland evolution are indistinguishable. Further, tests that account for phylogeny in the context of model (2) may lack power to detect the island rule under certain conditions. In his study of primate data, Welch (2008) found, frustratingly, that the island rule held for some measures of body size (skull length; body mass), but not for others (head-body length).

The debate continues, and yet it seems there will not be a clear-cut demonstration of the ubiquity of the island rule for large sets of examples. All seem to agree that certain clades of mammals, for example, show giantism or dwarfing, and so for those clades at least the island rule holds. The problem may lie in the term ‘rule’, which some might interpret to mean a regular law-like principle that always applies, whereas others might see a ‘rule’ as something that happens in many cases, but may be overwhelmed by other processes as well. In the latter ‘soft’ interpretation, the island rule is a useful generalization, analogous to others in biology, such as Cope’s rule or Dollo’s rule.

3.2. Hypotheses for size change on islands

There have been many explanations for the island rule, and these seek to explain either why large animals become smaller, why small animals become larger, why both relative size changes occur, and sometimes why the rule seems to be best expressed in warm-blooded animals such as mammals and birds.

Several of the hypotheses have been rejected, whether outright, or partially, and these are listed first.

(1) The *relict population* viewpoint (Hinton, 1926; Cowan, 1935) is that giant rodents on islands may be relicts of once more widespread populations. Tougher selection on the mainland perhaps led to the extinction of the majority of the larger species or morph, leaving only relict population on islands. Foster (1964) rejected the relict population model using several arguments: there are differences among the island giant forms of rodents (relicts of a formerly more widespread population ought to be more similar), there is limited evidence that the smaller mice had displaced the putative larger mainland forms, the size changes may be seen in many unrelated mammals, birds and lizards, and it is not clear that the large size of insular rodents is a conservative character.

(2) A further suggestion is that *reduced prey size on islands* could induce dwarfing among predators. This idea stems from the observation of a general correlation between predator and prey size. With few competitors, small-sized prey are abundant, and predators therefore might scale down in size in order to exploit it. Case (1978) rejected this idea because of limited evidence that island giants or island dwarfs are matched by either giant or dwarfed prey. Further, this hypothesis cannot explain the dwarfing of herbivores because they do not match their size to the size of the plants they eat.

(3) *Sexual selection* on islands could foster increases in body size where other selective pressures present on the mainland have been removed (Carlquist, 1965). This might be a contributing factor for some mammals, where sexual selection favours large size, but clear examples have not been demonstrated.

(4) Island dwellers might show a tendency to move towards an *optimal body size* (Brown *et al.*, 1993; Damuth, 1993; Lomolino, 2005; Palombo, 2007) when competition and

other pressures are relaxed. This optimum is the size at which energy capture from the environment is maximized, and for mammals this might be 0.1 kg (Brown *et al.*, 1993) or 1 kg (Damuth, 1993). Meiri *et al.* (2004) found less predictable results among carnivores than had been expected, and they were concerned that the predictions of optimal body size for mammals varied so much. Further, Raia & Meiri (2006) showed that related island species tend to different body sizes depending on local conditions, and so there is no tendency towards a single optimal body size. This is part of the wider observation that the body mass of the largest animals in a region depends on the maximum area available, which relates to the size of the required home range, which in turn reflects food requirements (Burness *et al.*, 2001).

(5) Some examples of giantism may stem from *selection of immigrants for large size* (Lomolino, 2005). The idea is that, in some cases, immigrants swam to the islands they colonize, and so the larger and stronger animals could swim further, or more successfully, based on size-related metabolic demand (Roth, 1992). The founding population then might consist of larger species, at least in the early stages of colonization. A criticism is that this might apply in some cases, but not all, and in any case would be hard to demonstrate.

Among the hypotheses for the island rule that still have currency are ecological release, niche expansion, resource limitation, and optimization of life history traits.

(1) *Ecological release* has been a key suggestion, that island species are freed from normal pressures from competitors, predators, and parasites on the mainland, and so may change body size as a result (Foster, 1964; Carlquist, 1965; Van Valen, 1973; Azzarolli, 1982; Lomolino, 1985, 2005; Raia & Meiri, 2006). Islands typically have fewer species than equivalent mainlands, an example of the species-area effect

(Williams, 1943). Those species that on the mainland are typically present at low abundance, such as large herbivores and top predators, are the most likely to be absent from an island. Absence of larger mammals or birds means there is generally less competition for food and shelter, and less predation, and tiny, furtive mammals can become larger and bolder. Birds on islands often become flightless perhaps for the same reasons, as well as to conserve energy (McNab, 1994). For large animals that become smaller on islands, the absence of predators removes one of the benefits of large size (escaping predation by being big), and so animals can become smaller without risking being picked off from the herd by a predator (Van Valen, 1973; Lomolino 1985). In their study of Pleistocene and modern mammals, Raia & Meiri (2006) found strong evidence for size decreases among island herbivores in the absence of competitors and predators.

(2) A linked explanation may be *niche expansion* (Grant, 1965; Van Valen, 1973; Heaney, 1978), where the lower numbers of species on islands open possibilities for the expansion of normal niches to take in new diets and opportunities. So, animals constrained to small size on the mainland can allow their overall size range to expand, and in certain cases take over the roles of absent middle-sized animals.

(3) *Resource limitation* on islands has often been suggested (Foster, 1964; Case, 1978; Lomolino, 1985, 2005; Burness *et al.*, 2001; Raia & Meiri, 2006) as a selective pressure on larger mammals and birds. Large herbivores, such as elephants, rhinoceroses, deer, or cattle often require large foraging areas, and they may traverse hundreds or thousands of kilometres in order to find appropriate food supplies at different seasons of the year. Such long treks are impossible on islands, and so a large herbivore might either become reduced in size so as to accommodate

itself to the size of the island, or go extinct. Raia & Meiri (2006) found that body sizes of island carnivores are influenced by resource limitation, and little else.

(4) Other models suggest that size changes depend on *optimization of life-history traits* such as metabolic rate, gestation time, size at birth, age and size at maturity, birth and death rates, trophic level, home range size, and population density (Palkovacs, 2003; Palombo, 2007). It is likely that in certain cases, r-selected animals, through their earlier sexual maturity, and hence smaller size, could simply make better island colonizers. Island mammals then change their size according to their *Bauplan*, the most appropriate empty niches, and presence or absence of potential competitors or predators. This relates to views expressed by Case (1978), Meiri *et al.* (2004, 2006) and others, that size change may be contingent on circumstances, and so regular predictable patterns of the island rule may not always be found.

In summary, Lomolino (2005) argued that dwarfing on islands may be maintained by ecological release from predators and resource limitation, whereas giantism on islands may be promoted by ecological release from large competitors and predators and immigrant selection (Fig. 1). In both cases, intensified natural selection promotes the directional shifts in mean body size.

	Smaller	Same	Larger	Size ratio[1]	Slope[1]	Size ratio[2]	t test[2]
All mammals	131	44	190	1.01***	0.95***	1.00	-2.98**
Marsupials	3	0	5	1.08	0.88***	[1.07]	[0.77]
Insectivores	15	8	15	1.07	1.01	1.00	-1.05
Lagomorphs	12	3	5	1.03*	0.81**	1.03	-1.73
Rodents	53	26	151	1.10**	0.91***	1.08	-1.09
Chiroptera	-	-	-	-	-	0.99	0.77

Primates	-	-	-	-	-	0.92	-0.72
Scandentia	-	-	-	-	-	0.94	-0.62
Carnivores	33	7	14	0.90*	0.88**	0.94	1.14
Artiodactyls	15	0	0	0.71**	0.84*	0.88	-1.66

Table 1. Results from a census of 365 island races or species of mammals, from Lomolino (1985, 2005) and Meiri *et al.* (2008). Size ratio is the sum of insular weights divided by mainland weights for each group, and slope is based on a regression of island/ mainland comparisons for all 71 species (365 races), where slopes less than one indicate a trend from giantism in the smaller species to dwarfing in the larger species. The t-test assesses whether the distribution of size ratios is significantly different from one; * significant difference from ratio of 1.00, at $p < 0.05$; ** $p < 0.01$; ***$p < 0.001$; [1] from Lomolino (1985); [2] from Meiri *et al.* (2008), non-phylogenetic values for whole sample (marsupial data for Diprotodontia only).

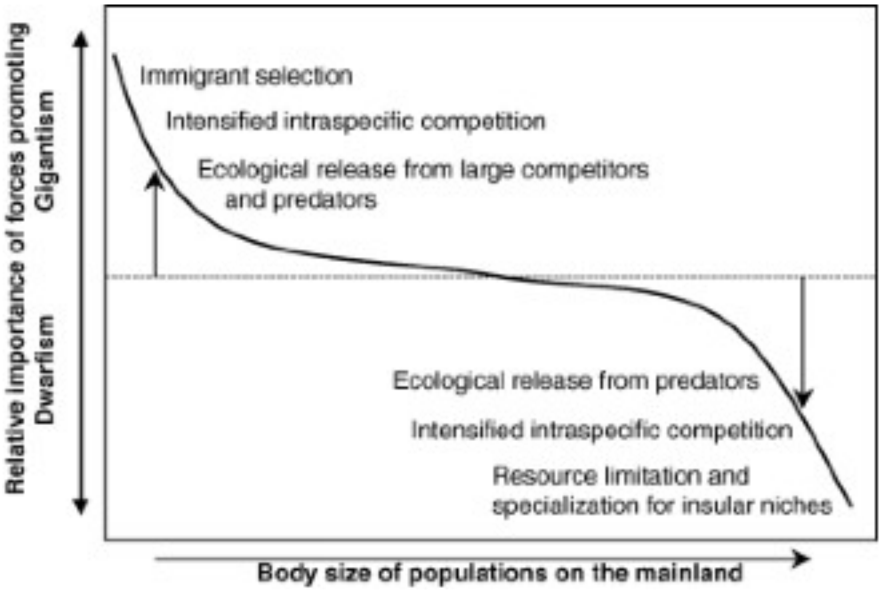


Fig. 1. The island rule is an emergent pattern resulting from a combination of selective pressures whose importance and influence on insular populations change in a predictable manner along a gradient from relatively small to relatively large species. (Based on Lomolino, 2005).

3.3 Dwarfing in Pleistocene and Holocene mammals

Island faunas of mammals, most studied from the Pliocene, Pleistocene, and Holocene of the Mediterranean islands (Boekschoten & Sondaar, 1966; Azzarolli, 1982; Lister, 1996; Marra, 2005; Raia & Meiri, 2006; De Vos *et al.*, 2007; Palombo, 2007, 2008), show low diversity. The Pleistocene mammalian faunas of Mallorca and Menorca include only three genera of mammals, the bovid *Myotragus*, the glirid rodent *Hypnomys*, and the shrew *Nesiotites*. The faunas of the same age from Malta include pigmy elephants, pigmy hippo, pigmy deer, and the giant dormouse *Leithia*. Sicily shows a similar fauna, and other Mediterranean islands, including Cyprus, Corsica, Capri, and many Aegean islands yield similarly depauperate faunas.

Two larger Mediterranean islands, Crete and Sardinia, have yielded slightly more diverse mammalian faunas. Crete was populated by pigmy elephant, pigmy hippo, and pigmy deer, as well as two genera of giant rodents and giant insectivores. The Pleistocene faunas of Sardinia were most diverse of all, but still less so than those of the mainland, with pigmy elephant, pigmy hippo, and pigmy deer, as well as large rodents and shrews, as on the other islands. In addition, Sardinia was populated by moles, rabbits (*Prolagus*), carnivores (the dog *Cynotherium* and the weasel *Enhydrictis*), a pig, and a macaque.

Islands elsewhere show similar patterns. The late-surviving dwarf mammoths of Wrangel Island, lying offshore from the north of Russia, are well known, but there were some even smaller, and younger, mammoths on islands in the North Pacific that existed 7900 years ago. These tiny mammoths became larger and smaller as sea level fell and rose, suggesting a direct connection between body size and resource use (Guthrie, 2004). In a further example, the Greater Antilles in the Caribbean were home to dwarfed ground sloths.

The Pleistocene of the Channel Islands off the California coast (Johnson, 1978) has yielded specimens of dwarfed elephant (*Mammuthus*) and over-sized species of the rodent *Peromyscus*. Low diversity of dwarfs and giants are seen on larger islands, such as Java and Borneo, where pigmy elephants and large rodents are known from the Pleistocene, and the pigmy buffalo *Bubalus depressicornis* survives today in Sulawesi. The pigmy human species, *Homo floresiensis* from Flores Island, Indonesia is a remarkable example of possible Pleistocene dwarfing of humans on an island (Bromham & Cardillo, 2007). Madagascar, an even larger island, still had less diverse faunas than in neighbouring parts of Africa, and these included in the Pleistocene and Holocene giant lemurs, giant insectivores, a pigmy hippo, and the giant flightless bird *Aepyornis*.

Size may reduce in line with the expectations of allometry. Azzaroli (1982) showed an example of *Megaceros giganticus*, the great Irish deer, up to 2.1 m tall at the shoulder. The smaller species *M. algarensis* from Sardinia is 0.8-1 m at the shoulder, while the tiny *M. cretensis* from Crete is 55-65 cm at the shoulder (Fig. 2). In tracking back through these dwarfs, the antlers become smaller and simplify in a negatively allometric manner. So the smallest deer has relatively tiny and simple antlers with only a couple of points, and barely as long as the skull, whereas *M. giganteus* has antlers with up to ten points and four times the length of the skull. The middle-sized *M. algarensis* shows intermediate antlers, three times the length of the skull and with four or five points. Other deer show similar negative allometry in the reduction of limb length: a dwarf deer from Pianosa in the Tyrrhenian Sea has relatively short legs in comparison to its full-sized relatives.

Fig. 2. Artist's reconstruction of the dwarfed Mediterranean island deer from the Pleistocene, *Megaceros* cretensis from Crete (shoulder height 55-65 cm) and *Megaceros algarensis* from Sardinia (shoulder height 80-100 cm), compared to their presumed ancestor *Megaceros verticornis* from continental Europe. Drawing by Cristina Andreani.

Pleistocene island mammals are generally said to show paedomorphosis. Size reduction through dwarfing implies a process of heterochrony, or size change during development, and in particular paedomorphosis (retention of juvenile conditions in the adult). Paedomorphosis can occur by one of three processes: an overall reduced rate of development (neoteny), a postponed onset of development (post-displacement), or achievement of sexual maturity early (progenesis). The first two can lead to adults of the same size as the unaffected relatives, whereas progenesis usually leads to adults of reduced size (Gould, 1977; Alberch *et al.*, 1979). Indeed, selection for small body size is likely the driver, and morphological change through progenesis the consequence.

In their study of the dwarfed elephant *Elephas falconeri* (Fig. 3) on Sicily, Raia *et al.* (2003) argued for paedomorphosis by progenesis. They also presented an ecological model based on the preponderance of juveniles, nearly 60% of the 104 specimens studied. From this they argued that food supplies were good for such a small elephant (weighing 100 kg rather than 5 tonnes), and that reproductive rates may have increased, but that calf mortality was also high – together a rather more r-selected strategy than seen in modern elephants. Perhaps this was a response to a highly seasonal environment, as experienced by elephants today, but without the space to migrate when climate and food supply become harsh. Their measurements of tibiae suggest (Fig. 4) distinct size classes and highest numbers of the smallest size category. This they interpret as evidence that *E. falconeri* bred at discrete times of the year, instead of all year round as modern elephants do, and that juvenile mortality was high perhaps during seasonal droughts.

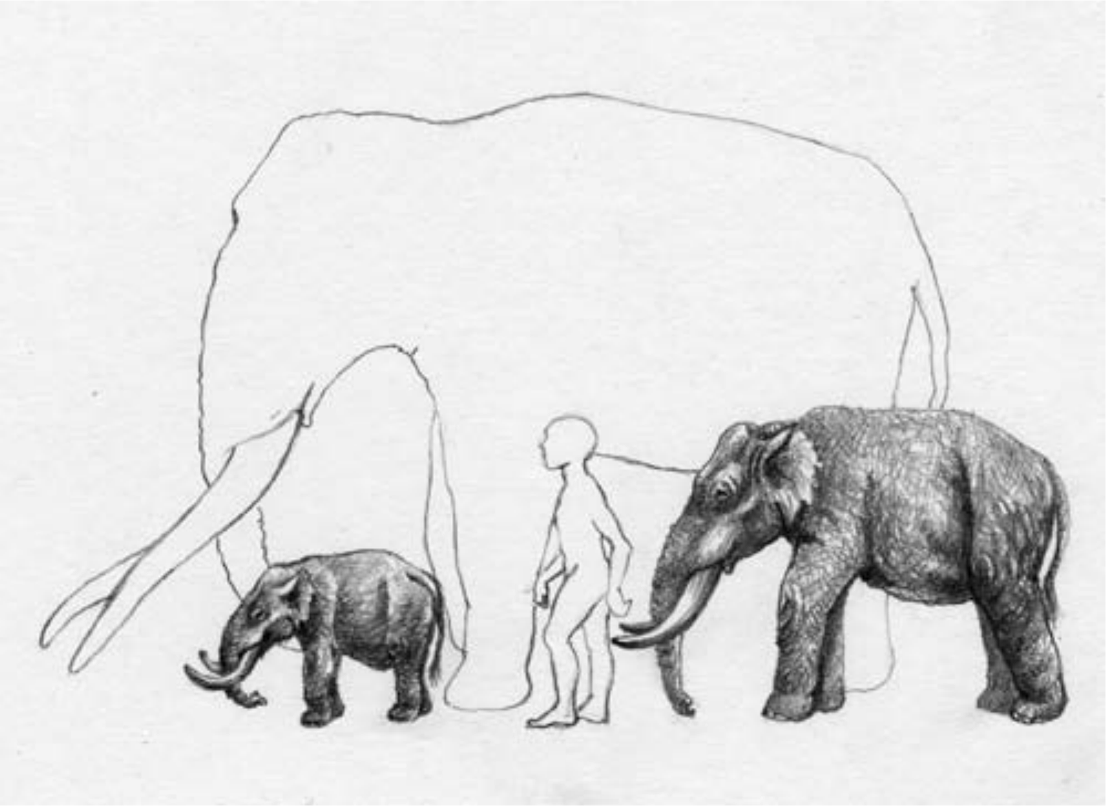


Fig. 3. Artist's reconstruction of the extinct dwarf elephant, *Elephas falconeri* (left), from the late Pleistocene of Sicily and Malta (height at shoulder, 0.9-1.0 m), *Elephas mhaidriensis* (right) from the middle and late Pleistocene of Sicily and Malta (height at shoulder, 1.6-1.8 m), and *Elephas antiquus* (behind) from the middle and late Pleistocene of continental Europe, the supposed ancestor of the two dwarfed forms (height at shoulder, 3.0-3.5 m). Drawing by Cristina Andreani.

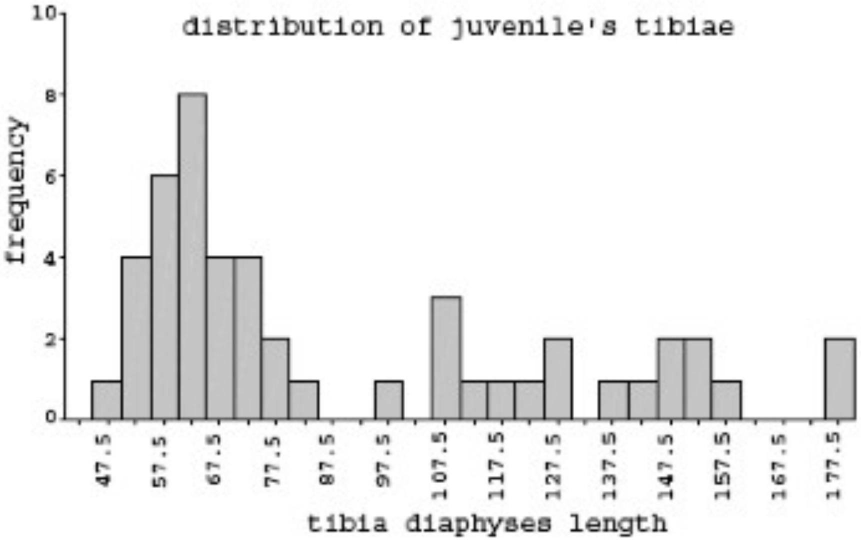


Fig. 4. Frequency histogram of tibia lengths in a sample of 104 specimens of *Elephas falconeri*, the island dwarf form from Sicily, showing the occurrence of three size classes, and highest numbers in the most juvenile. This is possible evidence of year classes and seasonal breeding and/ or seasonal mortality. From Raia *et al.* (2003).

In other cases, the dwarfed mammals have become adapted morphologically to their new size and changed habits. The pigmy *Hippopotamus creutzburgi* from the late Pleistocene Crete has longer legs than its larger relatives from Africa, presumably an adaptation to getting around on a rugged island where there were no large rivers or lakes (Boekshoten & Sondaar 1966). The bovid *Myotragus balearicus* from the Balearic Islands has evolved modified teeth and eyes: the lower incisors are reduced to one pair and these grow continuously, as in rodents, and the orbits have shifted forward to give the animal improved stereoscopic vision. Further, as the animal became dwarfed, its limbs became relatively very short and stocky, and these changes may all be followed through successions of fossils through the Pliocene, Pleistocene and Holocene (Moyà-Solà & Pons-Moyà, 1980). All these changes suited the unusual *Myotragus* to a life in rugged, hilly terrain, leaping from rock to rock (need for stereoscopic vision), and without fear of predators (less need for lateral vision).

Studies of dwarfed Pleistocene and Holocene mammals have shown peculiar anatomical features. For example, Azzaroli (1982) notes that dwarfed mammals often retain over-sized dentition because the teeth sometimes do not reduce in proportion to the rest of the body or skull, and he notes as an example a small macaque from Sardinia in which the cheek teeth are relatively large in comparison to those of related species of normal size.

Two well-known 'fossil islands' are southern Calabria and the Gargano promontory (Azzarolli, 1982; De Vos *et al.*, 2007), the toe and heel of the boot of Italy, which were surrounded by seawater when sea levels were higher before the Pleistocene ice ages began. The southern Calabrian island was home to a small elephant and a small deer, *Megaceros*, in the Pleistocene. The Gargano island in the Miocene was populated by the gigantic hedgehog *Deinoglaerix* and large mice, among other mammals, and these were preyed on by giant owl, *Tyto gigantea*, and a giant eagle, *Garganoaetus freudenthali*, which were twice the size of their nearest relatives.

The ages of the various islands in the Mediterranean may be determined with some precision, and so the likely rates of size change among the mammals and birds may be estimated. Mammals in particular can have reached the various islands only when the sea retreated and left a land bridge. Likely dates when the endemic faunas reached the islands are: Sardinia (0.9-1.0 Ma), Crete (0.7 Ma) Sicily (0.4 or 0.23 Ma). The dwarfs and giants largely became extinct from the end of the Pleistocene and beginning of the Holocene (0.01 Ma), so dwarfing or giant size must have happened in hundreds of thousands of years (Azzarolli, 1982; Guthrie, 2004; De Vos *et al.*, 2007); indeed, fast morphological evolution of mammal faunas seems to be a common phenomenon (Millien, 2006).

Advantages of dwarfing for elephants and other large taxa on islands might include the reduction in food requirement and so of the time spent searching for and processing food, and the reduction in size of home range and so of competition with other populations (Palombo, 2007). Size reduction among Mediterranean island elephants was achieved by paedomorphosic processes, and probably a shorter period of pregnancy (Palombo, 2007). Life cycles may have been generally shortened and fecundity increased, both features associated with size reduction.

4. Was Haţeg an island?

Nopcsa (1914b, 1923) was the first to suggest that the Haţeg dinosaurs lived on an island, but his reasoning was that the animals had undergone island dwarfism and so must have lived on an island. As Grigorescu (2005) noted, little independent geological evidence was adduced until late in the twentieth century.

Modern tectonic and palaeogeographic studies (Csontos and Vörös, 2004; Dercourt *et al.*, 1993, 2000; Sandulescu 1990) have shown an archipelago of islands over much of southern Europe in the Late Cretaceous (Fig. 5). The Haţeg island has been estimated to have had an area of 7500 km^2 (Weishampel *et al.*, 1991) or about 200,000 km^2 (based on Dercourt *et al.*, 1993, 2000). The first, smaller estimates represent the strict size of the Haţeg Basin, some 45 km long (E-W) and 15 km wide (N-S). However, the Haţeg island was more extensive than this, and included other areas of the Transylvanian Basin and surrounding areas with preserved Maastrichtian continental deposits (Codrea & Dica, 2005; Codrea & Godefroit, 2008) as well as uplifted areas of the Carpathian chain with no net deposition (e.g. Bojar *et al.*, 1998; Willingshofer *et al.*, 2001). According to this, more reliable estimates show an area of approximately 80,000 km^2, corresponding to an island of about the size of Haiti (Csiki, 2005). Paleogeographic reconstructions also suggest that the Haţeg island was located at least some 200-300 km from the nearest landmasses in all directions, the Franco-Iberian land to the west, the Bohemian Massif to the northwest, and the Balkan-Rhodope Massif to the southeast; probably the closest continental areas were the emerged segments of the Adriatic-Dinaric Carbonate Platform to the south. The surrounding marine areas were represented by mainly deep-marine basins with flysch sedimentation, eventually passing into shallower epicontinental seas covering the nearby Moesian and East-European platforms (e g. Pamic, 1998; Sandulescu, 1984, 1990; Willingshofer *et al.*, 1999). Based on palaeomagnetic studies, Hațeg lay at a latitude of about 27^{o}N (Grigorescu, 2005; Panaiotu & Panaiotu, this vol.), so just within the equatorial belt.

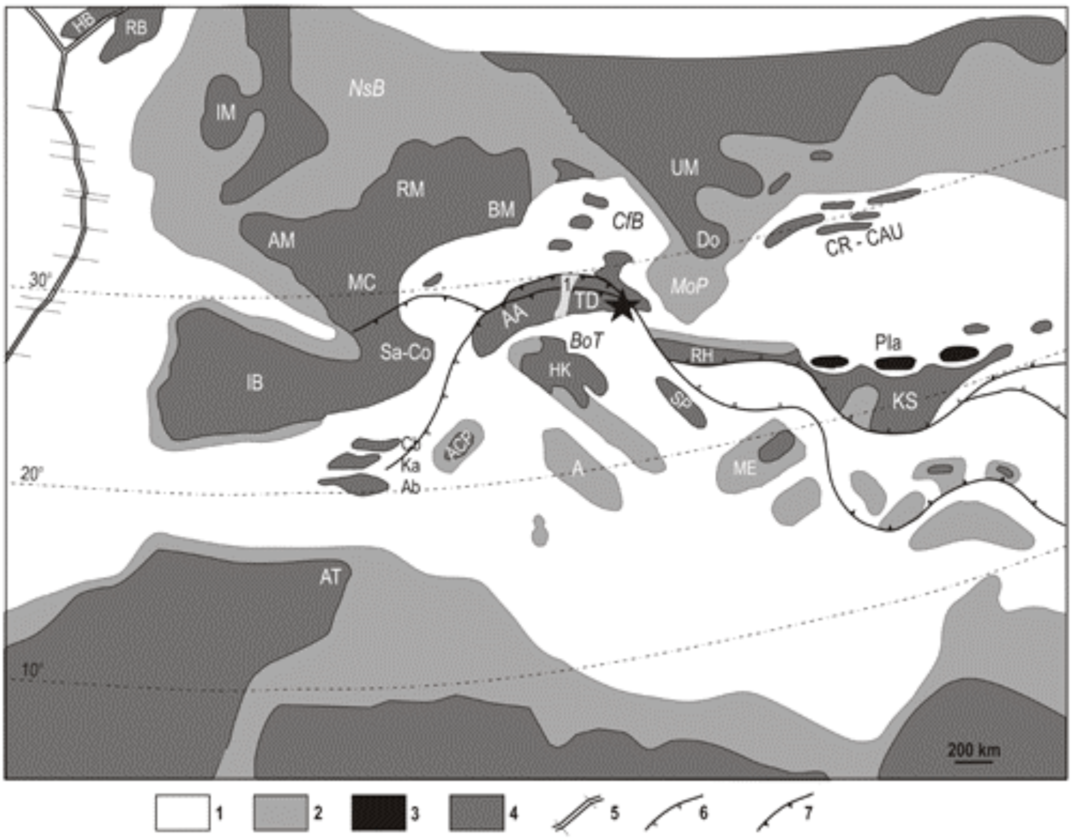


Fig. 5. Palaeogeographic map of the Mediterranean sector of Tethys during the Maastrichtian, showing the location of Hațeg (after Camoin *et al.*, 1993). Palaeolatitude revised based on Panaiotu and Panaiotu (this volume). Abbreviations: A, Apulia; AA, Austro-Alpine Domain; Ab, Alboran Block; ACP, Apennine Carbonate Platform; AM, Armorican Massif; AT, Atlas; BM, Bohemian Massif; BoT, Bosnian Trough; Cb, Calabrian Block; CfB, Carpathian Flysch Basin; CR-CAU, Crimea-Caucasus; Do, Dobrogea; HB, Hatton

Bank; HK, High Karst (Dinaric Carbonate Platform); IB, Iberia; IM, Irish Massif; Ka, Kabylia; KS, Kirshehir; MC, Massif Central; ME, Menderes; MoP, Moesian Platform; NsB, North Sea Basin; PIa, Pontides Island arc; RB, Rockall Bank; RH, Rhodope Massif; RM, Rhenish Massif; Sa-Co, Sardinian-Corsican Block; SP, Serbo-Pelagonian Massif; TD, Tisia-Dacia Block (the position of "Haţeg Island" is marked by a black star); UM, Ukrainian Massif; 1, the western boundary of Tisia-Dacia, as marked by the Maramureş-Szolnok Trough and the Mid-Hungarian Line. Legend: 1, deep marine basins (pelagic carbonates, flysch); 2, shallow marine basins (mainly siliciclastic and carbonate shelf deposits); 3, island arc; 4, emergent land; 5, active speading ridge; 6, thrust; 7, subduction.

Jianu & Boekschoten (1999) suggested that Haţeg had not been an island based on tectonic, sedimentological, and palaeontological evidence. They argued that some palaeogeographic reconstructions show continuity from the proto-Carpathians to other European landmasses, and that the alluvial sediments on Haţeg are too extensive and too thick (up to 2.5 km thick) to have been deposited on an isolated volcanic island. Such a thickness of sediment points rather to a large-scale subsiding basin as part of a mainland. Finally, with dozens of species, the fauna would have been much too diverse, especially for an island only 7500 sq. km in dimensions. Jianu & Boekschoten (1999) then preferred to regard Haţeg as an outpost, a remote tip of the mainland, rather than an island.

However, when considering the larger dimensions of the island, as outlined above, most of the arguments presented by Jianu & Boekschoten (1999) are significantly weakened. The emergent area of Haţeg was only part of a larger tectonic block with continental crust (the Tisia - Dacia block; Sanders, 1998; Sandulescu, 1990; Csontos and Vörös, 2004) assembled during the late Early to latest Cretaceous orogenic phases affecting the Carpathian areas (Willingshofer, 2000; Willingshofer *et al.*, 2001). Large parts of this block were actively uplifting during the latest Cretaceous, synchronously with the deposition of the Sânpetru and Densuş-Ciula formations (Bojar *et al.*, 1998; Willingshofer, 2000) and this coincidence between strong uplift, marked subsidence within an extensional basin and subtropical, seasonally variable climate represents probably the explanation for the deposition of thick sequences of continental deposits. The estimated size of the emergent Haţeg area was also probably large enough to support a relatively diverse palaeofauna. Palaeogeographic evidence still strongly indicates the existence of an archipelago of islands bordering the northern margin of Tethys. Although, admittedly, the changing palaeogeography of this archipelago was influenced by tectonic events such as extension within oceanic basins, continental convergence and collision, as well as eustatic sea-level changes (see e.g. Dercourt *et al.*, 2000), possibly leading to formation of occasional land bridges or shallow-marine dispersal corridors to the European mainland or other emergent landmasses of southern Europe, the presence of a larger Haţeg Island seems relatively well supported.

A final question should address the how long the Haţeg Island existed. Evolving within the dynamic framework of the Mediterranean Sill (Dercourt *et al.*, 2000), besides the continuously changing geographic extent of the Haţeg Island, its evolution was circumscribed by its duration. It is noteworthy, that an emergent landmass corresponding to the position of the Haţeg Island can be followed continuously from the latest Early Cretaceous onward on the palaeogeographic maps of Dercourt *et al.* (1993, 2000).

As noted above, coalescence of former continental blocks (pre-Apulian, Rhodope) to form the Tisia-Dacia Block took place during the late Early to earliest Late Cretaceous (Sanders, 1998; Sandulescu, 1984; Willingshofer, 2000; Willingshofer *et al.*, 1999), and this time period is marked by continental sediments in the Haţeg area (Stilla, 1985; Grigorescu, 1992) as well as other Carpathian areas (Grigorescu, 1992). The presence of an earliest Late

Cretaceous emergent area can thus be suggested, representing the earliest identifiable occurrence of the Haţeg Island.

Subsequent collisional events during the late Turonian (pre-Gosau or Subhercynian tectogenetic phase) was followed by enlargement of the continental area, and deposition of continental or littoral deposits in several areas of the Apuseni Mountains and Southern Carpathians during the Coniacian and Santonian (Dragoş, 1971; Petrescu and Huică, 1972). Tree trunks found in marine deposits of Campanian age in southwestern Transylvania (Iamandei *et al.*, 2005) also suggest the proximity of emergent areas. Nopcsa (1902) reported an isolated theropod tooth from Coniacian - early Santonian littoral deposits (Csiki and Grigorescu, 1998) of the Borod Basin, northern Apuseni Mountains, northwestern Romania, as '*Megalosaurus hungaricus*' (now a nomen nudum, as the specimen seems to be lost from the collections of the Geological Institute of Hungary, Budapest). This theropod tooth suggests that colonization of the Haţeg Island had already occurred by the Coniacian.

Based on this evidence, the continuity of an emergent landmass can be suggested from the Cenomanian to the Maastrichtian, this landmass occupying more or less the same area as the larger surroundings of the present-day Haţeg Basin.

5. Sedimentary setting, palaeoclimate, and fauna

While Nopcsa (1914a) interpreted the Haţeg deposits as fluvial-lacustrine, but representing mainly lakes and swamps subject to periodic inundations, more recent work (e.g. Grigorescu, 1983; Van Itterbeeck *et al.* 2004; Bojar *et al.*, 2005; Therrien, 2005, 2006) has identified a wider range of sedimentary settings. Further, the stratigraphy has been clarified since Nopcsa's day. There are two successions of Maastrichtian, the Densuş-Ciula and Sânpetru formations, both representing molasse-type deposits (Grigorescu, 1992);

whether these represent also the lower Paleogene is yet to be substantiated. The Densuş-Ciula Formation occurs in the northwestern part of the Haţeg Basin, and it has a total thickness of some 4 km. The Sânpetru Formation crops out mainly along the Râul Mare and Sibisel valleys, and it is up to 2.5 km thick.

The Densuş-Ciula Formation is divided into three members. The lower member consists of volcano-sedimentary sequences interlayered with lacustrine marls. The thick middle portion consists of matrix-supported conglomerates, massive and cross-bedded sandstones, and massive red, brown, and green-grey mudstones. These deposits have yielded diverse microvertebrates (Grigorescu *et al.*, 1999; Csiki *et al.*, 2008) including multituberculates (Csiki and Grigorescu, 2000), dinosaur bones and eggs (Grigorescu *et al.*, 1990), as well as mollusc shells and plants (Antonescu *et al.*, 1983). The upper part of the formation, possibly Paleogene in age, lacks volcaniclastic sediments and dinosaur remains.

The Sânpetru Formation is almost devoid of volcaniclastic sediments, but dinosaur and other vertebrate bones are common at certain levels. The sedimentology is primarily alluvial, including coarse, unsorted debris flows, channels, and overbank deposits (Grigorescu, 1992). Stream types were mainly braided, yielding gravel and sandy channel infills, gravel and sandy bars, sandy and silty levees and crevasse splays, and silty and clayey overbank deposits. Meandering streams are rarer, in association with well-drained and poorly drained floodplain deposits, the first associated with calcrete paleosols, and the second with hydromorphic paleosols (Therrien, 2005). Vertebrate fossils are found mainly in the lower part of the Sânpetru Formation, and their rarity in the upper part of the formation led some previous workers to mistakenly place the Cretaceous-Tertiary boundary at the transition between the lower and upper Sânpetru Formation (Therrien, 2006). In the upper Sânpetru Formation, the sediments indicate an episode of rapid uplift of the sediment source area and the creation of extensive wetlands, not conducive to preservation of large-scale bone accumulations (Csiki *et al.*, this volume). Climates were subtropical, with rainy and dry seasons, as indicated by study of paleosols (Van Itterbeeck *et al.*, 2004; Therrien, 2005), the mixed floras of ferns and *Normapolles-Postnormapolles* angiosperms (Antonenscu *et al.*, 1983), and stable isotopes. Carbon isotopes indicate 'dry woodland' conditions (Bojar *et al.*, 2005), and oxygen isotopes suggest a mean annual temperature of about 20-25^{o}C. Rainfall estimates differ between evidence from plants and from paleosols. The paleosols indicate a climate characterized by seasonal precipitation in which evapotranspiration exceeded precipitation, and where the water table fluctuated during the year (Therrien, 2005). Geochemistry of the paleosols indicates that palaeoprecipitation was less than 1000 mm/year, significantly lower than estimates from the tropical palaeoflora of the region (1300–2500 mm/year year – Pop and Petrescu, 1983). The difference in estimates may be explained by the fact that tropical plants can live in warm, monsoonal climates if they have access to sufficient water during the dry seasons to satisfy their metabolic needs, and so the palaeobotanical value was perhaps an overestimate (Therrien, 2005).

Over 70 vertebrate taxa make up the Haţeg faunal assemblage, divided into aquatic (rare fishes), semi-aquatic (frogs, albanerpetontids, turtles, crocodilians), aerial (pterosaurs, birds), and terrestrial (lizards, snakes, dinosaurs, mammals) forms (e.g. Grigorescu *et al.*, 1985, 1999, 2002; Folie & Codrea, 2005; Grigorescu, 2005; Therrien, 2005). The fauna shows connections with the generalized Euramerican fauna of the Early Cretaceous (Weishampel *et al.*, this volume). Intermittent connections with surrounding landmasses were probably established during low sea-levels, and species may have passed between the Franco-Iberian landmass and Haţeg, as suggested for the second part of the Campanian by Csiki and Grigorescu (1998).

6. Dwarfed dinosaurs on islands

6.1. Background

The large size of dinosaurs has posed intriguing questions about their growth rates: did they have low metabolic rates and take decades or centuries to reach adult size, or did they have high metabolic rates and growth to full size very fast? Current work on bone histology suggests the latter (Sander, 2000; Erickson *et al.*, 2001; Padian *et al.*, 2001), and that growth followed a sigmoid curve, with a relatively slow growth rate in their first 1-5 years, an accelerated rate for 2-6 years, and then a levelling-off when sexual maturity and adult size were attained. Sexual maturity might have been achieved at 40-70% adult size in sauropods (Sander, 2000). Estimates of the time to achieve maximum size for the largest dinosaurs range from ten (Erickson *et al.*, 2001) to 26 (Sander, 2000) years, and the lower estimate would imply maximum growth rates of more than 5 tonnes per year during the juvenile growth spurt. Estimates of how long dinosaurs lived include 8-18 years and 13-14 years for the medium-sized theropods *Oviraptor* and *Deinonychus* (Erickson *et al.*, 2007), up to 28 years for *Tyrannosaurus* (Erickson *et al.*, 2004), and 38 years for the sauropod *Janenschia* (Sander, 2000). The estimates of age are made by counting lines of arrested growth (LAGs, sometimes called 'growth lines') that are best seen in fibrolamellar primary bone, indicating fast growth. As growth slowed in adult individuals, lamellar-zonal bone was laid down in the outer cortex, followed by denser bone with narrowly spaced LAGs in the outermost cortex.

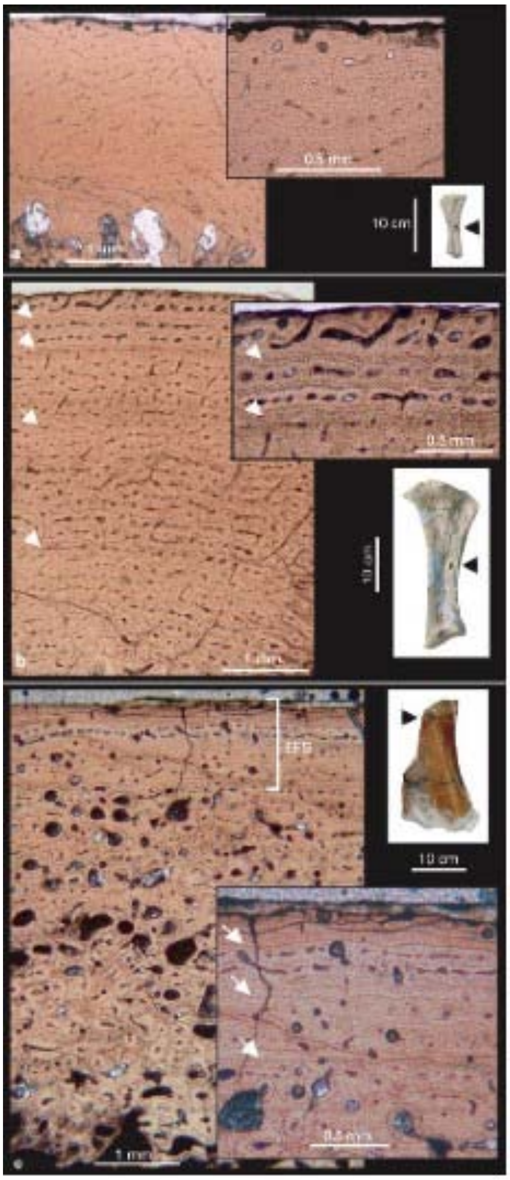
An island dwarf should be smaller than its closest mainland relative and show demonstrable evidence that it is a small adult and not a small juvenile. Such evidence includes morphological indicators of adulthood (fusion of the suture between the centrum and neural arch in vertebrae; firm junction or fusion of cranial sutures, especially of the braincase; long bones of adults should also have a surface of smooth lamellar bone (Callison & Quimby, 1984), well developed surface structures for muscle and ligament attachment (Coombs, 1986), well ossified articular ends, sharp and well developed processes, as well as bone histological indicators of slowing growth (transition to lamellar-zonal bone, and outer cortical bone with many closely spaced LAGs).

The best reported example of an insular dwarfed dinosaur analysed in detail is *Europasaurus* from the Kimmeridgian of northern Germany (Sander *et al.*, 2006). Individuals ranging in body length from 1.7 to 6.2 m show bone histological characteristics of juveniles to adults (Fig. 6). The 6.2 m long adult is one-third the length of its close relative *Camarasaurus* (18 m long). The largest *Europasaurus* shows these histological characters of adulthood:

(1) the inner cortical fibrolamellar bone is extensively remodelled by secondary osteons that nearly obliterate the primary bone;

(2) the outer cortex has closely-spaced LAGs indicating radical slow-down in growth rate;

(3) and the outer zone shows characteristics of being an external fundamental system (outer cortex is avascular and consists of lamellar bone).



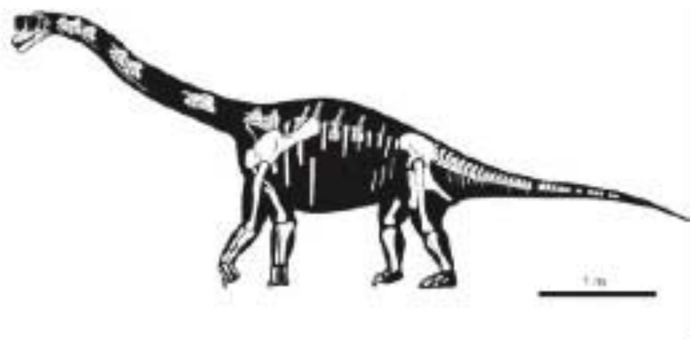


Fig. 6. Skeleton of *Europasaurus holgeri* Sander *et al.*, 2006, showing body outline (black) and known skeletal elements (white) against a 1 m scale, and histological growth series with sampled bones. a, Tibia from the smallest individual (body length 1.75 m). The reticular fibrolamellar tissue, which grades into laminar fibrolamellar tissue (inset), and the absence of growth marks indicate its juvenile status. b, Tibia from a mid-sized individual (body length 3.7 m). The cortex consists of laminar fibrolamellar bone interrupted by growth marks (arrows). Wide vascular canals opening to the outer bone surface (inset) indicate that this animal was still actively growing. c, Distal femur from the largest individual (body length 6.2 m). The external fundamental system (ESF; inset) indicates that it was fully grown. Bone surface is at the top of all photomicrographs. Black arrows indicate sample locations; white arrows indicate growth marks.

Palaeogeographic maps show that there were islands across northern Germany less than 200,000 km^2 in area, and these could have been large enough to support populations of dinosaurs, but small enough to induce dwarfing. The growth marks in the bones of *Europasaurus* suggest it achieved small size by a slowing of its growth rate, in contrast to the accelerated growth of giant sauropods (Padian *et al.*, 2001; Sander, 2004).

Further evidence for dinosaur dwarfing has also been reported for the ornithopods of the lowermost Cretaceous fissure fillings of Cornet, near Oradea, northwestern Romania (Benton *et al.*, 1997, 2006). First, the Cornet dinosaur fauna is more depauperate than a typical Wealden assemblage from England or continental western Europe, consisting of only four or five species, instead of ten or more, and the ornithopods are smaller on average than their European and North American counterparts. Insular adaptations are shown in the Romanian *Camptosaurus* sp., the iguanodontids, and dryosaurids, which are smaller than their west European and North American counterparts. The dryosaurid is at the lower end of the size ranges of *Dryosaurus* and *Valdosaurus*, the camptosaurid is two-thirds the size of its American and British relatives, and the iguanodontid is one-quarter to one-third the size of western European *Iguanodon*. Scaling to weight, an animal that is half the overall length of another of identical shape and proportions, weighs one-eighth as much (0.5 x 0.5 x 0.5 = 0.125). So, if a large American *Camptosaurus* weighed 700 kg (Peczkis, 1994), then the Cornet camptosaurid weighed only 87.5 kg. Likewise, if the range of body masses for different species of *Iguanodon* was 4 - 7 tonnes (Peczkis, 1994), the Cornet iguanodontid, at say one-third the length, weighed only one–twentyseventh (0.037) as much, a mere 150 - 260 kg. Dwarfing in the Cornet ornithopods may have occurred via progenetic paedomorphosis, i.e. retention of ancestral juvenile characteristics by earlier maturation in the descendant (Benton *et al.*, 2006), but skeletochronological study is still required.

Several other ancient island faunas have been identified. Some of the oldest come from the Late Triassic and Early Jurassic fissure faunas of Bristol and South Wales (Whiteside & Marshall, 2008). Palaeogeographic reconstructions show numerous small islands in the area, most only a few km across, and these were populated by small faunas, ranging from 2-

16 species, of basal reptiles, dinosaurs, and early mammals. Body sizes were small, normally less than 30 cm body length, and including rarely the 1.5 m-long prosauropod dinosaur *Thecodontosaurus*, which might be a dwarfed relative of larger, 7-m long prosauropods such as *Plateosaurus* and *Lufengosaurus*. Dalla Vecchia (2002) has reported a further example from the mid Cretaceous Adriatic Carbonate Platform, where he found a sauropod half the length of close relatives from North America, and postulated that this was a dwarfing phenomenon brought about by a rise in sea levels and temporary formation of islands. Dinosaurs from the Early and Late Cretaceous of the area were of more normal size, linked with more extensive landmasses at those times, and suggesting a temporary dwarfing phenomenon. Island-dwelling dinosaurs have also been reported from the Late Cretaceous of New Zealand (Molnar & Wiffen, 1994) and the Chatham Islands nearby (Stilwell *et al.*, 2006). The area consisted of small islands at the time, and and some of these taxa may also represent dwarfed dinosaurs, although more specimens and histological studies are required to be sure.

6.2. Impoverishment of the Haţeg dinosaur faunas

Nopcsa (1914a) and others have suggested that the Haţeg dinosaurian faunas were impoverished, and this has been used as key evidence that they lived on an island. The low diversity Nopcsa detected was partly a result of limited collecting, and totals of dinosaurian species from Haţeg are much higher now: 13 (Sânpetru Formation) and 10 (Densuş-Ciula Formation), some of them as yet unnamed (Weishampel *et al.*, 2004; Table 2). It turns out that the Haţeg dinosaurian fauna is impoverished on a global scale, but that all European faunas of the age are similarly impoverished.

Sânpetru Formation	Densuş-Ciula Formation
1. *Bradycneme draculae* (?troodontid)	1. Dromaeosauridae indet.
2. *Elopteryx nopcsai* (?troodontid or alvarezsaurid?)	2. Unnamed theropod
3. *Heptasteornis andrewsi* (?troodontid)	3. Oviraptorosauria indet.
4. cf. *Saurornitholestes* sp.	
5. cf. *Euronychodon* sp. (?troodontid)	4. cf. *Euronychodon* sp.
6. cf. *Paronychodon* sp.	
7. cf. *Richardoestesia* sp.	5. cf. *Richardoestesia* sp.
8. *Magyarosaurus dacus* (titanosaurid)	6. *Magyarosaurus dacus*
9. Titanosauria n. gen et sp.	7. Titanosauria n. gen et sp.
10. *Struthiosaurus transylvanicus* (nodosaurid)	
11. *Zalmoxes robustus* (euornithopod)	8. *Zalmoxes robustus*
12. *Zalmoxes shqiperorum* (euornithopod)	9. *Zalmoxes shqiperorum*
13. *Telmatosaurus transsylvanicus* (hadrosaurid)	10. *Telmatosaurus transsylvanicus*

Table 2. The dinosaurs of the two stratigraphic units, of equivalent age that comprise the Hațeg faunas. Data from Weishampel *et al.* (2004).

Latest Cretaceous (Campanian, Maastrichtian) dinosaurian faunas from North America and Asia frequently exceed 30 or 40 taxa (e.g. Hell Creek Formation of South Dakota, Wyoming, and Montana, Lance Formation of Wyoming, Scollard and Horseshoe Canyon formations of Alberta, Djadokhta and Nemegt formations of Mongolia). Within Europe, the Sânpetru Formation yields the richest Late Cretaceous dinosaurian fauna, somewhat more diverse than French and Spanish dinosaur sites of similar age. The nearest contenders are the Grès à Reptiles Formation of Bouches-du-Rhône and Var in France, with ten taxa, the Aren Formation in Lleida, Spain, with eight, and the Grès de Saint-Chinian in Hérault, France, with seven, and others have as few as two or three reported taxa (Weishampel *et al.*, 2004). Some of these western European dinosaurian faunas may be particularly limited because they have not been so thoroughly collected as those from Romania, and some are sampled from only one site.

European latest Cretaceous dinosaurian faunas are all impoverished in numbers, but also in ecological types and major clades. Missing groups throughout Europe include large theropods, ankylosaurids, pachycephalosaurs, ceratopsians (but see Godefroit & Lambert, 2007). The Haţeg faunas show closest similarities to Late Cretaceous faunas from Gosau in Austria, southern France, and Spain (Holtz *et al.*, 2004) as well as Hungary (Ősi and Rabi, 2006), all of which share rhabdodontid ornithopods and ankylosaurs of the *Struthiosaurus*-group (Weishampel *et al.*, 1991). However, other taxa such as the sauropods *Ampelosaurus* and *Lirainosaurus*, the ?dromaeosaurid *Variraptor*, abelisaurs, and the bird *Gargantuavis*, known from the western European faunas, are absent from Haţeg, possibly indicating the need for more collecting, but not really showing that Haţeg island was depauperate in comparison to other European faunas. All these dinosaurian faunas throughout Romania, southern France, and Spain are depauperate on a global scale, and all apparently occupied islands.

6.3. Primitiveness of the taxa

The Haţeg dinosaurs generally occupy basal positions in cladograms. *Magyarosaurus* requires anatomical revision, and it has not yet been included in a cladistic analysis. *Telmatosaurus* is the basalmost hadrosaur, sister taxon to Euhadrosauria (Weishampel *et al.*, 1993). This position so low in the phylogeny is unexpected for a Maastrichtian hadrosaur, and it places *Telmatosaurus* phylogenetically below hadrosaurids from the Santonian and

Campanian of North America and Asia, so introducing a ghost lineage of some 15 myr.

Zalmoxes is even more of a 'living fossil' in the Haţeg fauna. In their cladistic analysis, Weishampel *et al.* (2003) found that the two species of *Zalmoxes* pair with *Rhadodon*, known from the Campanian and Maastrichtian of France and Spain, as the new family Rhabdodontidae, sister clade of Iguanodontia, confirming a long-held assumption. Iguanodontians are primarily from the Early Cretaceous, and this implies a ghost lineage of 73 My, connecting the latest Cretaceous rhabdodontids with their closest, Early Cretaceous, relatives. In this case, *Zalmoxes* is not uniquely primitive to Haţeg Island, but the whole ornithopod fauna of Europe in the latest Cretaceous appears to be relictual, consisting of derived non-iguanodontian ornithopods, with rare hadrosaurids, rather than the hadrosaurids that dominated elsewhere in the Northern Hemisphere.

Finally, *Struthiosaurus* shows the same kind of low phylogenetic position, interpreted either as a basal nodosaurid (e.g. Ösi, 2005) or as a basal ankylosaurian (Vickaryous *et al*., 2001). The basal position of *Struthiosaurus* would result in a similarly long (about 55 My) ghost lineage in the first case, or even a more extended one (at least 85 My) as its the sister-taxa are Late Jurassic (possibly even Middle Jurassic) in age. The relictual status of *Struthiosaurus* appears similar to that of *Zalmoxes.*

The common occurrence of relictual taxa across Europe in the latest Cretaceous suggests that there must have been communication earlier than the late Campanian and Maastrichtian: dinosaurian genera have durations estimated at typically 5-10 myr (Dodson, 1990). Whether these genera were formerly ubiquitous and their range was split by the division of a larger landmass into islands, so stimulating vicariant phylogenetic events, or whether the Haţeg dinosaurs dispersed from the mainland by island hopping, as first suggested by Nopcsa (1923), is not clear (Weishampel *et al.*, 1991).

6.4. Dwarfing in the Haţeg dinosaurs

Dwarfing has been suggested in *Magyarosaurus*, *Zalmoxes*, and *Telmatosaurus* from Haţeg, but not in the other taxa. The ankylosaur *Struthiosaurus* and the unnamed pterosaur (Jianu *et al.*, 1999) are smaller than expected compared to their close relatives, but it has yet to be shown that the specimens come from mature adults or subadults. In contrast, the dromaeosaurid and troodontid theropods from Haţeg, although small, are no smaller than are their close relatives in Asia and North America. Only the pterosaur *Hatzegopteryx* (Buffetaut *et al.* 2002) is large, with an estimated wingspan of 12 m, in the range of the largest members of the azhdarchid clade. The crocodilians, turtles, and mammals are no smaller or larger than their relatives elsewhere.

The titanosaurid sauropod *Magyarosaurus*, with an estimated body length of 5-6 m, was much smaller than its relatives, which ranged from the 7 m long *Saltasaurus* to the 25-20-m long *Argentinosaurus* (Jianu & Weishampel, 1999). From a study of humeri, these authors found that *Magyarosaurus* was the smallest of the adult neosauropods they studied, that the humeri appear to be more similar to those of subadults than to adults of other taxa, that the dwarfing is apomorphic, and that the scaling suggests *Magyarosaurus* achieved small size as an adult by paedomorphosis.

The hadrosaurid *Telmatosaurus* was about 4 m long, which contrasts with mean body lengths for other hadrosaurids of 7-10 m.

The two species of *Zalmoxes* achieved different estimated lengths, up to 3 m (*Z. robustus*), and 4.0-4.5 m (*Z. shqiperorum*), larger than some basal relatives, such as *Hypsilophodon* from the Early Cretaceous (2.3 m long), similar in length to others, such as *Thescelosaurus* from the Late Cretaceous (3-4 m), but smaller than other relatives, such as

Camptosaurus from the Late Jurassic (5-7 m) and *Tenontosaurus* from the mid Cretaceous (7-8 m). Weishampel *et al*. (2003) presented an analysis of growth and heterochrony, based on measurements of femoral length and midshaft width in ontogenetic samples of *Zalmoxes robustus*, *Z. shqiperorum*, *Rhabdodon priscus*, *Tenontosaurus tilletti*, *Hypsilophodon foxii* and *Orodromeus makelai*. Pair-wise comparisons of the growth series of these euornithopods (e.g. between *Zalmoxes* and *Orodromeus*, between *Zalmoxes* and *Tenontosaurus*, etc.) revealed that the relative ontogenetic change in femoral shape is statistically indistinguishable between taxa ($P > 0.2$), except between *T. tilletti* and *Z. robustus* ($P = 0.03$). In other words, femoral proportions changed in the same fashion in ontogenetic series of *Hypsilophodon*, *Thescelosaurus*, *Rhabdodon* and *Zalmoxes*.

Weishampel *et al*. (2003) went on to evaluate heterochrony in *Zalmoxes* by plotting maximal (adult?) femoral length of terminal taxa on their cladogram of basal euornithopods and comparing each node from the base of the tree to *Tenontosaurus*. The femurs of both *Orodromeus* and *Hypsilophodon* reached a length of 17 cm. At the other extreme, the largest femora (54–58 cm) belong to *Tenontosaurus* and *Rhabdodon*. The femora of *Zalmoxes shqiperorum* and *Z. robustus* are intermediate in length (47 cm and 36 cm, respectively). Through optimization of maximal femur length onto the cladogram, Weishampel *et al*. (2003) identified a a peramorphic trend (peramorphocline) from basal euornithopods such as *Orodromeus* through more highly positioned taxa such as *Hypsilophodon*, *Rhabdodon* and *Tenontosaurus*. However, downsizing occured in *Zalmoxes shqiperorum* and more so in *Z. robustus* (a 36% decrease in length).

As adults, the sauropod *Magyarosaurus* and the ornithopod *Telmatosaurus* seem to have been about one half the length of their closest relatives from elsewhere, and a linear reduction of one half, corresponds to much reduced body masses, about one-eighth (0.5^3 =

0.125). Downsizing in *Zalmoxes robustus* to 64% corresponds to a reduction in mass to 26% (0.64^3), about one quarter. These reduced body masses are more meaningful biologically speaking in terms of metabolism and the amount of food required by the insular Hațeg dinosaurs.

6.5. Juvenile characters in the Hațeg dinosaurs

The Hațeg hadrosaurid *Telmatosaurus* is smaller than more basal iguanodontians like *Ouranosaurus* and *Iguanodon*, and its teeth retain features found in juveniles of these last-mentioned taxa (Weishampel *et al.*, 1993). The upper teeth of *Telmatosaurus* are narrow, diamond-shaped, and equipped with a single centrally placed ridge, most like the juvenile condition seen in non-hadrosaurid iguanodontians, but also similar to the typical maxillary teeth of more derived hadrosaurids. Its lower teeth, in contrast, are wider, asymmetrical, and bear several low ridges, making them intermediate between those of other hadrosaurids and more primitive iguanodontians. They too were small, but most resemble the shape of adults of non-hadrosaurid iguanodontians. Weishampel *et al.* (1993) regarded this juvenilization of the teeth in *Telmatosaurus* as marking an important step in the evolution of hadrosaurid dentitions from those of ancestral iguanodontians, but also as auxiliary evidence of dwarfing by paedomorphosis in this taxon.

The small adult size of most of the better-known Hațeg dinosaurs is also supported by osteological correlatives of growth cessation. Complete fusion of neurocentral sutures in vertebrae (Galton, 1981, 1982; Coombs, 1982; Britt and Naylor, 1994; Chure *et al.*, 1994) is a commonly observed phenomenon in small ornithopod dorsal vertebrae, around 30 mm in length. Even in a juvenile specimen of *Zalmoxes shqiperorum* (FGGUB specimen; Weishampel *et al*., 2003), the neurocentral suture is not fused only in the anterior dorsals

(15 mm in length), while it is fused, although still visible, in the more posterior ones. Some of the smallest known titanosaur dorsals from Haţeg (such as BMNH R.4896; centrum length about 80 mm) show the neurocentral suture completely obliterated, suggesting a post-juvenile developmental stage.

Specimens of *Zalmoxes* show changes in certain osteological features and in relative proportions with growth that match findings in other taxa, such as *Tenontosaurus*, and so confirm that the larger examples of the Haţeg taxon are indeed probably adults. For example, Weishampel *et al*. (2003) note several changes seen in ontogenetic series of *Zalmoxes*: increase in the number of tooth families in the dentary from 8 to 10, change in dentary shape from markedly convex ventrally in small individuals to more or less parallel-sided in adults, lengthening and increasing angularity of the deltopectoral crest, increasing prominence of the anterior trochanter and a slight distal shift in the position of the fourth trochanter in the femur, increasing robustness of the tibia, with a larger cnemial crest, and changes in hindlimb proportions from a relatively short femur in juveniles to one that equals or exceeds the length of the tibia in adults.

Of the three heterochronic processes that can produce paedomorphosis, neoteny and post-displacement can lead to adults of the same size as the unaffected relatives, whereas progenesis usually leads to adults of reduced size (Gould, 1977; Alberch *et al.*, 1979). In many, or most, cases of dwarfing in Pleistocene mammals, as noted above, selection for small body size was likely the driver, and morphological change through progenesis the consequence. Additional evidence for progenesis is the retention of anatomically juvenile characters in adult *Telmatosaurus*: this suggests that development of the dentition finished early by comparison with larger close relatives, and so this points to progenesis (early offset) as the heterochronic process.

6.6. Bone histological evidence for dwarfing in the Haţeg dinosaurs

We investigated the bone histology of all Haţeg dinosaur species for which long bone material from different-sized individuals is available. Two of the species show unequivocal evidence that they were fully grown at a small body size compared to their sister taxon. These are the largest members of the fauna, *Magyarosaurus dacus* and *Telmatosaurus transylvanicus*. The rhabdodontids *Zalmoxes robustus* and *Z. sqhiperorum* cannot unequivocally be interpreted as being fully grown, although they are certainly not juveniles. Stein et al (2010) sampled an ontogenetic series of *Magyarosaurus dacus* long bones (Fig. 7) and found that they show a histology that is only seen in very large and senescent individuals of other sauropod species. The cortex of even the smallest specimen in the *Magyarosaurus dacus* growth series (45% maximum size) is dominated by secondary osteons, with only a few islands of remnant primary fibrolamellar bone with a strong lamellar component in the bone matrix (Fig. 8a-d). Although the only unequivocal sign of a fully grown specimen is the presence of an external fundamental system (EFS) in the outermost bone cortex, none was observed in the entire sample set. However, the advanced secondary remodelling is typical of the oldest histological ontogenetic stages (HOS) (stage 12-13) of large-sized sauropods, when even the EFS has been remodelled (Klein and Sander, 2008). In addition, an EFS is easily destroyed by rough mechanical cleaning of the bone surface. In the many other sauropod taxa studied so far (Sander, 2000; Klein and Sander, 2008), a completely remodelled long bone cortex occurs only in the largest and fully grown specimens of e.g. *Apatosaurus* (femur length 1800 mm) and *Supersaurus* (ulna length 1250 mm). The largest *Magyarosaurus* long bones are only a fraction of that size (femur length 550 mm, humerus length 490 mm).

The secondary bone remodelling has continually and progressively obliterated the primary growth record in *Magyarosaurus dacus* long bones, and thus any growth marks or other indicators of the growth rate. Therefore, it is difficult to say how fast *Magyarosaurus* really grew. However, the strong lamellar component of the remnant primary bone suggests a slower growth rate than in large-sized sauropods (Fig. 8e), but still faster than ectothermic reptiles, which have a lamellar zonal bone dominated cortex (Fig. 8f). The extensive remodelling and remnant primary bone indicate that the largest *Magyarosaurus dacus* specimens had attained full size, and that the species achieved its small size by a slowing of the growth rate, analogous to *Europasaurus* (Stein et al, 2010).

Fig. 7. Photographs of some of the sampled titanosaur bones from the Maastrichtian of Romania. (a-d) *Magyarosaurus dacus* humeri, specimens: (a) MAFI Ob. 3092 (the smallest recorded body size, 45% maximum size); (b) FGGUB R.1246 (65% maximum size); (c) MAFI v.13492 (76% maximum size); (d) FGGUB R.1048 (largest known specimen). (e) "*Magyarosaurus*" *hungaricus*, MAFI Ob.3104. Scale bar equals 100 mm.

LeLoeuff (2005) questioned the dwarfing of *Magyarosaurus dacus*, based on the presence of large long bones in the collection of the BMNH and the MAFI. However, bone histological features (larger size, but earlier HOS than the largest *Magyarosaurus dacus* specimen) suggest that these bones belong to a different taxon (Stein *et al.*, 2010) and are listed in Table 2 as "titanosaurid indet." The presence of a larger sauropod species together with *M. dacus* may be explained by chance dispersal from the mainland.

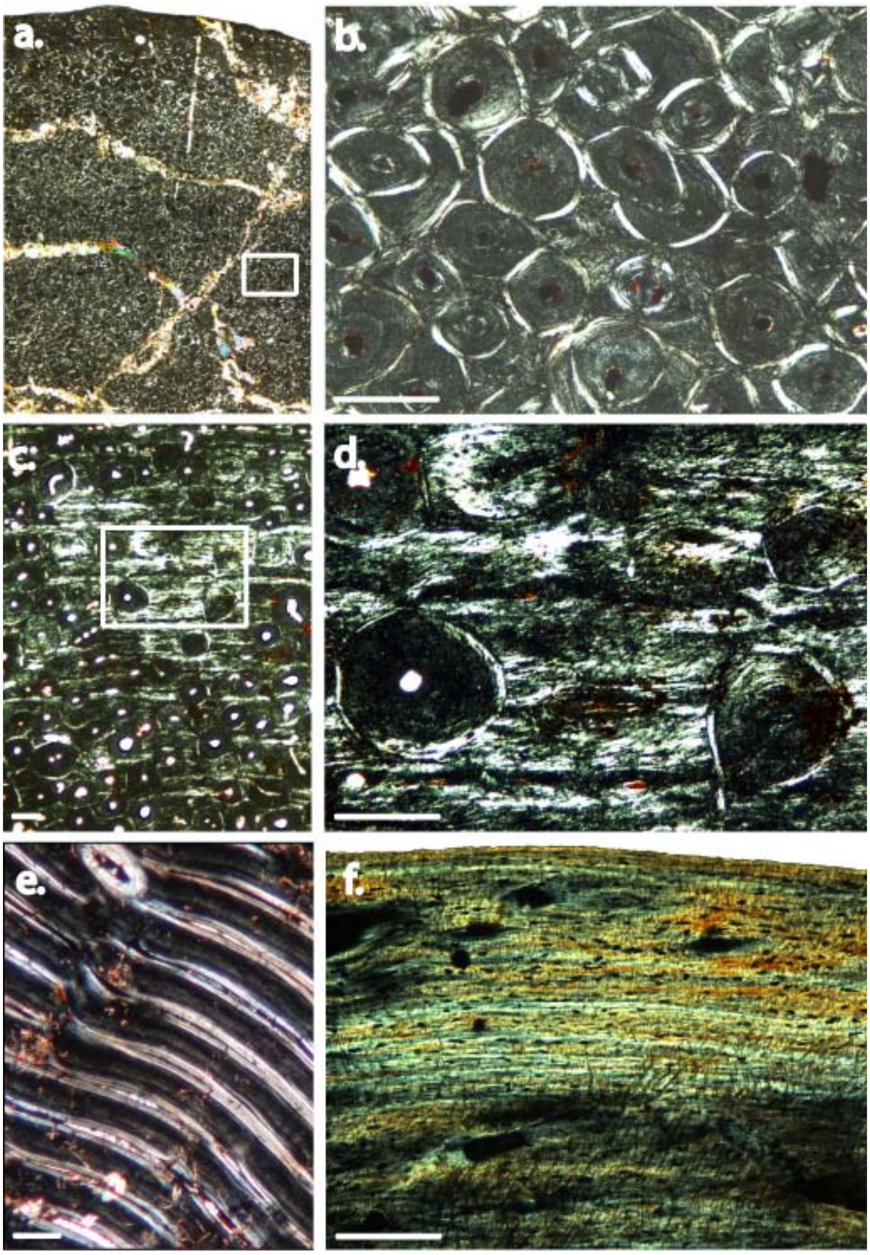


Fig. 8. Long bone histology of *Magyarosaurus dacus* compared with other vertebrates. (a-b) Long bone histology of *Magyarosaurus dacus*: *(*a) overview of cross section (specimen MAFI V.13492, 76% max size); (b) close-up of (a): cortex dominated by secondary remodelling. (c) Close-up of largely interstitial laminar primary bone in outermost cortex of the smallest available specimen of *Magyarosaurus dacus* (MAFI Ob.3092, 46% max size). The vascular canals are oriented circumferentially as in laminar fibrolamellar bone, but the bone matrix between the vascular canals consists largely of parallel-fibered and lamellar bone, with only a minute fraction of fibrous (or woven) bone tissue. (d) Close-up of (c). (e) Laminar fibrolamellar bone of *Apatosaurus*. (f) Alligator long bone histology showing lamellar-zonal bone for comparison. All scale bars equal 200 µm.

Long bones of the hadrosaurid *Telmatosaurus trannsylvanicus* were sampled from a wide range of ontogenetic stages, from hatchlings to fully grown specimens. The primary bone tissue consists of fibrolamellar bone with reticular primary osteon organisation in hatchlings and laminar organization in subadults (Fig. 9A, B) and adults (Fig. 9C). The fibrolamellar bone matrix has a strong lamellar component, analogous to *Magyarosaurus dacus*, suggesting a slow growth rate. Open vascular canals at and near the bone surface were only found in the subadult specimen and the hatchlings. These are an unequivocal sign of active growth at the time of death.

The largest long bones (Fig. 9C) show dense secondary remodelling in the inner cortex, but secondary osteons become more scattered closer to the surface of the bone, similar to remodelling of adult specimens of the hadrosaur *Maiasaura peeblesorum* (Horner *et al.* 2000). The largest specimens also preserve a high number of growth marks (eight in total). This supports the suggestion that the animal was fully grown. An EFS could not be observed due to abrasion of bone surfaces. The largest *Telmatosaurus* specimens (femur length, 460 mm) were thus most likely adult and significantly smaller than other adult hadrosaurs (*Maiasaura peeblesorum*: 1000 mm, Horner *et al.*, 2000).

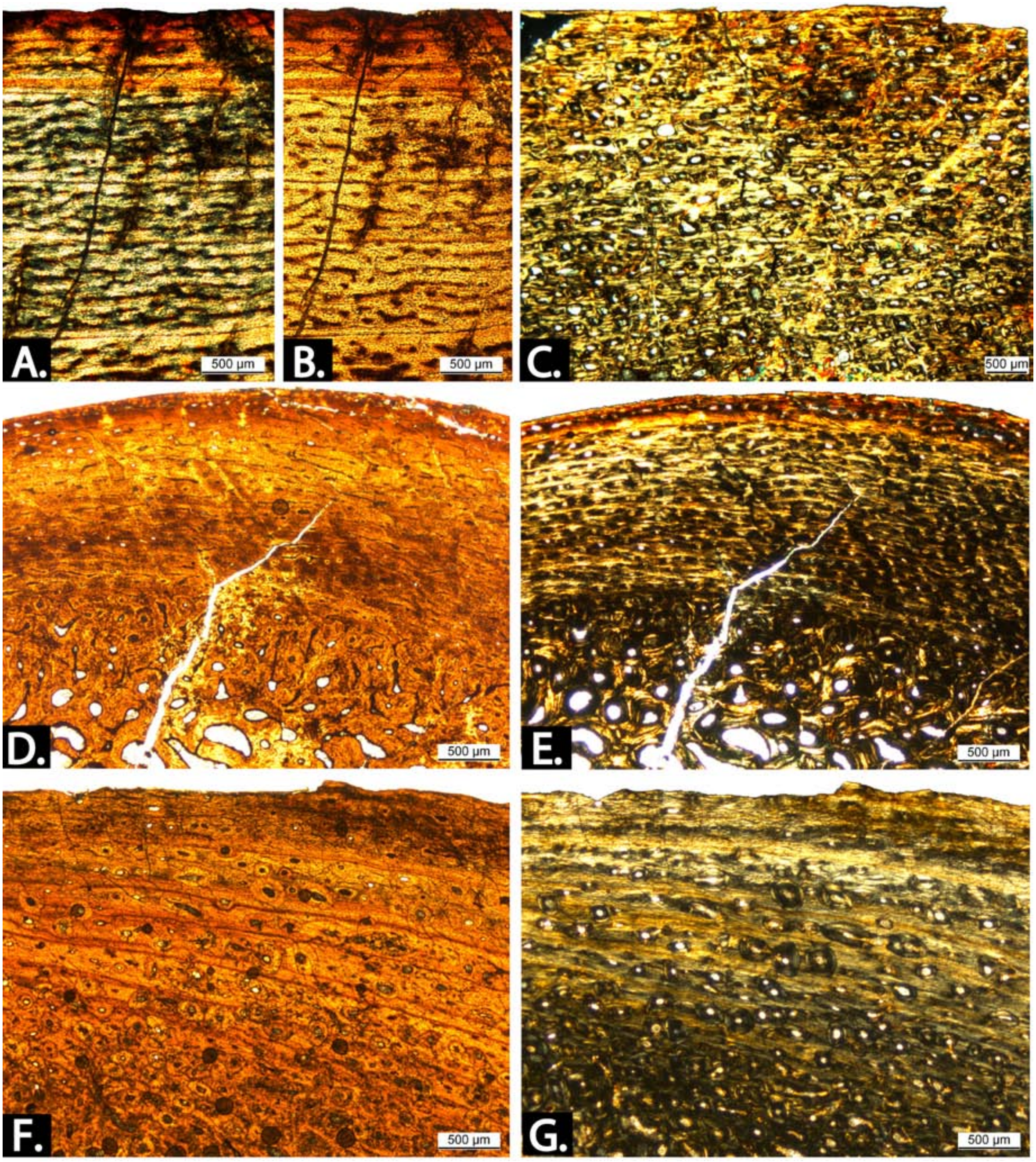


Fig. 9. Histology of the long bones of the ornithopods *Telmatosaurus trannsylvanicus* (A-C), *Zalmoxes shqiperorum* (D-E), and *Z. robustus* (F-G). (A) Overview of the bone histology of the femur of subadult specimen R1832 (femur length 25 cm). No secondary remodelling has altered the primary bone of the middle and outer cortex yet. Primary bone consists of fibrolamellar bone tissue with reticular organisation of the primary osteons. Growth marks occurr regularly spaced throughout the cortex. (B) Same view in polarized light. (C) The femur of the oldest adult specimen Ob.3130 (estimate of femur length around 46 cm = max size) reveals secondary remodelling of the primary bone up to the outer cortex. The bone surface is missing and thus no EFS was observed. View in polarized light. (D) Long

bone histology of *Zalmoxes shqiperorum*. The femur (length 16.4 cm) of the subadult specimen BMNH R1088 shows that secondary remodelling is restricted to the inner cortex indicating a late onset of remodelling. Primary osteons occur in longitudinal and reticular organization. Vascular canals open to the bone surface indicating active growth at the time of death. (E) Same view in polarized light. (F) Bone histology in the femur of *Zalmoxes robustus* BMNH R1392 (estimate of femur length 28 cm). The high number of growth marks (11; only 7 seen in this view) indicates an adult stage of this specimen. Remodelling is dense in the inner cortex and scattered in the middle cortex. (G) Same view in polarized light. Scale bars equal 500 µm.

The ornithopod *Zalmoxes* was sampled from subadult (Fig. 9D, E) and adult (Fig. 9F, G) femora and humeri. *Zalmoxes* has a remarkable histology in terms of a late onset of the secondary remodelling in the subadult stage (Fig. 9D, E). In the oldest specimens, dense remodelling is restricted to the inner cortex. Isolated to scattered secondary osteons occur in the middle cortex, and are absent in the outermost cortex (Fig. 9F, G). In addition, vascular canals opening to the bone surface in all sampled specimens indicate that a fully grown stage had not been reached yet. Nevertheless, from the high number of growth marks (up to 13 in *Z. robustus* and 7 in *Z. shqiperorum*), a juvenile stage can be excluded for these bones. Bone histology also shows that *Z. robustus* is smaller than *Z. shqiperorum* at the same ontogenetic stage, as has been suggested before by bone morphology (Weishampel *et al.*, 2003). Femur lengths between 250 and 320 mm were estimated for *Z. robustus*, and between 333 to 355 mm for *Z. shqiperorum*. The high number of growth marks compared to the femur lengths suggests a slow growth rate for *Z. shqiperorum* and very slow for *Z. robustus*. Although the sampled specimens had probably not reached their maximal size yet, femur lengths of fully grown *Z. shqiperorum* and *Z. robustus* would still be significantly smaller than those of other euornithopods (557 mm in *Tenontosaurus dossi*

(Winkler et al 1997); 400 mm in *Tenontosaurus tilletti* (Forster, 1990); 544 mm in the small iguanodontid *Camptosaurus dispar* (Paul, 2008), and 600 mm in *Rhabdodon priscus* (Garcia *et al*., 1999)). Dwarfing may therefore be suggested for *Zalmoxes* but cannot convincingly be confirmed by bone histology.

7. Conclusions

The island rule is hotly debated among evolutionary biologists, with some finding it is a general principle that applies to modern island-living mammals, birds, and some other groups, whereas others reject the rule, saying it is an artefact of poor statistical analysis. Both sides agree, however, that many large animals on islands have become small, while many small animals have become larger.

Many reasons for the size changes on islands have been proposed. The commonest explanation for size reduction (dwarfing) is shortage of resources, whereas size increase is explained most commonly as a response to the absence of larger competitors and the absence of predation.

Island dwarfing among Pliocene, Pleistocene, and Holocene mammals of the Mediterranean was noted first by Victorian palaeontologists, and many examples of dwarfing among elephants, deer, hippos, and other herbivores are noted. These studies of dwarfed elephants and giant dormice on Malta have entered school textbooks. Franz Nopcsa was first in 1914 to suggest that the dinosaurs from the latest Cretaceous of Haţeg had lived on an island, and had undergone dwarfing by comparison with the nearest relatives on larger landmasses, particularly in the area of France-Spain and in North America.

Our studies confirm that Haţeg was probably an island, and that at least two of the herbivorous dinosaurs, the sauropod *Magyarsaurus* and the ornithopod *Telmatosaurus*, an possibly also the ornithopod *Zalmoxes*, are indeed reduced in size as adults, and their dwarfing arose through paedomorphosis, and possibly progenesis. The evidence comes from measurements of relative limb lengths and evidence for allometric shape change in the small-sized adults, as well as bone histology.

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Chapter 7

Synthesis

1. Bone histology as a palaeontological tool

Bone histological research has in the last decade produced revolutionary knowledge about different aspects of the palaeobiology and evolution of sauropods and dinosaurs in general. Although the first explorative palaeohistological studies took place more than 50 years ago (Enlow and Brown 1957; Riqcles 1968a, b), only in the last decade, partly made possible by new methods (Chapter 2), have signals in the bone microstructure of extinct (and extant) vertebrates become fully appreciated, though not nearly fully understood. These signals may provide significant information about the evolutionary history, life history, ecology and biomechanics of the animals being studied. In this dissertation, mostly evolutionary history, life history and palaeoecological aspects of sauropodomorph bone histology were emphasized, and biomechanics were only briefly touched upon. A more meticulous appreciation of the biomechanical aspects of sauropodomorph bone histology can be found in Pyzalla et al. (2006) and Dumont et al. (2009; in press).

2. Sauropodomorph body size evolution

2.1 The evolution of gigantism and fibrolamellar bone

Sauropodomorphs evolved from small (facultative) bipedal animals, not larger than a dog, to the largest land living animals this planet has ever seen. How these animals were able to get so big is a question that has captured palaeontologists and evolutionary biologists through the ages (see Chapter 1). Part of the answer can be found in sauropod and sauropodomorph bone microstructure. Fibrolamellar bone (FLB) was one of several evolutionary innovations (Sander and Clauss 2008; Sander et al. 2010), allowing sauropods to grow at rates that rival those of mammals.

In the current work, FLB was documented in the long bones of the earliest sauropodomorphs (Chapter 4). The observation of growth marks in these early sauropodomorphs indeed suggests their growth was interrupted at certain time intervals. These growth marks are rarely observed in sauropods, indicating that early sauropodomorph bone microstructure was a preadaptation for the growth rate acceleration in sauropods, as already suggested by Sander and Klein (2005).

Through the evolution of sauropodomorph bone microstructure, another transition occurs in the organization of the vascular canals. Small early sauropodomorphs mostly have a longitudinal to plexiform vascular architecture. In larger sauropodomorphs more circumferential (laminar) vascular canals are formed, which culminates in the laminar FLB of even the earliest sauropods like *Isanosaurus* (Sander et al. 2004), *Spinophorosaurus* and *Jobaria* (K. Stein pers. obs.). This trend towards laminarity can also be observed throughout the ontogeny of single sauropod species (Klein and Sander 2008), suggesting that vascular architecture is a compromise between growth rate and biomechanical requirements (cf. De Margerie et al. 2004).

FLB convergently evolved in large mammals (Chapter 4). Preliminary data suggests that FLB in sauropodomorphs was formed with a different osteoblastic strategy than in mammals. Sauropodomopha have moderately sized cells (Chapter 3), but a high cell density, and mammals have big cells, but less per unit volume of bone. A high cell density in sauropodomorphs compared to mammals, and certainly to poikilotherms, indicates high rates of osteoblast mitosis. This translates into apposition rates that are only seen in endothermic vertebrates today.

Some poikilotherms, like the giant boid snake *Titanoboa* (Head et al. 2009) and the giant crocodylian *Deinosuchus* (Erickson and Brochu 1999) grew to a large size, but did so by prolonging life history in a greenhouse environment. *Deinosuchus* therefore was able to reach 8 to 10 m in length with a lamellar-zonal bone pattern (Erickson and Brochu 1999). Furthermore, sauropod growth rates, measured from rare growth mark data, indicate that body mass increased a few tons per year during the active phase of growth, and that full size was reached in less than four decades (Curry 1999; Sander 2000; Erickson et al. 2001; Sander and Tückmantel 2003; Wings et al. 2007; Lehman and Woodward 2008; Sander et al. *in press*). Such growth rates are not compatible with the metabolic rate of any modern bradymetabolic terrestrial vertebrate, and therefore points to tachymetabolic endothermy in sauropods (Sander et al. *in press*). It thus appears that for the evolution of sauropod gigantism, and large terrestrial vertebrate body size in general, a high growth rate, and a tachymetabolic physiology are prerequisites. This hypothesis is supported by the seminal observation of Case (1978a) that growth rate and metabolic rate are closely linked in extant amniotes, but also by a new paradigm in evolutionary ecology, the Metabolic Theory of Ecology (Gillooly et al. 2001; West et al. 2001; Brown 2004).

2.2 Dwarfism/nanism in sauropodomorphs

The presence of fibrolamellar bone in island dwarf sauropods suggests that they had a reduced growth rate, but still had a relatively high metabolic rate. The basal macronarian *Europasaurus* achieved to lower its growth rate by reintroducing inactive periods of growth (Sander et al. 2006). Calculating mass from limb bone circumference (Stein et al. 2010, Chapter 5) and using growth marks as a proxy for age (Sander et al. 2006), we were able to estimate its maximal growth rate. On the classic maximum growth rate vs. adult mass graphs of Case (1978a,b), *Europasaurus* plots on the trend line for dinosaurs, but returned to more basal sauropodomorph growth dynamics (Fig. 1).

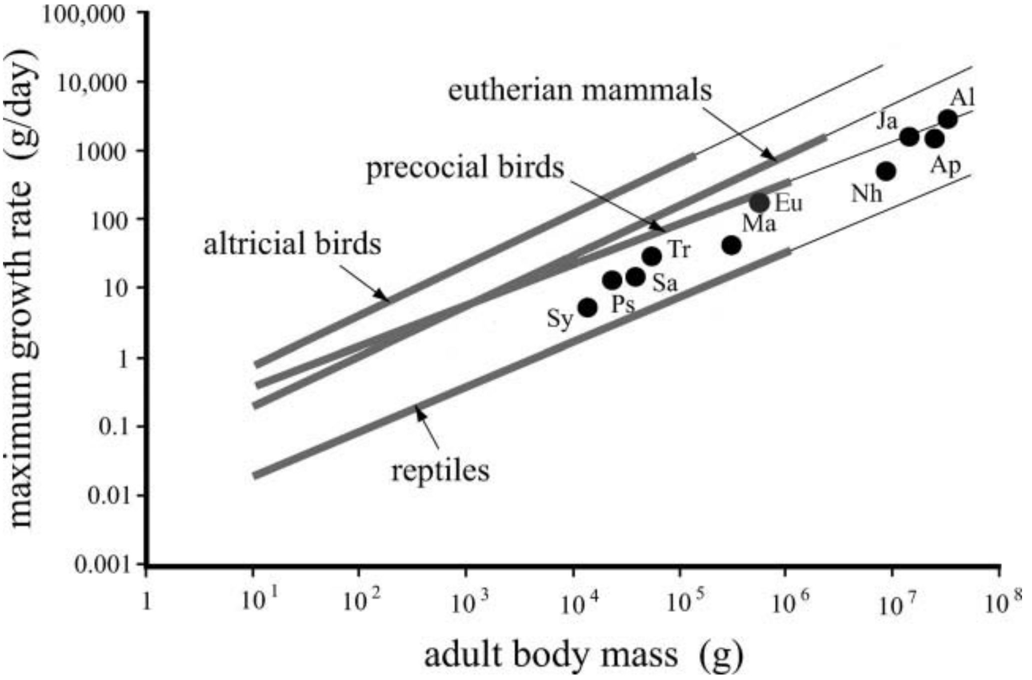


Fig. 1. *Europasaurus* (Eu) maximum growth rate compared with other dinosaurs and extant tetrapods. Note how *Europasaurus* shifted back from typical sauropod growth rates towards a prosauropod condition (cf. Ma, *Massospondylus*), and stays on a general dinosaur growth rates trend line. All the evidence suggests *Europasaurus* retained high metabolic rates. Sym.: *Syntarsus*; Ps:*Psittaosaurus*; Sa: *Saurornitholestes*; Tr: *Toodon*; Ma: *Massospondylus*; Nh: Norhtampton sauropod; Ap: *Apatosaurus*; Ja: *Janenschia*; Al: *Alamosaurus*. Modified from Lehman and Woodward (2008); data from Case (1978a), Erickson (2001), Lehman (2007) and Lehman & Woodward (2008).

The titanosaur *Magyarosaurus* is histologically different from *Europasaurus*, most remarkably by its intense secondary bone remodelling (Chapters 5 & 6). The remodelling makes it impossible to tell if there are growth marks in *Magyarosaurus*, but it certainly indicates skeletal maturity. The precise reasons for extensive remodelling in *Magyarosaurus*, with up to four generations of secondary osteons in the largest individuals, remain unclear.

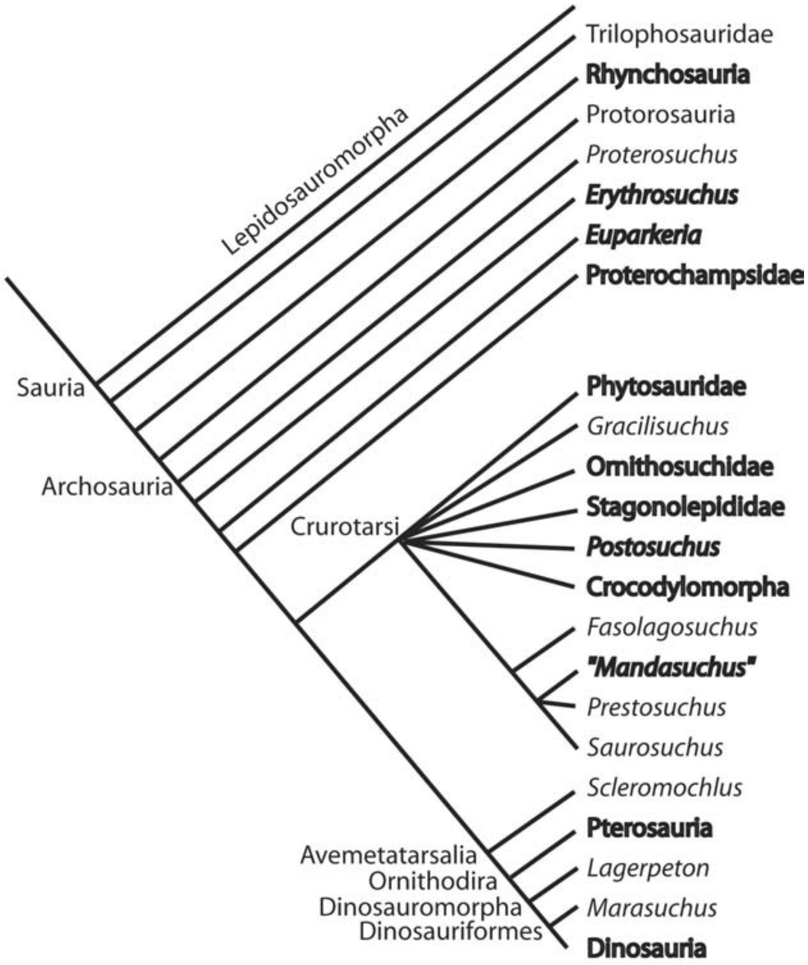
A possible explanation includes peramorphic processes in Titanosauria, with an increased resorption rate in certain parts of the skeleton, possibly coupled with increased pneumatization at the base of Titanosauria, as seen in *Saltasaurus* and *Alamosaurus* ribs and vertebrae (Wedel 2003; Woodward and Lehman 2009). Alternatively the remodelling may be correlated with a change in locomotion, as noted by different anatomical features, and so-called ‘wide-gauge’ trackways (Wilson 2005). However, titanosaur biology and evolution remains poorly studied (Curry Rogers 2005), and needs to be resolved before any hypothesis can be tested.

Nonetheless, the bone histology of *Magyarosaurus* resolved an almost one-century-old hypothesis (Nopcsa 1914), that the small dinosaurs from the Hatzeg Basin are dwarfed, analogous to Pleistocene insular elephants and hippos on the Mediterranean islands. Dwarfing in these island mammals was most likely caused by the same principles causing the dwarfing of these sauropods, which probably also lived on an island. Resource availability in island ecosystems is usually inadequate to support large predators, which releases predation pressure on the largest herbivores, which in turn reduce in size very rapidly (Millien 2006). The suspension of predation pressure, the main force driving the sauropod gigantism cascade (Sander et al. 2010), thus quickly led to the loss of gigantic size in the sauropods on these Mesozoic island systems. In fact most other dinosaurs with large mainland ancestors eventually reduced their body size on these islands (Chapter 6). Interestingly, in a Dollo-like trend, the sauropods did not change their general sauropod bauplan back to a more basal sauropodomorph condition.

3. Selective advantages and origin of FLB in Archosauromorpha

FLB most likely evolved in a dinosaur ancestor (Chapter 4). Most recent insights from basal archosauromorphs suggest that high growth rates were not the basal condition for the group (Werning 2010). However, the selective advantages of tachymetabolic endothermy are numerous in terrestrial environments (Clarke and Pörtner 2010). Therefore, as different terrestrial archosaur clades diversified, these selective advantages possibly led to one or several instances where FLB evolved in the wake of high metabolic rates (cf. Ricqles et al. 2003; 2008; Fig. 2, Table 1). Redispersal into aquatic and semi-aquatic niches also caused a reversal to poikilothermy in other clades, like in modern crocodylians (Seymour et al. 2004).

Fig. 2. Phylogeny of Archosauromorpha following Benton (2004). The pictured tree is a consensus tree of 18 most parsimonious trees. Bootstrapping revealed some weak nodes in Crurotarsi, which are shown collapsed in a polytomy on the cladogram. Taxa and groups marked in bold have been histologically sampled by De Ricqles et al. (2003, 2008) (see Table 1).



taxon	**sampled bones**	**cortical bone tissues**	**cyclical growth**	**publication**
Rhynchosauridae indet	ribs?	parallel fibred, lamellar zonal, longitudinal prim osteons, haversian	Annuli with LAG	Ricqles et al. 2008
Erythrosuchus TYPE	rib, metatarsal	FLB, longitudinal to radial dense vasc, ill defined EFS?	absent	Ricqles et al. 2008
Erythrosuchus	fibula shaft	plexiform vasc. FLB	absent	Ricqles et al. 2008
Teleocrater	metatarsal	FLB longit prim ost., dense endosteal spong. + free marrow cavity metaphyseal?	annulus close to periphery	Ricqles et al. 2008
"Mandasuchus"	femur shaft	FLB, ill defined prim ost.	2 annuli	Ricqles et al. 2008
	ribs	lamellar zonal, scarse secondary osteons	annuli and zones	
"Chanaresuchus"	indet long bone shaft	internal: FLB longit vc external: lamellar zonal	ext. cortex: lamellar zonal with cycles	Ricqles et al. 2008
Euparkeria	long bone (fem/hum) shaft, rib, scutes	FLB, longit vc, similar to *Orodromeus*	absent	Ricqles et al. 2008
Rutiodon	prox femur mid shaft section	internal: sub- plexiform FLB (atypical) external: lamellar zonal secondary osteons present	inner cortex: absent; outer: annuli with LAG	Ricqles et al. 2003
Phytosauria indet.	femur	lamellar zonal, scarse woven bone; lots of secondary remodeling	10 growth cycles preserved	Ricqles et al. 2003
Desmatosuchus	prox hum	lamellar zonal with one zone with FLB; scattered secondary osteons	annuli with one or more LAGs	Ricqles et al. 2003
Desmatosuchus	prox radius	longit prim ost, homogenous matrix, (?longit fibr., few circ fibr.?) internal cortex: dense haversian bone	annuli >10 cycles + many LAGs	Ricqles et al. 2003

Stagonolepis (‘Calyptosuchus’)	fem shaft	Deep cortex: atypical FLB (many circ.fib.) cf. Rutiodon; towards periph: local lamellar zones; scatt. sec. ost.	min 10 annuli with LAGs	Ricqles et al. 2003
Typothorax	prox hum	longitudinal section sec endosteal trabeculae calcified cartilage	NA	Ricqles et al. 2003
Typothorax	radius shaft	lamellar zonal; longit prim. ost.; radial FLB wedge	4-5 cycles; but up to 12 LAGs	Ricqles et al. 2003
Postosuchus	humerus shaft	-deep cortex: haversian -cortex: lamellar zonal with diminishing vasc towards periphery	+- 20 annuli with LAG	Ricqles et al. 2003
Postosuchus	tibia	-deep cortex: haversian and FLB -external cortex: lamellar zonal, almost no secondary remodeling	annuli with LAGs (at least 15) also LAGs within annuli	Ricqles et al. 2003
Terrestrisuchus	humerus	longit vc, FLB, slower circumferential fibres towards periphery	2 LAGs	Ricqles et al. 2003
Alligator (Pleistocene)	humerus	lamellar zonal	12 annuli	Ricqles et al. 2003
Ornithosuchus	rib frag.	FLB? Longit primary osteons, secondary osteons	one distinct annulus	Ricqles et al. 2008
Luperosuchus	large bone shaft	lamellar zonal; deep cortex: longit vc with local FLB	6-7 growth cycles	Ricqles et al. 2008
Hesperosuchus agilis	diaphyses frags	lamellar zonal; ‘islands’ with secondary osteons	plenty annuli	Ricqles et al. 2008
Herrerasaurus	large shaft frag	laminar to plexiform FLB	absent	Ricqles et al. 2008
Herrerasaurus	humerus, tibia	laminar to plexiform FLB	absent	Ricqles et al. 2003

Coelophysis	right femur	plexiform to reticular FLB	one possible LAG, minor modulations	Ricqles et al. 2003
Thecodontosaurus	?left tibia	FLB; secondary osteons	one LAG	Ricqles et al. 2008
Lesothosaurus		FLB; longit primary osteons	absent	Ricqles et al. 2008

Table 1. Archosauromorph taxa sampled by Ricqles et al. (2003, 2008) and their bone microstructure. FLB: fibrolamellar bone, LAG: Line of arrested growth, frag: fragment, vc: vascular canals.

Traditionally, tachymetabolic endothermy has been viewed as the adaptive trait, with a high metabolic rate being needed to produce endothermy, and a high growth rate and FLB being the by-product. Sander & Clauss (2008) noted that the high growth rate might in certain lineages be the major focus of selection, with a high metabolic rate as a prerequisite and endothermy and FLB as by-products. Yet another view, which has not appeared in print before to our knowledge, would be that the high growth rate is the major focus of selection, but with FLB being the key innovation allowing this high growth rate, and a high metabolic rate and endothermy having evolved in its wake. Further research will therefore focus on the origin or origins of FLB and high basal metabolic rates in different archosauromorph lineages.

4. Conclusion

Bone is a living tissue, and will adapt, shape change and heal to some extent throughout an animal's life. Fossil bone is therefore a largely untapped archive of natural history, allowing us to reconstruct the biology, ecology, biomechanics of extinct animals, and the evolution thereof through time. On an evolutionary scale, bone can undergo extreme changes. Depending on the nature and intensity of selective pressures, the bone tissue of an organism will change in accordance with its needs to deal with extrinsic (e.g. changing environments, climates, food availability) and intrinsic factors (e.g. physiology, body size, behaviour). However, sauropod FLB, prerequisite for high growth rates and gigantism, was already present in the earliest sauropodomorph ancestor. It only undergoes minor structural changes as sauropods become large, most likely to cope with high loads, and may have its origin in a more basal archosaur ancestor.

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